ANTIBODY THEORY

Introduction

The Meaning of Antibodies (satire)

by Tom Cowan, M.D.

1. Antibodies are very important for our immune system in getting over viral and bacterial diseases. We make non-specific IgM Antibodies (Ab) initially then specific IgG Ab later. Once we make IgG we are immune for life against that pathogen.

2. An example of this is with mumps, mumps is caused by a virus, we get the disease once, make IgG antibodies and are immune for life.

3. Another example is measles, we test for IgG and prove we are immune for life, well except that in a Supreme Court case in Germany it was shown that the measles virus doesn't exist, never mind.

4. Another example is chickenpox, we get chicken pox once and never again due to IgG antibodies, except if we get shingles which is also chickenpox, but well that's different.

5. If we have AIDS we test for the presence of Ab, if we have them we know for sure that we have a deadly virus which will soon kill us. That's because the HIV virus is "smart" and knows how to evade the Ab unlike the mumps virus which is stupid and doesn't. The measles virus doesn't exist and the chickenpox virus is smart-ish.

6. Another example is Hep C, again if your liver is falling apart you can be tested for Ab and if they are positive it means you have a deadly virus which is eating your liver. This is clearly another example of a smart virus.

7. With rhino virus, the cause of the common cold, we, naturally develop antibodies to the virus, but this virus is very smart (seeing as how it jumped to humans as a result of the unfortunate tendency of Africans to eat rhinos) and many of us get colds year after year in spite of the presence of antibodies.

8. Similarly the influenza virus is very, very smart and unlike the measles virus which not only doesn't exist but has remained constant in its non-existence for centuries, the flu virus changes

its form yearly. This is undoubtedly because the flu virus is so smart it has included flu vaccine companies in its stock market portfolio. I wish I were as smart as the flu virus.

7. If you have symptoms of Lyme's disease and you test for the presence of Ab and show them to your infectious disease doctor and tell him you tested positive for antibodies which means you have Lyme disease, he will throw you out of the office and call you a fucking lunatic. That's because the presence of Ab don't mean anything in Lyme's disease

8. If you have Covid and you test positive for Ab it means you either had the virus or you didn't have the virus. Also, it is clear evidence that you were either sick or not sick. That's because the corona virus is so smart that it can trick you into either making Ab or not to throw off the immunologists.

Hope this makes it perfectly clear.

By Dr. T.C.

REPRODUCIBILITY CRISIS IN ANTIBODY RESEARCH:

Over the last few decades, it has been shown that there is a huge reproducibility crisis going on in science. This was brilliantly pointed out by John Ioannidis, a very well-respected physician/scientist and professor at Stanford, who concluded in a 2005 essay he wrote that most of the published scientific literature is wrong. One of the reasons for this comes down to a lack of reproducibility:

"Several methodologists have pointed out [9–11] that the HIGH RATE OF NON REPLICATION (lack of confirmation) OF RESEARCH discoveries is a consequence of the convenient, yet ill-founded strategy of CLAIMING CONCLUSIVE RESEARCH FINDINGS SOLELY ON THE BASIS OF A SINGLE STUDY"

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1182327/

Over the years, it has been shown that the lack of reproducibility has been discovered in many areas relating to Virology including cell cultures, genomics, and PCR. So knowing that most of the scientific literature is wrong and extends to many areas Virology incorporates, it shouldn't come as any surprise that this reproducibility crisis is happening in the world of antibodies as well.

Below are highlights from 4 articles detailing the antibody reproducibility crisis. The evidence

was first presented in 2015 and I've included articles from the last few years to show there hasn't been any improvement since. Rather than summarize each article independently (thus making this post even longer than it already is), I will summarize key areas of information from each at the end:

REPRODUCIBILITY CRISIS: BLAME IT ON THE ANTIBODIES (2015):

"In 2006, things were looking pretty good for David Rimm, a pathologist at Yale University in New Haven, Connecticut. He had developed a test to guide effective treatment of the skin cancer melanoma, and it promised to save lives. IT RELIED ON ANTIBODIES — large, Y-shaped proteins that bind to specified biomolecules and can be used to flag their presence in a sample. Rimm had found a combination of antibodies that, when used to 'stain' tumour biopsies, produced a pattern that indicated whether the patient would need to take certain harsh drugs to prevent a relapse after surgery. He had secured more than US$2 million in funding to move the test towards the clinic.

But in 2009, everything started to fall apart. When Rimm ordered a fresh set of antibodies, HIS TEAM COULD NOT REPRODUCE THE ORIGINAL RESULTS. The antibodies were sold by the same companies as the original batches, AND WERE SUPPOSED TO BE IDENTICAL — BUT THEY DID NOT YIELD THE SAME STAINING PATTERNS, EVEN ON THE SAME RUMOURS. Rimm was forced to give up his work on the melanoma antibody set. “We learned our lesson: WE SHOULDN'T HAVE BEEN DEPENDENT ON THEM,” he says. “That was a very sad lab meeting.”

"Antibodies are among the most commonly used tools in the biological sciences — put to work in many experiments to identify and isolate other molecules. BUT IT IS NOW CLEAR THAT THEY ARE AMONG THE MOST COMMON CAUSES OF PROBLEMS, too. THE BATCH-TO-BATCH VARIABILITY that Rimm experienced CAN PRODUCE DRAMATICALLY DIFFERENT RESULTS. Even more problematic is that ANTIBODIES OFTEN RECOGNIZE EXTRA PROTEINS IN ADDITION TO THE ONES THEY ARE SOLD TO DETECT. This can cause projects to be abandoned, and waste time, money and samples.

MANY THINK THAT ANTIBODIES ARE A MAJOR DRIVER OF WHAT HAS BEEN DEEMED A 'REPRODUCIBILITY CRISIS', a growing realization that the results of MANY BIOMEDICAL EXPERIMENTS CANNOT BE REPRODUCED AND THAT THE CONCLUSIONS BASED ON THEM MAY BE UNFOUNDED. POORLY CHARACTERIZED ANTIBODIES PROBABLY CONTRIBUTE MORE TO THE PROBLEM THAN ANY OTHER LABORATORY TOOL, says Glenn Begley, chief scientific officer at TetraLogic Pharmaceuticals in Malvern, Pennsylvania, and author of a controversial analysis SHOWING THAT RESULTS IN 47 OF 53 LANDMARK CANCER RESEARCH PAPERS COULD NOT BE REPRODUCED."

"BUYER BEWARE

Take the example of Ioannis Prassas, a proteomics researcher at Mount Sinai Hospital in Toronto, Canada. He and his colleagues had been chasing a protein called CUZD1, which they thought could be used to test whether someone has pancreatic cancer. They bought a protein-detection kit and wasted two years, $500,000 and thousands of patient samples before they realized that THE ANTIBODY IN THE KIT WAS RECOGNIZING A DIFFERENT CANCER PROTEIN, CA125, and did not bind to CUZD1 at all2. In retrospect, Prassas says, A RUSH TO GET GOING ON A PROMISING HYPOTHESIS MEANT THAT HE AND HIS GROUP HAD FAILED TO DO ALL THE RIGHT TESTS. “If someone says, 'Here is an assay you can use,' you are so eager to test it you can forget that what has been promised is not the case.”

Most scientists who purchase antibodies believe the label printed on the vial, says Rimm. “AS A PATHOLOGIST, I WASN'T TRAINED THAT YOU HAD TO VALIDATE ANTIBODIES; I WAS JUST TRAINED THAT YOU ORDERED THEM.”

"For a more consistent product, the B cells can be retrieved, fused with an 'immortalized' cell and CULTURED TO PROVIDE A THEORETICALLY UNLIMITED SUPPLY.

Three decades ago, SCIENTISTS WHO NEEDED ANTIBODIES FOR THEIR EXPERIMENTS HAD TO MAKE THEM THEMSELVES. But by the late 1990s, reagent companies had started to take over the chore.

Today, more than 300 companies sell over 2 million antibodies for research. As of 2011, the market was worth $1.6 billion, according to global consultancy Frost & Sullivan."

"DEVASTATING EFFECTS

THERE ARE SIGNS THAT PROBLEMS WITH ANTIBODIES ARE HAVING BROAD AND POTENTIALLY DEVASTATING EFFECTS ON THE RESEARCH RECORD. In 2009, one journal devoted an entire issue to assessing the antibodies that are used to study G-protein-coupled receptors (GPCRs) — cell-signalling proteins that are targeted by drugs to treat various disorders, from incontinence to schizophrenia. In an analysis of 49 commercially available antibodies that targeted 19 signalling receptors, MOST BOUND TO MORE THAN ONE PROTEIN, MEANING THAT THEY COULD NOT BE TRUSTED TO DISTINGUISH BETWEEN THE RECEIPTS.

The field of epigenetics relies heavily on antibodies to identify how proteins that regulate gene expression have been modified. In 2011, an evaluation of 246 antibodies used in epigenetic studies found that ONE-QUARTER FAILED TESTS FOR SPECIFICITY, MEANING THAT THEY OFTEN BOUND TO MORE THAN ONE TARGET. Four antibodies were perfectly specific — BUT TO THE WRONG TARGET."

"Scientists often know, anecdotally, that some antibodies in their field are problematic, but it has been difficult to gauge the size of the problem across biology as a whole. Perhaps the largest assessment comes from work published by the Human Protein Atlas, a Swedish

consortium that aims to generate antibodies for every protein in the human genome. It has looked at some 20,000 commercial antibodies so far and found that less than 50% can be used effectively to look at protein distribution in preserved slices of tissue. This has led some scientists to claim that UP TO HALF OF ALL COMMERCIALLY AVAILABLE ANTIBODIES ARE UNRELIABLE.

BUT RELIABILITY CAN DEPEND ON THE EXPERIMENT. “Our experience with commercial antibodies is that they are USUALLY OKAY IN SOME APPLICATIONS, BUT THEY MIGHT BE TERRIBLE IN OTHERS,” says Mathias Uhlén at the Royal Institute of Technology in Stockholm, who coordinates the Human Protein Atlas.

Researchers ideally should check that an antibody has been tested for use in particular applications and tissue types, BUT THE QUALITY OF INFORMATION SUPPLIED BY VENDORS CAN VARY TREMENDOUSLY. A common complaint from scientists is that companies DO NOT PROVIDE THE DATA REQUIRED TO EVALUATE A GIVEN ANTIBODY'S SPECIFICITY OR ITS LOT-TO-LOT VARIABILITY. Companies might ship a batch of antibodies with characterization information derived from a previous batch. AND THE DATA ARE OFTEN DERIVED UNDER IDEAL CONDITIONS THAT DO NOT REFLECT TYPICAL EXPERIMENTS. Antibody companies contacted for this article said that it is IMPOSSIBLE TO TEST THEIR PRODUCTS ACROSS ALL EXPERIMENTAL CONDITIONS, but they do provide reliable data and work with scientists to improve antibody quality and performance.

Many academics use Google to find products, so OPTIMIZING SEARCH RESULTS CAN SOMETIMES MATTER MORE TO A COMPANY THAN OPTIMIZING THE ACTUAL REAGENTS, says Tim Bernard, head of the biotechnology consultancy Pivotal Scientific in Upper Heyford, UK. Christi Bird, a Frost & Sullivan analyst based in Washington DC, says that researchers are often more interested in how quickly reagents can be delivered than in searching for antibodies with appropriate validation data. “It's the Amazon effect: they want it in two or three days, with free shipping.”

Researchers who are aware of the antibody problem say that scientists need to be more vigilant. “Antibodies are not magic reagents. YOU CAN'T JUST THROW THEM ON YOUR SAMPLE AND EXPECT THE RESULT YOU GET IS 100% RELIABLE without putting some critical thinking into it,” says James Trimmer, head of NeuroMab at the University of California, Davis, which makes antibodies for neuroscience. Like many suppliers, NeuroMab explicitly states the types of experiment that an antibody should be used for, but scientists do not always follow the instructions."

"By necessity, many researchers rely on word of mouth or the published literature for advice. But that creates a self-perpetuating problem, in which better-performing antibodies that become available later are rarely used, says Fridtjof Lund-Johansen, a proteomics researcher at the University of Oslo. “We have very good antibodies on the market,” he says, “BUT WE DON'T

KNOW WHAT THEY ARE.” Lund-Johansen is trying to change that by developing high-throughput assays that could compare thousands of antibodies at once."

"Some scientists are calling for much more radical change. In a Comment in Nature in February7, Andrew Bradbury of Los Alamos National Laboratory in New Mexico and more than 100 co-signatories proposed a massive shift in the way antibodies are produced and sold. They suggested using only antibodies that have been defined down to the level of the DNA sequence that produces them, and then manufactured in engineered 'recombinant' cells. This would circumvent much of the variability introduced by production in animals. BUT THE PROPOSAL DEMANDS INFORMATION ABOUT INDIVIDUAL ANTIBODIES THAT MANY COMPANIES CONSIDER TO BE TRADE SECRETS — and the antibody marketplace and its millions of products would have to be essentially demolished and reconstructed."

"THE PRESSURE TO CHARACTERIZE CURRENTLY AVAILABLE ANTIBODIES IS SURGING. As part of efforts to improve reproducibility, some researchers have started to discuss enlisting an independent body to establish a certification programme for commercial antibodies. And several journals (including Nature) ask authors to make clear that antibodies used in their papers have been profiled for that particular application.

The quality will creep, rather than leap, forward, says Trimmer, who hopes to see a positive-feedback loop: AS SCIENTISTS BECOME AWARE OF ARTEFACTS, THEY WILL BE MORE LIKELY TO CHALLENGE RESULTS AND UNCOVER MORE ARTEFACTS. Already, he says, THE WIDESPREAD INSOUCIANCE ABOUT ANTIBODY VALIDATION has started to fade. “It's turning around a little bit,” he says. “We need to keep talking about it.”

https://www.nature.com/news/reproducibility-crisis-blame-it-on-the-antibodies-1.17586

ANTIBODY VALIDATION…IS THERE STILL A CRISIS? (2019):

"Over the years there has been much debate regarding who should take responsibility for antibody validation. Is it the responsibility of the antibody supplier to offer a fully characterised and validated antibody, or should the researcher validate the antibody specifically for their own needs? Initially there was a greater onus on the researcher to check an antibody performed as it should in their assays. CURRENT OPINION PLACES A LARGER OBLIGATION ON THE ANTIBODY MANUFACTURERS TO ENSURE LOT TO LOT CONSISTENCY AND SPECIFICITY FOR THE ANTIBODY TARGET. As part of our State of the Industry survey published in our 2019 antibody market report, the manufacturers themselves were polled for their opinions on antibody validation. A LARGER PERCENTAGE OF MANUFACTURERS, COMPARED TO THE PREVIOUS YEAR, BELIEVED ANTIBODY VALIDATION IS A PROBLEM that should be tackled by the industry. Only 45.8% of the companies polled in the 2018 survey thought it was an industry problem compared to 57.6% in 2019. When asked to give suggestions on how to improve quality standards, 37% SUGGESTED

THE IMPLEMENTATION OF GLOBAL STANDARDS."

"A bigger shake up of the research reagent market was presented in the publication by Bradbury and Pluckthun in the scientific journal Nature. Bradbury, Pluckthun and 110 co-signatories called for funding to allow the genetic sequencing of all hybridoma-produced monoclonal antibodies to key targets. DESCRIBING INCONSISTENT LEVELS OF CHARACTERIZATION BETWEEN MANUFACTURERS, AND POTENTIAL PROBLEMS ASSOCIATED WITH HYBRIDOMAS. They proposed that replacing these antibodies with recombinant versions would ensure a never-ending supply of highly-characterised antibodies. Thus, PROMOTING THE TRANSITION TOWARDS THE EXCLUSIVE USE OF RECOMBINANT ANTIBODIES, AND EVEN SUGGESTING THE PHASING OUT OF POLYCLONAL ANTIBODIES ALTOGETHER!"

"At the 3rd International Antibody Validation Meeting held in Bath, THE IMPORTANCE OF ANTIGENS IN ANTIBODY GENERATION, CHARACTERIZATION AND VALIDATION WAS ALSO STRESSED. Speakers highlighted the need for them to be full-length, correctly-folded, 'AUTHENTIC' proteins for effective antibody generation. Furthermore, discussions included factors such as antigen abundance and availability, which should be taken into account when planning an antibody characterisation and validation strategy. The role of the scientists purchasing these antibodies was not ignored, with an emphasis on correct usage, as antibody performance is application dependent."

https://pivotalscientific.com/scientific-library/antibody-validation-is-there-still-a-crisis

WHEN ANTIBODIES MISLEAD: THE QUEST FOR VALIDATION (2020):

"Commercial antibodies are commonplace in biology laboratories. Researchers use these giant Y-shaped proteins to detect specific molecules in cells, tissues and test tubes. BUT SOMETIMES THE PROTEINS DETECT OTHER MOLECULES, TOO — OR EVEN INSTEAD. When that happens, confusion can snowball."

"Antibodies work by binding to specific parts of a protein, according to the protein's shape and chemical properties, BUT AN ANTIBODY PRODUCED TO BIND TO ONE PROTEIN CAN OFTEN BIND TO ANOTHER, AND SOMETIMES WITH BETTER AFFINITY."

"That's borne out in McPherson's work. He and his team bought 16 antibodies marketed to detect CR9ORF72. Then they took a cell line that produces the protein at high levels and used the genome-editing tool CRISPR–Cas9 to make a line in which CR9ORF72 was knocked out, so the protein would not be present. THEY THEN ASSESSED HOW THE ANTIBODIES PERFORMED IN THE TWO LINES IN A SERIES OF COMMON TESTS AND FOUND THAT THE ANTIBODY THAT HAD BEEN USED IN THE MOST PUBLICATIONS (and cited most often) FOUND THE PROTEIN EVEN WHEN IT WASN'T THERE. Those that worked best for each assay had not appeared in the literature at all.

Others have reported comparable experiences. Cecilia Williams, a cancer researcher at the KTH Royal Institute of Technology in Stockholm, tested 13 antibodies to try to untangle conflicting data about estrogen receptor β, a protein discovered in 1996 that is a potential anticancer target. TWELVE OF THE ANTIBODIES, INCLUDING THE TWO MOST POPULAR, GAVE EITHER FALSE POSITIVES OR FALSE NEGATIVES, OR BOTH, she and her team reported. “DON’T TAKE EITHER THE LITERATURE OR THE ANTIBODY FOR GRANTED,” she warns."

"Researchers often buy antibodies according to the number of times the product has been cited in the literature, but that strategy can overlook newer products that have been put through more rigorous tests. THEY ALSO TEND TO ASSUME THAT OTHERS WHO USED THE ANTIBODY BEFORE THEM CHECKED THAT IT WORKED AS INTENDED, AND THAT IT WILL THEREFORE WORK IN THEIR OWN EXPERIMENTS, OPENING THE DOOR FOR SELF-PERPETUATING ARTEFACTS.

“WHEN I LOOK AT PAPERS IN GENERAL, I GET DEPRESSED BY THE QUALITY OF THE ANTIBODY CHARACTERIZATION,” says Simon Goodman, a science consultant at the Antibody Society, a not-for-profit professional association. Goodman is based in Darmstadt, Germany, and has organized a series of educational webinars on appropriate techniques for the society3. “If you ask ‘HOW DID YOU VALIDATE THE ANTIBODY?’, researchers will say, ‘WELL WE BOUGHT IT AND THE PRODUCER SAYS THAT IT BEHAVES LIKE THIS.’”

Often, the data that companies provide to show an antibody works COME FROM A CELL LINE THAT HAS BEEN ENGINEERED TO EXPRESS THE PROTEIN AT LEVELS SUBSTANTIALLY HIGHER THAN UNDER PHYSIOLOGICAL CONDITIONS."

"Not every antibody can be tested using knockout controls, Edwards admits. About 10% of genes are essential to life, so a knockout cell line is not viable for them. Also, AN ANTIBODY THAT PERFORMS WELL IN ONE CELL LINE COULD FALL SHORT IN ANOTHER."

"IDENTITY CRISIS

SOMETIMES IT'S NOT EVEN CLEAR WHICH ANTIBODY RESEARCHERS HAVE USED, especially in older studies. ONLY ABOUT 11% OF THE ANTIBODIES USED IN PAPERS PUBLISHED IN 1997 ARE IDENTIFIABLE, according to an analysis led by researchers at the University of California, San Diego (UCSD), and the data-sharing platform SciCrunch in La Jolla, California; nowadays, that figure has risen to 43%."

"The bottom line is: HOWEVER AN ANTIBODY-DRIVEN EXPERIMENT COMES OUT, RESEARCHERS WOULD BE WISE TO BE SCEPTICAL. When experiments fail, researchers often question their own technique, says Goodman. “Of course you blame yourself as a young scientist.” BUT THE SCIENTIFIC COMMUNITY SHOULD BE EQUALLY SCEPTICAL OF ANTIBODIES THAT SEEM TO WORK, says Edwards, and demand evidence that they do before relying on them. “WE BUY

ANTIBODIES, WE DON'T TEST THEM, AND THEN WE PUBLISH ARTICLES THAT SEND THE FIELD SIDEWAYS."

https://www.nature.com/articles/d41586-020-02549-1

ANTIBODY INACCURACY, NON-SPECIFICITY AND IRREPRODUCIBILITY AT THE ROOT OF RESEARCH EXTINCTION (2021):

"One of the most commonly used and critical reagents found in most biomedical research laboratories is central to what is known as "The Reproducibility Crisis". AN ALARMING NUMBER OF RESEARCHERS IN THE LIFE SCIENCES HAVE NOTED IRREPRODUCIBILITY IN THEIR ANTIBODY-BASED EXPERIMENTS, fueled by insights originally unearthed by A. Bradbury and C. Glenn Begley (1–3). Through their desire to highlight the unregulated state of the antibody industry, Bradbury and Begley have helped push the need for industry reform including the need for more rigorous testing and validation.

AT THE ROOT OF ANTIBODY IRREPRODUCIBILITY IS THE CHALLENGE OF BATCH VARIABILITY. This occurs when antibodies are sold under the same catalogue number and by the same vendor, however EXHIBIT DIFFERENT SPECIFICITY AND/OR AFFINITY. THIS IS OFTEN THE RESULT OF CELL-CULTURING ENVIRONMENTS AND/OR DIFFERENT PRODUCING ANIMALS. Researchers not only need to overcome the challenges associated with commercial antibody production, if they opt to produce their own, they can face a multitude of other obstacles. Notably, MAINTAINING THE HIGH-STANDARD PROCEDURAL CONTROLS TO MAINTAIN CELL LINE VIABILITY.

The challenges associated with antibody production and supply pose a significant problem for researchers, with the worst-case scenarios PREVENTING EXPERIMENTAL REPRODUCIBILITY."

"1. BATCH-TO-BATCH VARIABILITY – A SIDE EFFECT OF POLYCLONAL ANTIBODY DEVELOPMENT

There are different factors contributing to the antibody reproducibility crisis. The first comes from the nature of antibody production. In human and animal bodies, antibodies are secreted by B cells in a polyclonal mixture of heterogeneous molecules that recognize and bind to different epitopes of an antigen. In the lab, polyclonal antibodies (pAbs) are produced by injecting a particular antigen into an animal that elicits an immune response. The pAbs secreted are then harvested from the animal and used in different antigen-specific experiments.

THE REPRODUCIBILITY CRISIS OF POLYCLONAL-BASED DEVELOPMENT STEMS FROM BATCH-TO-BATCH VARIABILITY THAT COMMONLY OCCURS DURING PRODUCTION. The problem arises when different host animals are injected with the same antigen but then PRODUCE pAbs THAT

HAVE DIFFERENT SPECIFICITIES AND AFFINITIES. This means that even if one was to use a new batch of antibodies, THEY CANNOT REPRODUCE THE EXACT SAME EXPERIMENTAL RESULTS. Even more importantly, the same animal sometimes may have different immune responses to the same antigen and also exhibit time-dependent variation known as the affinity maturation process.

2. FRAGILE, UNSTABLE HYBRIDOMAS ARE ENDANGERING MONOCLONAL ANTIBODIES

Ever since the breakthrough discovery of hybridoma technology by Köhler and Milstein in 1975, it has remained to be the primary method for scientists to produce monoclonal antibodies (mAbs). The process starts by injecting animals with specific antigen that provokes an immune response. Individual B cells producing antibodies that bind to the injected antigen are then isolated from the animal and fused with myeloma cells to produce a hybrid cell line called a hybridoma. By fusing short-lived antibody-producing B cells with immortal myeloma cells, hybridoma cells are expected to provide a never-ending supply of identical mAbs.

However, in the laboratory, HYBRIDOMAS ARE OFTEN CONSIDERED UNSTABLE AND FRAGILE, so they require high-standard procedural controls to maintain cell line viability. ALTHOUGH RESEARCHERS SPEND A SIGNIFICANT AMOUNT OF TIME AND EFFORT ON CULTURING HYBRIDOMA CELL LINES, POOR GROWTH OR EVEN CELL DEATH STILL OCCURS REGULARLY. Under normal conditions, ANTIBODY CONTENTS CAN DECREASE CONTINUOUSLY DURING THE COURSE OF HYBRIDOMA CULTIVATION (4). Many other factors can lead to failure of hybridoma survival. Long-time storage, repeated freeze-thaw cycles, improper handling, and contamination CAN ALL CAUSE HYBRIDOMA DEATH AND PERMANENT LOSS OF IMPORTANT ANTIBODIES.

In addition, hybridoma cell lines are also known to undergo gene mutations and rearrangements over time, LEADING TO ANTIBODY HETEROGENEITY AND BATCH-TO-BATCH VARIABILITY (5).

A recent study of 185 clonal hybridoma cell lines identified NEARLY A THIRD CONTAINED ADDITIONAL HEAVY OR LIGHT CHAIN GENES, RESULTING IN IMPAIRED AFFINITY AND SPECIFICITY TO THE TARGET ANTIGEN. An investigation conducted by Rapid Novor in 2019 showed similar results – among 80 research-purpose mAbs analyzed, a significant portion (14%) were shown to have a second light chain present (Figure 1). In other cases, HYBRIDOMAS MAY EVEN LOSE THE CHROMOSOME CONTAINING ANTIBODY GENES COMPLETELY ARRESTING THE PRODUCTION OF ANTIBODIES (6).

3. LACK OF STANDARDIZED VALIDATION – WHEN ANTIBODIES MISLEAD

Despite the complexity and uncertainty of antibody production, THERE IS NO STANDARDIZED PROTOCOL OR REGULATION FOR THE VALIDATION OF ANTIBODIES, INCLUDING ASSESSING THEIR SPECIFICITY, AFFINITY, SEQUENCES, AND APPLICATIONS IN DIFFERENT ASSAYS.

Among over 2 million commercial antibodies provided by more than 300 vendors, the majority of them are polyclonal. THE QC DATA listed on the product sheets are often obtained from previous batches and NO LONGER VALID FOR THE ONES DELIVERED TO USERS. With regard to mAbs, there is also NO WAY OF KNOWING IF THE CURRENT HYBRIDOMA CELL LINE IS CHARACTERIZED AS STABLE AND FUNCTIONAL. This largely stems from the issue of inconsistent catalog numbers that have not accounted for new culturing environments, production animals or company merges. LACK OF A PROPER VALIDATION PROCESS LEADS TO THE RELEASE OF A LARGE NUMBER OF POORLY CHARACTERIZED ANTIBODIES ("bad antibodies") to users for research. It was estimated that despite there being 2 million antibodies on the market, only 12.5 – 25% are considered unique 'core' antibodies (7).

When it comes to selecting key antibodies, the process varies by user and laboratory. For example, some labs regularly purchase from their trusted vendors, some rely on word of mouth, and some buy a few from different vendors and then test which one works. One similarity tends to exist, many researchers will reference publications in which their antibodies were referenced or will refer to the information provided on the product sheets. However, this still does not guarantee a perfectly safe decision. Take for example the following antibodies that were used to identify therapeutically relevant clinical biomarkers. Despite all of these antibodies being regularly cited in industry publications and journals for their respective use cases, ALL WERE SHOWN TO EXHIBIT CROSS-REACTIVITY RESULTING IN SIGNIFICANT SUMS OF RESEARCH RESOURCES BEING LOST.

Thus, using antibodies as biomarker tools for detection can be potentially fatal if they have not been fully verified with rigorous scrutiny. The unfortunate reality is, IT'S NO LONGER SUFFICIENT TO RELY SOLELY ON VENDOR'S QUALITY ASSURANCE PROTOCOLS OR SCIENTIFIC PUBLICATIONS, it is necessary to independently assess and verify candidates."

https://www.rapidnovor.com/hybridoma/hybridoma-whitepapers/antibody-inaccuracy-non-specificity-and-irreproducibility-at-the-root-of-research-extinction

In Summary:

-numerous scientists have come forward claiming the inability to reproduce their own results using the same exact antibodies

-batch-to-batch variability has led to consistent differing results and proteins being bound that shouldn't be

-many believe antibodies are a main driver in the reproducibility crisis

-the lack of characterization of the antibodies has led to many false/unreproducible results

-the lack of specificity has been discovered with antibodies binding to more than one protein or not binding to the one they are supposed to

-at least half of the commercially available antibodies are unreliable

-vendors are not supplying validated data on their antibody products

-vendors are more concerned with protecting trade secrets than supplying validation

-current opinion is that the manufacturers should be in charge of addressing the problems of lot-to-lot variability, specificity, and characterization

-there is an increasing effort to move away from polyclonal and monoclonal antibodies in favor of recombinant antibodies in order to solve the crisis

(Quick side note: Recombinant Antibodies are mostly synthetic:

"Basically, recombinant antibodies are monoclonal antibodies GENERATED IN VITRO USING SYNTHETIC GENES. The technology involves recovering antibody genes from source cells, amplifying and cloning the genes into an appropriate phage vector, introducing the vector into a host (bacteria, yeast, or mammalian cell lines), and achieving expression of adequate amounts of functional antibody."

https://info.gbiosciences.com/blog/recombinant-antibodies-an-overview

-researchers often use antibodies cited by other researchers and ASSUME they are checked/validated

-many are warning that researchers should be skeptical of antibody research and any which show that antibodies work

-lack of antibody specificity/affinity is due to the cell culturing environment

-3 main problems contributing to the reproducibility crisis:

1. Batch-to-batch variability

2. Fragile, unstable hybridomas

3. Lack of standardized validation

There is quite a bit of great information in these articles which my highlights can not do justice so I highly recommend reading them for more in-depth information.

It should be clear now, with the lack of proof of purified/isolated antibodies over the last century as well as the alarming reproducibility crisis, why researchers/scientists/Dr.'s etc. have no clue how to answer questions about antibodies. They can't say how many antibodies are needed for protection. They can't say if they offer lifetime immunity or any immunity at all. They can't explain how antibodies can be a sign of protection in regards to vaccines yet are a sign of illness in regards to HIV. They can't interpret the test results accurately, explain what they mean, nor provide any reasons for the high rates of false-positives. They simply don't know anything about these hypothetical/theoretical particles because the "science" behind them is questionable at best.

ACP's NEW "COVID-19" ANTIBODY GUIDELINES:

Yesterday, the American College of Physicians put out new guidelines in response to "Covid-19" and immunity. What is clear after having read through them is that we are well over a year into this "pandemic" and they know absolutely nothing about the role of antibodies in regards to "Covid-19." These guidelines put a spotlight not only on the antibody scam but the "Coronahoax" as well. A nice overview was presented by Renal and Urology News:

"NEW GUIDANCE: AMERICAN COLLEGE OF PHYSICIANS DISCUSSES ANTIBODY RESPONSE IN COVID-19 IMMUNITY Because of the novelty of the coronavirus that causes COVID-19, THERE IS NOT ENOUGH EVIDENCE TO DETERMINE WHETHER ANTIBODIES PRODUCED AFTER EXPOSURE ARE PROTECTIVE AGAINST REINFECTION. As such, the American College of Physicians (ACP) published rapid, evidence-based living practice points in the Annals of Internal Medicine discussing the role of antibodies in, tests for diagnosing, and tests for estimating the prevalence of COVID-19.

PRACTICE POINT 1: ANTIBODY TESTS FOR COVID-19 DIAGNOSIS

The ACP DOES NOT RECOMMEND USING SARS-CoV-2 ANTIBODY TESTS to diagnose COVID-19. This recommendation is based on the limited evidence that suggests NOT ALL PATIENTS WITH COVID-19 DEVELOP ANTIBODIES early in the course of their infection, as the PRESENCE AND LEVELS OF ANTIBODIES CAN VARY ACROSS PATIENTS and be dictated by certain disease

characteristics.

PRACTICE POINT 2: ANTIBODY TESTS FOR ESTIMATING COMMUNITY PREVALENCE

STUDIES SUGGEST THAT PATIENTS DEVELOP IMMUNE RESPONSES FOLLOWING EXPOSURE TO THE NOVEL CORONAVIRUS. The evidence shows immunoglobulin (Ig)A and IgM antibodies are detectable in the majority of patients who are infected with the SARS-CoV-2 virus. Nearly all patients also demonstrate detectable IgG and neutralizing antibodies.

Over time, THE PREVALENCE AND LEVELS OF THESE ANTIBODIES MAY VARY BY DIFFERENT PATIENT CHARACTERISTICS, DISEASE SYMPTOMS, AND DISEASE SEVERITY. On average, the levels of each of the antibody types peak between 20 to 31 days following symptom onset or polymerase chain reaction diagnosis. Studies also show that the IgM antibodies may persist for up to 115 days and neutralizing antibodies may persist up to 152 days. Therefore, the ACP notes that antibody tests could be feasible options for estimating community prevalence of COVID-19.

PRACTICE POINT 3: THE PROTECTIVE EFFECT OF SARS-CoV-2 ANTIBODIES AGAINST REINFECTION

THERE IS A PAUCITY OF EVIDENCE TO SUGGEST THAT NATURAL IMMUNITY IS CONFERRED BY SARS-CoV-2 ANTIBODIES. THERE IS NO EVIDENCE TO SUGGEST SARS-CoV-2 ANTIBODIES CAN PREDICT THE PRESENCE, LEVEL, OR DURABILITY OF ANY CONFERRED NATURAL IMMUNITY, ESPECIALLY AS IT RELATES TO PROTECTION AGAINST REINFECTION.

Given that most patients exhibit detectable antibodies at least 100 days after infection, IT MAY BE PLAUSIBLE THAT NATURAL IMMUNITY CAN OCCUR. However, the panel reiterates that there is NO DIRECT EVIDENCE TO ANSWER THE QUESTION OF WHETHER THESE ANTIBODIES CAN PROTECT AGAINST REINFECTION.

Some literature indicates that both asymptomatic and symptomatic patients can develop an antibody response indicative of natural immunity following COVID-19, but variables such as disease severity, patient factors, type and amount of antibodies developed, as well as the longevity of those antibodies, play an important role.

The guideline panel cites a small study of hospitalized patients with COVID-19 that reported a single possible case of reinfection during the convalescence stage. This patient did not have IgM or IgG antibodies detected at the 4-week follow-up period.

LIMITATIONS OF THE PRACTICE POINTS

According to the guideline authors, THE PRACTICE POINTS PRESENTED CONCERN ONLY THE ANTIBODY-MEDIATED NATURAL IMMUNITY RESPONSE IN COVID-19 and do not particularly address the involvement of other natural immune responses, including cell-mediated immunity OR VACCINE-ACQUIRED IMMUNITY.

CURRENTLY, THE ONLY EVIDENCE-BASED RECOMMENDATIONS FOR INCREASING IMMUNITY TO THE SARS-CoV-2 VIRUS AND PRESENTING INFECTIONS IS TO RECEIVE AN AUTHORIZED COVID-19 VACCINE. Additional prevention strategies recommended in the guideline include social distancing, wearing a mask in public, quarantining, and regular hand washing.

“GIVEN LIMITED KNOWLEDGE ABOUT THE ASSOCIATION BETWEEN ANTIBODY LEVELS AND NATURAL IMMUNITY,” the guideline authors wrote, “patients with SARS-CoV-2 infection and those with a history of SARS-CoV-2 infection should follow recommended infection prevention and control procedures to slow and reduce the transmission of SARS-CoV-2.”

https://www.renalandurologynews.com/home/departments/mens-health-update/american-college-of-physicians-guidelines-antibody-response-in-sars-cov-2-reinfection

In Summary (Part 1):

-there is NOT ENOUGH EVIDENCE to determine if antibodies produced after exposure provide protection from reinfection

-they recommend not using antibody tests for "Covid-19" diagnosis

-not all patients with "Covid" develop antibodies early

-presence and levels of antibodies vary person to person

-studies SUGGEST patients develop an immune response after "Covid" exposure (except for asymptomatic I suppose...)

-prevalence and levels of antibodies may vary due to patient characteristics, disease symptoms, and disease severity

-there is a PAUCITY (i.e. scarcity) of evidence to suggest that natural antibodies from "Covid" infection confers immunity

-there is NO EVIDENCE to suggest that "Covid" antibodies can predict presence, level, or durability of any conferred immunity, especially regarding reinfection

-they state it MAY BE PLAUSIBLE that natural immunity can occur

-however, they reiterate that there is NO DIRECT EVIDENCE that the antibodies protect against reinfection

-the practice points are only in regards to ANTIBODY-MEDIATED NATURAL IMMUNITY

-they state the only "evidence-based" method to increase immunity is through vaccination (ARTIFICIAL IMMUNITY)

-they admit there is limited knowledge about the association between antibodies and natural immunity

Left out from the Renal and Urology News overview on the ACP's Guidelines were these gems:

"However, LIKE WITH OTHER VIRUSES, THE RELATIONSHIP BETWEEN ANTIBODIES AND NATURAL IMMUNITY MAY VARY ON THE BASIS OF DIFFERENCES IN THE LEVEL AND DURATION OF ANTIBODIES PRODUCED AS WELL AS VIRAL MUTATIONS OF THE INFECTION. When persons are infected with SARS-CoV-2, UNCERTAINTY EXISTS ABOUT WHETHER THE ANTIBODIES PRODUCED (IgM, IgG, IgA, or neutralizing) ARE PROTECTIVE AGAINST REINFECTION, and if so, FOR HOW LONG WHAT LEVELS OF ANTIBODIES ARE NEEDED FOR SUCH PROTECTION (1). In addition, because antibodies to other coronaviruses have been shown to decline over time, how long such protection against reinfection may last also needs to be determined."

"It is also important for clinicians and patients to keep in mind that SARS-CoV-2 antibody test results MAY BE FALSELY POSITIVE DUE TO CROSS-REACTIVITY WITH ANTIBODIES OF OTHER CORONAVIRUSES (74, 75). Furthermore, although a complete assessment of diagnostic accuracy of various antibody tests was beyond the scope of the evidence review, characteristics (for example, SENSITIVITY, SPECIFICITY, AND ACCURACY) VARIED SUBSTANTIALLY AMONG THE ANTIBODY TESTS USED in included studies (3, 4). SUCH VARIATION CAN CONTRIBUTE TO FALSE-NEGATIVE AND FALSE-POSITIVE TEST RESULTS AND ULTIMATELY WRONG CONCLUSIONS (76, 77)."

https://www.acpjournals.org/doi/10.7326/M20-7569

In Summary (Part 2):

-like with other "viruses," the relationship between antibodies and natural immunity MAY VARY

-UNCERTAINTY EXISTS as to whether the antibodies produced (IgM, IgG, IgA, or neutralizing) are

PROTECTIVE against reinfection

-they do not know for how long or at what levels these antibodies would be needed for protection

-the antibody tests may produce false-positives due to CROSS-REACTIVITY to other "Coronaviruses"

-test sensitivity, specificity, and accuracy VARIED SUBSTANTIALLY among all of the antibody tests

-the test variation leads to false-negatives, false-positives, and wrong conclusions

So here we are almost a year-and-a-half into this hoax and they have more certainty over the protective effects of the experimental mRNA jab than they do over how the body responds to their "virus" naturally. This is obviously by design. If they can keep people confused/scared about the uncertainty of the protection "natural immunity" does or does not afford, they will be more than happy to line up for the artificial "immunity" of the toxic gene therapy masquerading as a vaccine.

https://docs.google.com/document/d/e/2PACX-1vQw80KVLxFxRyLtgAM8XqYRfBQWkgYp1poOftiwgz8KyAbjLTtR8GhsNxbgNzQSyre7_g4iSCOxghOo/pub

Antibody Testing Is NOT CURRENTLY RECOMMENDED to Assess Immunity After COVID-19 Vaccination:

Did you want more evidence that unproven theoretical antibody measurements are absolutely useless? If so, here you go!

Antibody Testing Is NOT CURRENTLY RECOMMENDED to Assess Immunity After COVID-19 Vaccination: FDA Safety Communication

Date Issued: May 19, 2021

"The U.S. Food and Drug Administration (FDA) is reminding the public and health care providers that RESULTS FROM CURRENTLY AUTHORIZED SARS-CoV-2 ANTIBODY TESTS SHOULD NOT BE USED TO EVALUATE ANY PERSON'S LEVEL OF IMMUNITY OR PROTECTION FROM COVID-19 AT ANY TIME, and especially after the person received a COVID-19 vaccination."

"Recommendations for People Who Had or May Have a SARS-CoV-2 Antibody Test

Be aware that SARS-CoV-2 antibody tests help health care providers identify whether someone has antibodies to SARS-CoV-2, the virus that causes COVID-19, indicating a prior infection with the virus. However, MORE RESEARCH IS NEEDED TO UNDERSTAND THE MEANING OF A POSITIVE OR NEGATIVE ANTIBODY TEST, beyond the presence or absence of antibodies, including in people who received a COVID-19 vaccination, in people who have been exposed and have SARS-CoV-2 antibodies, and in people who are not fully vaccinated.

If you have not been vaccinated: Be aware that A POSITIVE RESULT FROM AN ANTIBODY TEST DOES NOT MEAN YOU HAVE A SPECIFIC AMOUNT OF IMMUNITY OR PROTECTION FROM SARS-CoV-2 INFECTION. If you have a positive test result on a SARS-CoV-2 antibody test, it means that IT IS POSSIBLE you were previously infected with the SARS-CoV-2 virus. Talk with your health care provider about the meaning of your SARS-CoV-2 antibody test results.

If you received a COVID-19 vaccination: Continue to follow the CDC's recommendations for fully vaccinated people. Be aware that if you have a positive test result on a SARS-CoV-2 antibody test, it is possible you were previously infected with SARS-CoV-2. A COVID-19 VACCINATION MAY ALSO CAUSE A POSITIVE ANTIBODY TEST RESULT FOR SOME BUT NOT ALL ANTIBODY TESTS. YOU SHOULD NOT INTERPRET THE RESULTS OF YOUR SARS-CoV-2 ANTIBODY TEST AS AN INDICATION OF A SPECIFIC LEVEL OF IMMUNITY OR PROTECTION FROM SARS-CoV-2 INFECTION. Talk to your health care provider or your state and local health departments if you have questions about whether an antibody test is right for you.

Recommendations for Health Care Providers

At this time, DO NOT INTERPRET THE RESULTS OF QUALITATIVE, SEMI-QUANTITATIVE, OR QUANTITATIVE SARS-CoV-2 ANTIBODY TESTS AS AN INDICATION OF A SPECIFIC LEVEL OF IMMUNITY OR PROTECTION FROM SARS-CoV-2 INFECTION AFTER THE PERSON HAS RECEIVED A COVID-19 VACCINATION. While a positive antibody test CAN indicate an immune response has occurred (seroconversion), and failure to detect such a response MAY SUGGEST a lack of immune response, MORE RESEARCH IS NEEDED. CURRENTLY AUTHORIZED SARS-CoV-2 ANTIBODY TESTS ARE NOT VALIDATED TO EVALUATE SPECIFIC IMMUNITY OR PROTECTION FROM SARS-CoV-2 INFECTION. SARS-CoV-2 antibody tests should be ordered only by health care providers who are familiar with the use and limitations of the test. For more information about antibody tests for SARS-CoV-2, see Serology/Antibody Tests: FAQs on Testing for SARS-CoV-2.

Be aware that vaccines trigger antibodies to specific viral protein targets. For example, currently authorized COVID-19 mRNA vaccines induce antibodies to the spike protein and not to nucleocapsid proteins that are likely detected only after natural infections. Therefore, COVID-19

vaccinated people who have not had previous natural infection WILL RECEIVE A NEGATIVE ANTIBODY TEST RESULT IF THE ANTIBODY TEST DOES NOT DETECT THE ANTIBODIES INDUCED BY THE COVID-19 VACCINE. If you are considering antibody testing in vaccinated individuals, follow the Centers for Disease Control and Prevention's guidelines for antibody testing. For more information about antibody test performance visit EUA Authorized Serology Test Performance."

"TEST RESULTS FROM CURRENTLY AUTHORIZED SARS-CoV-2 ANTIBODY TESTS SHOULD NOT BE USED TO EVALUATE ANY PERSON'S LEVEL OF IMMUNITY OR PROTECTION FROM COVID-19. If the results of the antibody test are interpreted as an indication of a specific level of immunity or protection from SARS-CoV-2 infection, there is a potential risk that people may take fewer precautions against SARS-CoV-2 exposure. Taking fewer precautions against SARS-CoV-2 exposure can increase their risk of infection and may result in increased spread of SARS-CoV-2."

https://www.fda.gov/medical-devices/safety-communications/antibody-testing-not-currently-recommended-assess-immunity-after-covid-19-vaccination-fda-safety

In Summary:

-results from currently authorized SARS-CoV-2 antibody tests SHOULD NOT BE USED TO EVALUATE A PERSON'S LEVEL OF IMMUNITY OR PROTECTION FROM COVID-19 AT ANY TIME, and especially after the person received a COVID-19 vaccination

-MORE RESEARCH IS NEEDED TO UNDERSTAND THE MEANING OF A POSITIVE OR NEGATIVE ANTIBODY TEST, beyond the presence or absence of antibodies, including in people who received a COVID-19 vaccination, in people who have been exposed and have SARS-CoV-2 antibodies, and in people who are not fully vaccinated

-a positive result from an antibody test DOES NOT MEAN YOU HAVE A SPECIFIC AMOUNT OF IMMUNITY OR PROTECTION from SARS-CoV-2 infection

-a COVID-19 vaccination may also cause a positive antibody test result FOR SOME BUT NOT ALL ANTIBODY TESTS

-YOU SHOULD NOT INTERPRET THE RESULTS OF YOUR SARS-CoV-2 ANTIBODY TEST as an indication of a specific level of immunity or protection from SARS-CoV-2 infection

-healthcare providers SHOULD NOT INTERPRET THE RESULTS OF QUALITATIVE, SEMI-QUANTITATIVE, OR QUANTITATIVE SARS-CoV-2 ANTIBODY TESTS as an indication of a specific

level of immunity or protection from SARS-CoV-2 infection after the person has received a COVID-19 vaccination

-while a positive antibody test CAN indicate an immune response has occurred (seroconversion), and failure to detect such a response MAY SUGGEST a lack of immune response, MORE RESEARCH IS NEEDED

-currently authorized SARS-CoV-2 antibody tests ARE NOT VALIDATED TO EVALUATE SPECIFIC IMMUNITY OR PROTECTION from SARS-CoV-2 infection

-COVID-19 vaccinated people who have not had previous natural infection WILL RECEIVE A NEGATIVE ANTIBODY TEST RESULT if the antibody test does not detect the antibodies induced by the COVID-19 vaccine

-one more time in case they didn't make it clear: Test results from currently authorized SARS-CoV-2 antibody tests SHOULD NOT BE USED TO EVALUATE A PERSON'S LEVEL OF IMMUNITY OR PROTECTION FROM COVID-19

Guess we can put all of this ridiculous antibody nonsense behind us now.

Related Collection on the Antibody Deception:

DO ANTIBODIES EQUAL PROTECTION? THEY DON'T KNOW:

How many antibodies equals protection/immunity? If you are vaccinated but have low or no detectable antibodies, did the vaccine not work? What about if you are vaccinated and still got

sick despite high levels of antibodies? If you have too many antibodies, does that mean you have an autoimmune disease due to an overactive immune system? If you have too few antibodies, do you have a weakened immune system?

The answer to all of these questions: **THEY DON'T KNOW**.

Take, for instance, the amount of antibodies present in a person. Do high or low antibody levels mean protection or rather an overactive or weakened immune system? Maybe...maybe not. It depends:

"If your IMMUNOGLOBULIN LEVEL IS HIGH, it MIGHT be caused by:

Allergies

Chronic infections

An autoimmune disorder that makes your immune system overreact, such as rheumatoid arthritis, lupus, or celiac disease

Liver disease

Inflammatory bowel disease

Cancer, such as multiple myeloma, lymphoma, or leukemia

LOW LEVELS OF IMMUNOGLOBULINS mean your immune system isn't working as well as it should. This can be caused by:

Medicines that weaken your immune system, such as steroids

Diabetes complications

Kidney disease or kidney failure

A weakened immune system that you were born with or developed (as with HIV/AIDS)

JUST BECAUSE YOUR IMMUNOGLOBULIN LEVEL IS HIGH OR LOW DOESN'T MEAN YOU HAVE ONE OF THESE CONDITIONS.

EACH PERSON'S TEST CAN DIFFER based on the method the lab uses to check the results. Talk to your doctor about your test results, and find out what you should do next."

https://www.webmd.com/a-to-z-guides/immunoglobulin-test

THEY DON'T KNOW.

Do antibodies equal protection from Covid-19?

“The risk [for immunocompromised patients] is that you may be operating under the false belief that you’re protected, AND YOU MIGHT NOT BE,” says rheumatologist Jeffrey Curtis, MD, MPH, a Professor of Medicine at the University of Alabama at Birmingham who led the task force that created the ACR COVID-19 Vaccine Clinical Guidance. “BUT IF AN ANTIBODY TEST COMES BACK WITH A CERTAIN NUMBER, NOBODY KNOWS WHAT THAT MEANS.”

In other words, if an antibody test result were to indicate that you had antibodies after the COVID-19 vaccine, THAT DOESN'T MEAN YOU CAN ASSUME YOUR’RE FULLY PROTECTED."

"Experts are still studying the effect of neutralizing antibodies for SARS-CoV-2 in humans. That means your doctor will not be able to tell you what your results mean in terms of your COVID-19 risk, SINCE IT’S NOT YET KNOWN WHAT LEVEL OF NEUTRALIZING ANTIBODIES WOULD BE NEEDED TO PREVENT INFECTION IN YOUR BODY."

"BECAUSE WE DON'T YET KNOW WHAT LEVEL OF ANTIBODIES ARE CORRELATED WITH COVID-19 PROTECTION, neither a “positive” qualitative or “high” quantitative test can tell you for sure if you’re adequately protected or not."

"First of all, NO GUIDE CURRENTLY EXISTS TO TRANSLATE THE RESULTS OF AN ANTIBODY TEST for what it means for your protection from COVID-19.

“At this point, WE DON'T KNOW HOW ANTIBODY PRESENCE OR LEVELS CORRELATE WITH IMMUNITY WELL ENOUGH TO SAY,” says Dr. Ramirez.

"Your antibody level will be a certain number, whether low or high, BUT YOU WON'T KNOW IF THAT’S A LEVEL THAT MEANS YOU’RE ACTUALLY PROTECTED. “NOBODY KNOWS THAT FOR HEALTHY PEOPLE, AND NOBODY KNOWS THAT FOR IMMUNOCOMPROMISED PEOPLE,” adds Dr. Curtis."

“Given the difficulties with interpreting antibody tests, and that we do not yet know if a specific antibody level post-vaccination can predict protection, it would be difficult to make definitive

conclusions," says Dr. Ramirez."

https://creakyjoints.org/living-with-arthritis/coronavirus/covid-19-vaccines/antibody-testing-covid-19-vaccine-immunocompromised

THEY DON'T KNOW.

How do antibody levels relate to protection in regards to other "viruses" and vaccines? Some have high antibody levels, some have low levels. Some are protected, others are not. It depends:

"Antibody levels to different vaccine and virus antigens were measured by their ELISA titer and normalized to the threshold of protection. THE THRESHOLDS FOR PROTECTION WERE TAKEN FROM THE LITERATURE, AND THERE ARE DIFFERENT STANDARDS FOR THE PROTECTION TO DIFFERENT INFECTIONS [26,27]. We calculated the time for antibody titers to fall to the defined threshold for protection for the given vaccine or virus antigen. DUE TO A LACK OF DATA, WE WERE NOT ABLE TO DETERMINE THE CONSEQUENCES OF VARIATION IN THE THRESHOLD FOR PROTECTION BETWEEN INDIVIDUALS. DIFFERENT LEVELS OF IMMUNITY ARE REQUIRED FOR DIFFERENT TYPES OF PROTECTION [27–30]. For example, HIGHER LEVELS of antibodies MIGHT BE REQUIRED TO PREVENT INFECTION, whereas LOWER LEVELS of antibodies MAY NOT PREVENT INFECTION per se BUT MAY STILL AMELIORATE DISEASE or PROTECT AGAINST LETHAL INFECTION."

"We examined the heterogeneity in both the magnitude and decay rate of antibody responses to different virus and vaccine antigens and used simple models to quantify how this heterogeneity affected the duration of protective immunity to a panel of vaccines and viruses. We found that VARIATION IN MAGNITUDE AND DECAY RATES OF RESPONSES CONTRIBUTE COMPARABLY TO THE DIFFERENCES IN ANTIBODY TITERS, that SOME INDIVIDUALS TEND TO MAKE HIGHER RESPONSES and these individuals also tend to have slower decay rates, and that DIFFERENT PATTERNS OF DURATION OF PROTECTIVE LEVELS OF ANTIBODIES WERE ELICITED BY REPLICATING VIRUSES AND PROTEINS."

https://journals.plos.org/plosbiology/article?id=10.1371%2Fjournal.pbio.2006601

THEY DON'T KNOW.

Mumps and "Antibodies"

To further illustrate the point that they do not know at what level or even if antibodies provide protection, here is a study on Mumps and antibody "protection" from 2015:

"DYNAMICS OF THE SEROLOGIC RESPONSE IN VACCINATED AND UNVACCINATED MUMPS CASES DURING AN EPIDEMIC"

"ALTHOUGH IT WAS ASSUMED THAT MUMPS VACCINATION INDUCED LIFE-LONG PROTECTION, several mumps outbreaks, ESPECIALLY AMONG VACCINATED STUDENT POPULATIONS, have been reported during the last decade in various countries where mumps vaccination has been implemented into their national immunization programs. In 2009-2012, a mumps epidemic (genotype G) arose that spread across multiple locations within the Netherlands, IN WHICH ALSO PRIMARILY VACCINATED STUDENTS WERE AFFECTED. Waning of vaccine-induced immunity HAS BEEN SUGGESTED to play a role in these outbreaks. When compared to the other components in the MMR vaccine, the mumps component SEEMED TO BE the least effective in eliciting good (high avidity) antibody responses, which were shown to wane to lower levels and in avidity index twenty years after a second MMR vaccination."

"YET OUR UNDERSTANDING OF THE NATURAL SEROLOGIC RESPONSE AGAINST THE MUMPS VIRUS REMAINS INCOMPLETE. Insight is into the ranges in mumps-specific antibody concentrations, their virus-neutralization capacity, as well as in the antibody dynamics seen in time after mumps virus exposure is lacking. The recent epidemic of the Netherlands provided an opportunity to evaluate such aspects of the antibody response following a clinical mumps virus infection."

"The course of anti-mumps IgG and virus-neutralizing (VN) antibody concentrations in 23 clinical mumps cases was investigated, 1-2 months and 7-10 months after onset of disease. SEVEN OF THESE CASES WERE NOT MMR VACCINATED, which made it possible to investigate the difference in the course of antibody response between MMR vaccinated and unvaccinated persons after recent mumps virus infection. In addition, a control group was included in the study, to be able to represent THE LOWER RANGE OF ANTI-MUMPS ANTIBODY LEVELS AS CAN BE EXPECTED IN HEALTHY (non-infected) VACCINES that were age–matched."

"In the Netherlands, a high overall MMR vaccination coverage of 96% and 93% for respectively

the first and second dose at 14 months- and 9 years-aged children has been reported. In a large cross-sectional cohort (n=7900) of the Dutch population (2006-2007), it was demonstrated that mumps seroprevalence appeared to be 91%, THEREBY REACHING THE HERD IMMUNITY THRESHOLD OF 86-92% (i.e. threshold percentages of mumps herd immunity combined from two studies, 86–88% and 88–92%). A moderate reduction in seroprevalence, i.e. below or approaching the herd immunity threshold, was observed in several age groups, including the (vaccinated) age group of 15-21 years. THE RELATIVELY LOW MUMPS-SPECIFIC SERUM ANTIBODY LEVELS IN 15-21 YEAR-AGED PERSONS CONFIRMED THE VULNERABILITY OF THIS GROUP WITH RESPECT TO MUMPS VIRUS INFECTION, and may explain the occurrence of the recent epidemic in the Netherlands (2009-2012), five years after this seroprevalence study. This epidemic has been described to count a total of 1,254 laboratory-confirmed mumps cases. THE

MAJORITY OF THE MUMPS CASES was male (59%), university student (47%), 18-25 years of age (68%), and VACCINATED TWICE WITH THE MMR VACCINE (68%). In the present study, two clear response patterns in IgG and VN antibody levels against the mumps vaccine strain could be detected in consecutive blood samples obtained from mumps virus infected persons during this epidemic. In previously vaccinated mumps cases, specific IgG concentrations as well as the ND50 values were significantly

higher shortly (1-2 months) after onset of disease than at 7-10 months. This pattern in antibody response characterizes a secondary response, i.e. rapid production of antibodies upon subsequent encounter with the same antigen. Alternatively, UNVACCINATED MUMPS CASES ALSO MOUNTED A SERORESPONSE, BUT OF GENERALLY LOWER IgG ANTIBODY CONCENTRATIONS AND ND50 VALUES AT BOTH SAMPLING POINTS THAN CASES WHO HAD RECEIVED 2 MMR VACCINE DOSES. The overall IgG concentrations or ND50 values of the unvaccinated cases did not differ significantly between the two time points, and two out of the seven unvaccinated mumps cases had a lower IgG antibody response 1-2 months compared with 7-10 months after onset of disease. This antibody pattern is illustrative of a primary response. It is striking that FOUR OUT OF THE SEVEN UNVACCINATED MUMPS CASES WERE AGED $\geq$ 40 YEARS, and it is likely, although not certain, that these persons had encountered wild-type mumps virus earlier in life. However, although NATURAL INFECTION WITH MUMPS IS THOUGHT TO CONFER LIFELONG PROTECTION, INCIDENTALLY CASES OF REINFECTIONS HAVE BEEN DESCRIBED, and with the absence of circulating wild-type mumps virus also naturally-acquired immunity against mumps may diminish. In addition, it must be kept in mind that in our assays ANTIBODIES WERE MEASURED AGAINST THE MUMPS VACCINE STRAIN, which may result in a relatively lower response in persons with naturally-acquired immunity induced by other mumps virus strain(s). One of the two persons who showed a rise in mumps-specific IgG antibody level in time, SUGGESTIVE OF A PRIMARY RESPONSE, WAS AGED > 50 YEARS, but the observed virus-neutralizing (VN) antibody response of this particular person was NOT TYPICAL FOR A PRIMARY RESPONSE."

"The study design, i.e. observational study with limited sample size, DID NOT ALLOW DETERMINATION OF WHY MUMPS CASES BECAME INFECTED DESPITE VACCINATION."

"Notwithstanding its limited sample size, our

present study describes how mumps-specific antibody concentrations and their virus-neutralization capacity develop in time after mumps virus infection; the antibody response was significantly higher in the vaccinated mumps cases compared to the unvaccinated cases at both sampling times,

SUGGESTIVE OF A BETTER AND MORE PRONOUNCED IMMUNITY AGAINST MUMPS. Although the humoral response certainly plays a role in protection against mumps, IT SHOULD BE CONSIDERED THAT IN VITRO MEASURED VN ANTIBODY CONCENTRATIONS, BUT ALSO IgG CONCENTRATIONS, MAY NOT BE FULLY PREDICTIVE OF IMMUNOLOGICAL ANTIBODY ACTIVITY IN VIVO, given that Fc-mediated phagocytosis, antibody-dependent cell-mediated cytotoxicity, and other processes that occur in the host are not reflected in the corresponding assays. Additionally, other immune mechanisms, such as cellular immunity, are likely involved in the protection against mumps disease as well as in the viral clearance. THE CELLULAR IMMUNITY AGAINST MUMPS HAS ONLY BEEN SCARCELY EXPLORED AND DESERVES MORE ATTENTION."

"Summarizing, mumps patients developed high levels of both mumps-specific IgG concentrations and mumps VN antibodies; vaccinated patients had higher antibody levels than unvaccinated patients. Antibody dynamics of vaccinated versus unvaccinated mumps cases differed, i.e. vaccinated mumps cases had higher antibody levels 1-2 months after onset of disease that declined at 7-10 months, which is characteristic of a secondary response. PREVIOUS MMR VACCINATION RESULTED IN HIGHER (functional) ANTIBODY LEVELS IN THE MUMPS CASES, probably by pre-existing B cell memory, ALTHOUGH IT WAS NOT EFFECTIVE ENOUGH TO PREVENT MUMPS VIRUS INFECTION."

"Seven out of the 23 mumps cases were unvaccinated and 16 CASES WERE PREVIOUSLY VACCINATED WITH TWO DOSES OF THE MMR VACCINE. In addition, 20 healthy control persons (25 yrs (22-29 yrs; 35% male) were included who had no symptoms of mumps or evidence of recent mumps virus infection based on serologic data. Two out of the 20 healthy controls (aged 55 and 57 years) were unvaccinated, and 18 CONTROLS WERE PREVIOUSLY VACCINATED WITH TWO DOSES OF THE MMR VACCINE."

"MUMPS-SPECIFIC IgG CONCENTRATIONS WERE ANALYZED USING THE MUMPS VACCINE

STRAIN (Jeryl Lynn (JL)) AS ANTIGEN. The fluorescent bead-based multiplex immunoassay (MIA) using Luminex technology was performed as described before. Briefly, plasma samples were diluted 1/200 and 1/4,000 in phosphate buffered saline containing 0.1% TWEEN 20 AND 3% BOVINE SERUM ALBUMIN."

Mumps virus-neutralizing (VN) antibodies were detected by focus reduction neutralization test (FRNT), partly based on the protocol described by Vaidya et al.34 Mumps vaccine virus (JL strain; stored at -80°C) was thawed and mixed with heat-inactivated (45 min 56°C) plasma samples (both 37.5 µl) to be incubated for 2 hours at 37°C. CULTURE MEDIUM (DULBECCO'S MODIFIED EAGLE MEDIUM (Life Technologies) SUPPLEMENTED WITH 5% FETAL CALF SERUM, PENICILLIN, STREPTOMYCIN, and L-glutamine) WAS REMOVED FROM VERO CELLS (2 x 104 cells/mL) AND 50 µl OF VIRUS/PLASMA MIXTURE WAS ADDED TO EACH WELL of a 96 wells plate (i.e. > 20 plaques mumps virus per well). Plates were incubated for 4 hours at 36°C, wells were emptied and 200 µl of 0.8% carboxymethylcellulose medium was added to each well. Plates were incubated for 40 hours at 36°C with 5% CO2, before they were washed with PBS and subsequently fixed with a mixture of aceton and methanol (2:3). After 10 min, plates were washed with ice-cold PBS, and incubated with block buffer (PBS containing 1% BSA) for 30 min at 36°C. ANTI-MUMPS NUCLEOPROTEIN ANTIBODY (Abcam) WAS IN BLOCK BUFFER (1:3000) AND 100 µl WAS ADDED TO EACH WELL. After incubation for 1 hour at 36°C, plates were washed with PBS CONTAINING 0.1% TWEEN-20 (PBST). Subsequently, 100 µl OF GOAT-ANTI-MOUSE IgG-HRP (DAKO) IN BLOCK BUFFER (1:2000) WAS ADDED TO EACH WELL and plates were incubated for one hour at 36°C. Plates were washed with PBST and wells were stained with 50 µl of TrueBlue peroxidase substrate (KPL, Inc.). The numbers of plaques were counted and the 50% VN antibody dose (ND50) of each sample was calculated. The WHO international standard RubI-1-94 (NIBSC) was used as positive control in each assay run and to calculate relative ND50 value IN ORDER TO ADJUST FOR INTER-ASSAY DIFFERENCES.``

doi: 10.1080/21645515.2015.1040967

In Summary:

-it was ASSUMED mumps vaccination led to life-long immunity

-this assumption has been challenged by several mumps outbreaks in highly vaccinated populations among those who have received 2 doses of the MMR vaccine

-vaccinated students were primarily affected

-they presume waning vaccine-induced immunity/antibody levels are at fault

-the mumps component of the MMR seemed to be the least effective at eliciting high antibody response

-they admit that the knowledge of the serologic response to mumps remains incomplete

-they looked at 23 mumps cases, 7 of which were not vaccinated with MMR while the rest were

-a "control" group of healthy vaccinated individuals were included to show low-level antibody protection

-the Dutch population had reached the 91% vaccination threshold for "herd immunity" yet were still experiencing outbreaks among the vaccinated

-low levels of antibodies in the 15-21 year-old age group meant they were vulnerable even with the "herd immunity" threshold achieved

-the majority (68%) of the mumps cases were among those vaccinated with the MMR vaccine 2x

-vaccinated mumps cases had higher antibody levels than the unvaccinated

-4 of the 7 unvaccinated mumps cases were above 40 years of age which SUGGESTED they encountered mumps earlier in life

-while natural infection with mumps is supposed to confer lifelong immunity, they admit reinfections do occur

-antibodies in this study were measured against the VACCINE strain

-an over 50 unvaccinated individual had a IgG response suggestive of a primary response but the neutralizing antibody response was not suggestive of a primary response

-the limited sample size did not allow for them to determine why mumps cases occurred in the vaccinated

-the higher antibody response in the vaccinated mumps cases were SUGGESTIVE of a better immune response than the unvaccinated group -however, they admit that results from experiments done IN VITRO (outside the body) do not necessarily equate to what actually occurs immunologically IN VIVO (in the body)

-they admit cellular immunity against mumps is scarcely explored and needs further research

-they conclude that previous MMR vaccination resulted in higher antibody levels but this did

not offer protection against mumps

In other words:

THEY DON'T KNOW.

https://www.google.com/url?q=https://docs.google.com/document/d/e/2PACX-1vTb8BUHtDFpKVSX70xrHNSYoK9rmRSZE2pHUfwDQNEJeXHs6rivgnG8oKSb-V-5R8YsHgxzOp5SQbmQ/pub&sa=D&source=editors&ust=1629138117981000&usg=AOvVaw30vTbZJStCfZnhzOAG7f36

ANTIBODY MADNESS!

MISSING ANTIBODY,

MISSING PROTECTION?:

SEROCONVERSION: The development of detectable antibodies in the blood that are directed against an infectious agent.

https://www.medicinenet.com/seroconversion/definition.htm

On April 20th, 2021 a study was published which attempted to look at the antibody response in regards to influenza infection. They were trying to decipher why some people mount a strong antibody response while others do not. Within the study is a great deal of information on the lack of seroconversion (detectable antibodies) in many cases of disease.

Unfortunately for the researchers, instead of uncovering why this lack of seroconversion occurs, their results showed that airway monocytes, which are typically associated with severe disease, were somehow linked to a protective effect. Obviously wanting to have their cake and eat it too, they concluded that sometimes immune factors play a protective role and other times they play a pathogenic role:

“THE IMMUNE FACTORS THAT EXPLAIN WHY SOME PEOPLE MOUNT A STRONG ANTIBODY RESPONSE AND OTHERS DON'T HAS NOT BEEN WELL UNDERSTOOD,” said Richard Webby, PhD, of the Infectious Diseases Department. “This study identifies some of those factors, providing information needed to design more effective vaccines for flu and other viruses.”

“Airway monocytes often show up AS CORRELATES OF SEVERE DISEASE, so it was interesting here to see them also associated with PROTECTIVE IMMUNE RESPONSES,” said Paul Thomas, PhD, of the Immunology Department. “IT POINTS TO THE FACT THAT MANY IMMUNE FACTORS PLAY PROTECTIVE AND PATHOGENIC ROLES AT THE SAME TIME.”

https://blogs.stjude.org/progress/flu-clues-antibody-production-immune-markers

While their conclusion regarding the simultaneous opposing nature of immune system factors is absolutely absurd, this study had some very insightful admittances along with some great links to other relevant research. Below are highlights from the 2021 study followed by a few quick highlights from some of the related studies:

ACTIVATED CD4+ T CELLS AND CD14hiCD16+ MONOCYTES CORRELATE WITH ANTIBODY RESPONSE FOLLOWING INFLUENZA VIRUS INFECTIONS IN HUMANS

"THE FAILURE TO MOUNT AN ANTIBODY RESPONSE following viral infection or seroconversion failure IS A LARGELY UNDERAPPRECIATED AND POORLY UNDERSTOOD PHENOMENON."

"INTRODUCTION

AN INCREASE IN ANTIGEN-SPECIFIC ANTIBODY TITER IN THE SERUM, known as seroconversion, HAS LONG BEEN ACCEPTED TO BE A SEROLOGICAL HALLMARK OF A RECENT INFECTION OR ANTIGEN EXPOSURE. However, the advent of molecular diagnosis has led to the observation that SOME INFECTIONS DO NOT ALWAYS RESULT IN THE SUBSEQUENT PRODUCTION OF DETECTABLE ANTIBODIES, PARTICULARLY THOSE WITH NEUTRALIZING AND PROTECTIVE ACTIVITY. This has been documented in infections with INFLUENZA virus,1, 2, 3, 4, 5 HUMAN CORONAVIRUSES,6 the MIDDLE EAST RESPIRATORY SYNDROME (MERS) coronavirus, 7 and the recently emerged SEVERE ACUTE RESPIRATORY SYNDROME-CORONAVIRUS-2 (SARS-CoV-2)8,9 infections. THE IMMUNOLOGICAL MECHANISMS UNDERLYING SEROCONVERSION FAILURE ARE NOT WELL UNDERSTOOD, but a better understanding is important, particularly for vaccine design."

"In humans, THE MAJORITY OF OUR UNDERSTANDING OF WHAT IS REQUIRED FOR THE

GENERATION OF A ROBUST ANTIBODY RESPONSE HAVE BEEN DERIVED FROM VACCINATION STUDIES. Such studies have the advantage of having temporally defined pre- and post-antigen exposures that facilitate sample and data acquisition and have used targeted or systems-wide approaches to identify correlates of robust antibody production."

"However, given the differences in antigenic composition and exposure route, POST-VACCINATION RESPONSES MAY NOT NECESSARILY REFLECT THE POST-INFECTION IMMUNE RESPONSE, particularly for respiratory viral infections."

"For influenza viruses, antibodies that target the major surface viral glycoproteins, hemagglutinin (HA) and neuraminidase (NA) are induced after infection. In terms of sero responses to influenza virus infection, HA antibodies measured in a HEMAGGLUTINATION-INHIBITION (HAI) ASSAY ARE CONSIDERED TO BE THE GOLD STANDARD. Antibodies detected by HAI assay primarily bind to the HA globular head, which contains the receptor-binding domain and the major antigenic sites. These antibodies confer high antigenic specificity and can provide potent protection from infection in humans and animal studies.21,22 THE HAI ASSAY DOES NOT STRICTLY MEASURE VIRUS NEUTRALIZING ACTIVITY, but its relative ease of use and ESTABLISHED CORRELATION WITH PROTECTION 21, 22, 23 have justified its use as a major serological endpoint in influenza vaccine and infection studies. SERUM HAI-ANTIBODY TITERS $\geq$ 40 HAVE BEEN SHOWN TO BE ASSOCIATED WITH PROTECTION FROM SEASONAL INFLUENZA VIRUS INFECTIONS 21 AND HAVE BEEN ADOPTED AS THE MINIMAL IMMUNOGENICITY REQUIREMENT for the licensure of seasonal influenza vaccines.24 Compared to HA, NA ANTIBODIES ARE LESS WELL STUDIED, although they have also been recently identified as a potential additional correlate of protection from severe influenza disease."

"THE FAILURE TO SEROCONVERT BY THE STANDARD HAI ASSAY AFTER LABORATORY-CONFIRMED INFLUENZA VIRUS INFECTION HAS BEEN REPORTED IN SEROEPIDEMIOLOGICAL,1,2 VACCINATION,27 AND EVEN CHALLENGE STUDIES.3, 4, 5,28 A review of human challenge studies showed that BETWEEN 50% AND 90% OF INDIVIDUALS WITH PCR CONFIRMATION OF INFLUENZA VIRUS INFECTION FAILED TO SEROCONVERT by HAI assay.3, 4, 5,28 THE UNDERLYING IMMUNOLOGIC FACTORS DRIVING THE MAGNITUDE OF THE ANTIBODY RESPONSE FOLLOWING INFLUENZA VIRUS INFECTION IS STILL, however, POORLY DEFINED."

"In the SHIVERS cohort, participants were enrolled according to the World Health Organization (WHO) case definition for influenza-like illness (ILI) or severe acute respiratory illness (SARI) from general practices or hospitals in Auckland between August and October 2013, which coincided with the peak influenza season in New Zealand.29 Of those with PCR-confirmed infection (N = 66), 21 (32%) PARTICIPANTS MET THE DEFINITION OF HAI SEROCONVERSION (at least a 4-fold increase in antibody titer in the paired sera)."

"Having determined the correlations between CD16+ monocytes and serum antibody responses, we next asked whether similar patterns were seen in the mucosal compartment. To address this, we measured the influenza-specific IgA and IgG titers in the nasal washes of influenza-confirmed individuals from a cohort in which we had previously characterized their monocyte subpopulations.31 AS THERE IS NO EQUIVALENT SEROCONVERSION STANDARD FOR MUCOSAL ANTIBODY TITERS, we identified 10 individuals with influenza-specific IgA titer >40 in their nasal wash samples within 2 weeks of symptom onset as SCs (GMT, 95% CI: 186.6, 98.4–354.1) (Figure 4A) AND ANOTHER 9 INDIVIDUALS, WITH NO DETECTABLE IgA TIGERS DURING THE COURSE OF STUDY, as non-SCs (Table S2). To ensure the validity of our classification of SCs and non-SCs, we also evaluated the influenza-specific IgG responses. Nine of the 10 SCs had influenza-specific IgG titers >40 (GMT, 95% CI: 28.9, 2.8–292), WHILE NONE OF THE NON-SCS HAD DETECTABLE TITERS AT ANY OF THE TIME POINTS TESTED (Figure 4B)."

"DISCUSSION

Seroconversion in influenza is typically defined by measuring the strain-specific HAI antibody response and is GENERALLY THOUGHT TO FOLLOW INFECTION. Somewhat unexpectedly, WE FOUND THAT ONLY 32% OF INDIVIDUALS WHO WERE PCR CONFIRMED TO BE INFECTED WITH INFLUENZA VIRUS MET THIS STANDARD DEFINITION OF SEROCONVERSION in SHIVERS."

"THE HIGHER INCIDENCE OF SEROCONVERSION IN HOSPITALIZED INDIVIDUALS SUPPORTS A CORRELATION BETWEEN THE SEVERITY OF INFECTION AND ANTIBODY RESPONSIVENESS in the periphery and nasal mucosa.5,41 Although our data cannot preclude the possibility of antibody-dependent enhancement (ADE) of influenza disease, we think it is unlikely within the context of our study design (antibody increases were detected post-hospitalization) and other available evidence. ADE of influenza disease has mostly been described during the 2009 H1N1 pandemic 42,43 and within critically ill or fatal cases.44,45 In our cohort, although they were hospitalized, most of the patients were on the “milder” end of the severity spectrum.36 Furthermore, in the present and previous SHIVERS studies on the immunological correlates of disease severity, WE FOUND NO SIGNIFICANT DIFFERENCES IN THE ACUTE-PHASE ANTIBODY TITERS BETWEEN MILD (outpatient) AND SEVERE (hospitalized) PATIENTS. Thus, it appears that, paradoxically, A STRONG PROINFLAMMATORY RESPONSE THAT IS ASSOCIATED WITH SYMPTOMATIC INFECTIONS 31,36 COULD ALSO HELP INDUCE A GOOD ANTIBODY RESPONSE, WHILE A WEAK SIGNAL MAY NOT PROVIDE ADEQUATE STIMULATION.46 This is a particularly important consideration for the ongoing coronavirus disease 2019 (COVID-19) pandemic, in which A PROPORTION OF MILD OR ASYMPTOMATIC COVID-19 CASES FAIL TO MOUNT DETECTABLE ANTIBODY RESPONSES DESPITE EVIDENCE OF ACTIVE INFECTION, WHILE SEVERELY ILL PATIENTS HAD COMPARABLY STRONGER ANTIBODY RESPONSES.8,9,47 Incidentally, the lack of association between age and seroconversion indicates that disease severity may be a stronger factor

driving post-infection antibody response than age, suggesting a strong proinflammatory response may compensate for the decline in immune function in the elderly. However, it should be noted that THE ASSOCIATION BETWEEN ANTIBODY RESPONSE AND SEVERITY OF INFECTION SHOULD NOT BE BROADLY INTERPRETED, AS IT HAD ALSO BEEN SHOWN THAT FATAL H7N9 AND COVID-19 PATIENTS FAILED TO MOUNT ROBUST ANTIBODY RESPONSES IN SOME CASES.47,48

"In summary, our data provide immunological evidence that RESPIRATORY VIRAL INFECTIONS DO NOT ALWAYS LEAD TO SUCCESSFUL SEROCONVERSIONS. These data have implications for our understanding of post-infection immunity and on studies that rely on such metrics."

"Finally, IT IS UNKNOWN WHETHER THE PRESENT FAILURE TO MOUNT AN ANTIBODY RESPONSE WOULD HAVE ANY IMPACT ON THE MEMORY B CELL RESPONSES and their capacity to mount a recall response."

https://www.sciencedirect.com/science/article/pii/S2666379121000537

In Summary (Part 1):

-the failure to mount an antibody response is an underappreciated and poorly understood phenomenon

-an increase in antibody titer in the serum has long been the hallmark of evidence for a recent infection or antigen exposure

-however, it has been observed that not all infections result in detectable antibody production, particularly those with neutralizing and protective activity

-this lack of antibodies has been repeatedly observed in influenza, human coronaviruses, MERS, and SARS-COV-2 cases

-the immunological mechanism for antibody failure is not well understood

-the majority of the understanding on what is required to get a robust antibody response comes from vaccination studies

-post-vaccination antibody responses may not reflect post-infection antibody responses

-hemagglutination-inhibition (HAI) assay is considered the gold standard for measuring antibody activity

-HAI does not strictly measure "virus" neutralizing antibody activity yet it is CORRELATED with protection

-serum antibody titers >40 are associated with protection and are the minimal immunogenicity requirement for vaccines

-NA antibodies are less well studied

-failure to seroconvert has been shown in seroepidemiological, vaccination, and challenge studies

-between 50 to 90% of PCR CONFIRMED influenza cases failed to seroconvert

-the underlying immunologic factors driving the magnitude of the antibody response following influenza infection is still poorly defined

-only 32% (21 of 66) of participants in the study met the seroconversion definition

-there is no seroconversion standard for mucosal antibody titers

-9 individuals had no detectable IgA levels

-9 of 10 seroconverters had detectable IgG while none of the non-seroconverters ever had detectable IgG

-seroconversion is generally thought to occur after infection

-they reiterate that only 32% of the participants seroconverted with detectable antibody levels in their study

-they state that a higher rate of seroconversion in hospitalized patients CORRELATES with severity of disease and increased antibody levels

-they found no differences in antibody levels of those with mild or severe illness

-they think a strong proinflammatory response may lead to greater antibody production over a weaker response

-with "Covid-19," mild and asymptomatic cases had no antibody response while those with severe illness had higher levels

-however, they state that the association between disease severity and antibody production should not be broadly interpreted as there were fatal H7N9 and "Covid-19" cases which also failed to mount antibody responses

-they conclude that respiratory "viruses" do not always lead to antibody production

-they do not know whether the failure to mount an antibody response has any impact on B cell

response

Here is a quick roundup of highlights from a few of the linked studies stating infections do not always result in detectable antibodies:

"Appropriately timed paired serology detects 80-90% RT-PCR confirmed H1N1-2009; ANTIBODIES FROM INFECTION WITH H1N1-2009 CROSS-REACTED WITH SEASONAL INFLUENZA VIRUSES."

https://pubmed.ncbi.nlm.nih.gov/20814575/

"Conclusion: Following the June-September 2009 wave of 2009 influenza A(H1N1), 13% OF THE COMMUNITY PARTICIPANTS SEROCONVERTED, and most of the adult population likely remained susceptible."

https://pubmed.ncbi.nlm.nih.gov/20388894/

"Although NO CORRELATION BETWEEN THE INFLUENZA VIRUS–SPECIFIC B-cell RESPONSE AND SEROLOGICAL HI ANTIBODY LEVEL WAS FOUND, these data clearly supported that acute infections could quickly induce the recalling response of virus-specific B cells, EVEN WHERE THE PREEXISTING ANTIBODY LEVEL IS UNDETECTABLE."

https://academic.oup.com/jid/article/209/9/1354/886344

"THIS STUDY REPRESENTS THE FIRST TIME THE CURRENT GOLD STANDARD for evaluating influenza vaccines as set by the U.S. Food and Drug Administration and the European Medicines Agency Committee for Medicinal Products for Human Use, A "PROTECTIVE" hemagglutination inhibition (HAI) titer of ≥1:40, HAS BEEN EVALUATED IN A WELL-CONTROLLED HEALTHY VOLUNTEERS CHALLENGE STUDY SINCE THE CUTOFF WAS ESTABLISHED. We used our established wild-type influenza A healthy volunteer human challenge model to evaluate how well this antibody titer predicts a reduction in influenza virus-induced disease. We demonstrate that although higher HAI titer is predictive of some protection, there is stronger evidence to suggest that neuraminidase inhibition (NAI) titer is more predictive of protection and reduced disease."

"The U.S. Food and Drug Administration and the European Medicines Agency Committee for Medicinal Products for Human Use both define “protective” titers as a hemagglutination

inhibition (HAI) titer of ≥1:40 (7).

THE EVIDENCE FOR THIS CUTOFF COMES FROM A SEMINAL LIVE INFLUENZA VIRUS CHALLENGE TRIAL CONDUCTED IN 1972 by Hobson et al., which established that a pre challenge serum HAI titer of 18 to 36 was ASSOCIATED WITH 50% PROTECTION from infection (and a similar study demonstrating a 29% infection rate in those with HAI titers of 1:40 to 60 (9). OTHER STUDIES IN THE SETTING OF LIVE AND ATTENUATED INFLUENZA VIRUS CHALLENGE HAVE BEEN LESS CONCLUSIVE (10,–12). A more recent study, conducted using live attenuated viruses, demonstrated 50% protection from intranasal infection in those with titers of ≥1:40 (13), BUT A RECENT EPIDEMIOLOGICAL STUDY OF VACCINES PERFORMANCE HAS BROUGHT THIS INTO QUESTION, WITH ONLY 22% PROTECTION OF CHILDREN WITH POST VACCINATION TITERS OF ≥ 1:40 (14). Even more concerning are data from the recent influenza seasons suggesting that CURRENT SEASONAL VACCINES HELD TO THESE STANDARDS ARE GREATLY UNDERPERFORMING, as overall seasonal vaccine effectiveness over the past 10 years HAS RANGED FROM 10 TO 56% WITH A MEAN OF 40% (15)."

"ANTIBODY RESPONSES WERE MEASURED POSTCHALLENGE DEMONSTRATING NO SIGNIFICANT INCREASE IN THE HAI GMT IN THE HIGH-HAI-TITER GROUP after challenge (week 😎, but a clear increase was noted in the low-HAI-titer group (Fig. 7A). THE RESPONSE IN THE LOW-TITER GROUP WAS VARIABLE, WITH APPROXIMATELY 50% OF THOSE PARTICIPANTS SHOWING A VERY SIGNIFICANT >4-FOLD RISE IN TITER, WHILE THE OTHER 50% OF PARTICIPANTS SHOWED NO SIGNIFICANT INCREASE IN HAI TITER (Fig. 7B)."

"IF THESE RESULTS ARE CONSISTENT WITH OUTCOMES FOLLOWING NATURAL INFLUENZA INFECTION, THE PROTECTION AFFORDED by an HAI titer of ≥1:40 WOULD NOT NECESSARILY INCLUDE THE PREVENTION OF A CLINICAL INFLUENZA-LIKE ILLNESS"

"APPROXIMATELY 50% OF THOSE WITH LOW BASELINE HAI TITERS HAD NO SIGNIFICANT RISE IN TITER. Those who demonstrated no rise in titer were JUST AS LIKELY TO DEVELOP MMID AND THE SEVERITY OF DISEASE OF THESE INDIVIDUALS WAS SIMILAR TO THOSE WHO DID RESPOND, ruling out the possibility of no infection in these cases."

"ALTHOUGH A CHALLENGE STUDY OF THIS TYPE DOES NOT NECESSARILY REPLICATE HOW HUMANS BECOME INFECTED WITH INFLUENZA NATURALLY, it still points out the importance of careful consideration of how protection is defined and suggests a strategy by which evaluation of the correlates of protection to influenza can lead to improved evaluation of vaccine efficacy."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4959521

"NO EVIDENCE FOR TRANSMISSION OF the ca or ts-1A2 recombinant VIRUS TO CONTROLS WAS OBSERVED. A SERUM hemagglutination inhibition RESPONSE WAS DETECTED IN LESS THAN 50% OF THE INFECTED VACCINES."

https://pubmed.ncbi.nlm.nih.gov/7216417/

"We evaluated serologic response of 42 Middle East respiratory syndrome coronavirus (MERS-CoV)-infected patients according to 4 severity groups: asymptomatic infection (Group 0), symptomatic infection without pneumonia (Group 1), pneumonia without respiratory failure (Group 2), and pneumonia progressing to respiratory failure (Group 3). NONE OF THE GROUP 0 PATIENTS SHOWED SEROCONVERSION, while the seroconversion rate gradually increased with increasing disease severity (0.0%, 60.0%, 93.8%, and 100% in Group 0, 1, 2, 3, respectively; P = 0.001). Group 3 patients showed delayed increment of antibody titers during the fourth week, while Group 2 patients showed robust increment of antibody titer during the third week. AMONG PATIENTS HAVING PNEUMONIA, 75% OF DECEASED PATIENTS DID NOT SHOW SEROCONVERSION BY THE THIRD WEEK, while 100% of the survived patients were seroconverted (P = 0.003)."

https://pubmed.ncbi.nlm.nih.gov/28821364/

"For the eight patients in the asymptomatic/mild symptom group, NONE HAD POSITIVE anti-SARS-CoV-2 IgM RESULTS AND THREE (cases 11-13) HAD NEGATIVE anti-SARS-CoV-2 IgG RESULTS (Fig. 1B)."

"Several limitations were found in our study. First, the sample size is small, as we included only 14 COVID-19 patients. Second, A FURTHER WESTERN BLOT ASSAY for detecting the anti-SARS-CoV-2-specific protein IgG or IgM antibodies WAS NOT PERFORMED TO VALIDATE THE PERFORMANCE of the ALLTEST 2019-nCoV IgG/IgM Rapid Test AND UNDERSTAND THE INCONSISTENCY IN THE PRESENCE OF anti-SARS-CoV-2 IgM AND IgG. Third, CROSS REACTION OF SERUM SPECIMENS from the acute phase of different viral infections (e.g., influenza, respiratory syncytial virus, and rhinovirus.) in the IgM portion of this SARS-CoV-2 assay WAS NOT PERFORMED."

"Patients with symptoms and development of anti-SARS-CoV-2 IgM antibodies had a shorter duration of positive rRT-PCR result and no worsening clinical conditions COMPARED TO THOSE WITHOUT THE PRESENCE OF anti-SARS-CoV-2 IgM ANTIBODIES."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7177139

"Serological antibody tests were performed at different times post–disease onset. The overall antibody positivity was 93.75% (105/112). Fifty-eight of 112 patients (51.79%) were positive for IgM (20.93 ± 45.94 AU/mL, mean ± SD) and IgG (122.26 ± 60.94 AU/mL), 7 (6.25%) WERE NEGATIVE FOR BOTH ANTIBODIES, 1 (0.89%) WAS POSITIVE FOR IgM WITH NO RESPONSE TO IgG, AND 46 (41.07%) WERE POSITIVE FOR IgG BUT NOT FOR IgM."

https://academic.oup.com/jid/article/222/2/183/5828055

In Summary (Part 2):

-antibodies from H1N1 cross-reacted with those of seasonal influenza "virus" (i.e. they were NOT SPECIFIC)

-only 13% of H1N1 patients seroconverted (i.e. had detectable antibodies)

-no correlation between B cell and HI antibody levels were found yet it was concluded that even in the absence of antibodies, B cell recall occurred

-the gold standard "PROTECTIVE" HAI of >40 was evaluated by human challenge trials

-the HAI >40 cutoff was established off of one study in 1972 with results only showing 50% were protected

-more recent studies have challenged the HAI cutoff

-one study found only 22% of children with HAI > 40 were "protected" after vaccination

-seasonal vaccines held to this standard consistently fail and fall anywhere from 10% to 56% in effectiveness with a mean of 40%

-postchallenge, there was no increase in antibody levels in the high HAI group

-postchallenge, only 50% of the low HAI group saw an increase in antibody levels while the other 50% saw none

-if the challenge results are consistent with real world infections, the HAI of >40 would NOT PREVENT clinical Influenza-like illness

-those with low HAI were just as likely to develop mild moderate Influenza disease and the severity to those with high HAI levels was comparable

-the challenge trials do not replicate how humans are infected naturally

-no evidence of transmission of recombinant "virus" to controls was observed

-an HAI response was detected in less than 50% of those infected by vaccine

-no one in the asymptomatic MERS group seroconverted

-among MERS patients with pneumonia, 75% of fatal cases did not seroconvert

-in asymptomatic/mild "SARS-COV-2" group, none had IgM levels and three tested negative for IgG

-a confirmatory Western Blot was not performed in order to understand the INCONSISTENCY of the presence of IgM and IgG

-cross-reaction tests were not performed

-only 58 of 112 "SARS-COV-2" patients had both IgM and IgG antibodies, whereas 7 had neither of them, 1 had IgM but no IgG, and 46 had IgG but no IgM

Taken all together, these studies provide indirect evidence that the unseen hypothetical/theoretical particles known as antibodies can be found in some cases of disease. These studies also provide evidence that these unseen hypothetical/theoretical particles are not found in many cases of disease. Antibodies may be associated with more severe disease but are also not present in cases of severe disease. They may be present in asymptomatic/mild cases of disease but are also not present in many asymptomatic/mild cases of disease. The HAI gold standard of antibody titers > 40 has been correlated with "protection" in a few studies but this level has also been shown to be completely ineffective at providing "protection" in many studies post vaccination.

The problem for Immunology and "antibodies" is that, just as in the case with Virology and "viruses," they rely primarily on correlation equaling causation. However, these correlations are inconsistent at best and many times non-existent and/or contradicted in future studies. Instead of admitting they may be wrong by throwing out the old theories and starting over, they try to cram new, contradictory findings/theories into the existing theory thus creating a nearly incomprehensible and overly confusing mosaic made up of pieces from completely unrelated puzzles.

This is why they can claim antibodies are "protective" even though they are not found in many cases of disease. This is how they can state that antibodies are a sign the immune system is working in regards to vaccinations yet they are also a sign one has a weakened immune system with HIV. This is why the presence of antibodies means one has successfully fought off a disease and will be "protected" from future infections yet in the case of Dengue Fever, antibodies will make a reinfection worse and potentially fatal. This is how we end up with multiple immune systems with factors that can be simultaneously protective and pathogenic.

https://docs.google.com/document/d/e/2PACX-1vThXfZwg1Os5HEfIZE_ThkUohR_I-t4AnhBlCC9Dct6vOzVb8v38IW0KSvQ5tHOwp1lALKwWTLtrrA0/pub

ANTIBODIES ARE NOT REQUIRED FOR IMMUNITY: (?)

In 2012, a study came out that completely contradicted the theory that antibodies are required for survival and immunity from "viruses." Knowing what we know about the lack of proof regarding these unseen hypothetical/theoretical particles, it shouldn't come as any surprise that results such as these eventually came about. It is very similar to what Merrill Chase "discovered" in 1942 when his findings broke immunity into the Innate (Cellular) system and the Adaptive (Antibody) system. However, whereas Chase theorized that antibodies still played a role in survival/immunity, newer findings are showing that this is not the case. Here are some excerpts from the ScienceDaily press release on the findings from 2012:

"ANTIBODIES ARE NOT REQUIRED FOR IMMUNITY AGAINST SOME VIRUSES

A NEW STUDY TURNS THE WELL ESTABLISHED THEORY THAT ANTIBODIES ARE REQUIRED FOR ANTIVIRAL IMMUNITY UPSIDE DOWN and reveals that an unexpected partnership between the specific and non-specific divisions of the immune system is critical for fighting some types of viral infections. The research, published online on March 1st in the journal Immunity by Cell Press, may lead to a new understanding of the best way to help protect those exposed to potentially lethal viruses, such as the rabies virus."

"THE RESEARCH TEAM STUDIED VSV INFECTION IN MICE THAT HAD B CELLS BUT DID NOT PRODUCE ANTIBODIES. Unexpectedly, although the B cells themselves were essential, SURVIVAL AFTER VSV EXPOSURE DID NOT REQUIRE ANTIBODIES OR OTHER ASPECTS OF TRADITIONAL ADAPTIVE IMMUNITY. "We determined that the B cells produced a chemical needed to maintain innate immune cells called macrophages. The macrophages produced type I interferons, which were required to prevent fatal VSV invasion," says co-author Dr. Matteo Iannacone.

Taken together, the results show that the essential role of B cells against VSV DOES NOT REQUIRE ADAPTIVE MECHANISMS, but is instead directly linked with the innate immune system. "OUR FINDINGS CONTRADICT THE CURRENT VIEW THAT ANTIBODIES ARE ABSOLUTELY REQUIRED TO SURVIVE INFECTION WITH VIRUSES LIKE VSV, and establish an unexpected

function for B cells as custodians of macrophages in antiviral immunity," concludes Dr. von Andrian.

https://www.sciencedaily.com/releases/2012/03/120301143426.htm

Below are highlights from this study:

B CELL MAINTENANCE OF SUBCAPSULAR SINUS MACROPHAGES PROTECTS AGAINST A FATAL VIRAL INFECTION INDEPENDENT OF ADAPTIVE IMMUNITY

ABSTRACT

"NEUTRALIZING ANTIBODIES HAVE BEEN THOUGHT TO BE REQUIRED FOR PROTECTION AGAINST ACUTELY CYTOPATHIC VIRUSES, such as the neurotrophic vesicular stomatitis virus (VSV). UTILIZING MICE THAT POSSESS B CELLS BUT LACK ANTIBODIES, we show here that survival upon subcutaneous (s.c.) VSV challenge was INDEPENDENT OF NEUTRALIZING ANTIBODY PRODUCTION OR CELL-MEDIATED ADAPTIVE IMMUNITY. However, B cells were absolutely required to provide lymphotoxin (LT) α1β2, which maintained a protective subcapsular sinus (SCS) macrophage phenotype within virus draining lymph nodes (LNs). Macrophages within the SCS of B cell-deficient LNs, or of mice that lack LTα1β2 selectively in B cells, displayed an aberrant phenotype, failed to replicate VSV, and therefore did not produce type I interferons, which were required to prevent fatal VSV invasion of intranodal nerves. Thus, although B cells are essential for survival during VSV infection, their contribution involves the provision of innate differentiation and maintenance signals to macrophages, RATHER THAN ADAPTIVE IMMUNE MECHANISMS.

INTRODUCTION

"ADAPTIVE IMMUNITY, ESPECIALLY NEUTRALIZING ANTIBODY PRODUCTION, IS THOUGHT TO PLAY A CRITICAL ROLE IN CONTROLLING CYTOPATHIC VIRAL INFECTIONS IN MAMMALS (Hangartner et al., 2006). However, external barrier breach by rapidly replicating viruses can place a host at risk long before adaptive immune components can be mobilized. Indeed, mice infected with VSV, an acutely cytopathic neurotropic rhabdovirus, CAN SUFFER FATAL NEUROINVASION DESPITE HIGH NEUTRALIZING ANTIBODY TITERS (Iannacone et al., 2010). This observation led us to revisit the contribution of humoral immune responses to survival after VSV infection.

Intravenous (i.v.) infection of mice with VSV elicits neutralizing T cell-independent IgM and T

cell-dependent IgG responses that become detectable by days 4 and 7 postinfection, respectively (Bachmann et al 1994, 1996; Charan and Zinkernagel, 1986; Karrer et al., 1997; Thomsen et al., 1997). Because B cell-deficient or CD4+ T cell-deficient mice die after i.v. VSV infection, IT HAD BEEN THOUGHT THAT NEUTRALIZING T CELL-DEPENDENT ANTIBODIES WERE ABSOLUTELY REQUIRED FOR SURVIVAL (Bründler et al., 1996)."

"We have shown recently that the susceptibility to VSV neuro-invasion upon LN macrophage depletion has a fatal outcome in ~60% of infected mice, WITH BOTH DYING AND SURVIVING ANIMALS PRODUCING SIMILAR NEUTRALIZING ANTIBODY TITERS (Iannacone et al., 2010). THUS, HUMORAL IMMUNITY WAS APPARENTLY NOT SUFFICIENT FOR MOST INDIVIDUALS' SURVIVAL OF s.c. VSV INFECTION, although it remained possible that antibodies afforded viral clearance in the surviving ~40% of mice. To clarify the role of B cells and antibodies and to re-examine the requirements for protection against VSV, we undertook the present study. By utilizing animals that selectively lack antibodies but retain B cells, WE FOUND THAT NEITHER HUMORAL NOR CELL-MEDIATED ADAPTIVE IMMUNITY WERE REQUIRED FOR PROTECTION AGAINST VSV."

"RESULTS

ANTIBODIES, but Not B Cells, ARE DISPENSABLE for Protection against Subcutaneous VSV Infection

Previous studies have shown that B cell-deficient mice are highly susceptible to acutely cytopathic viruses, including VSV (Bachmann et al., 1995; Bründler et al., 1996; Gobet et al., 1988; Hangartner et al., 2006). ALTHOUGH THIS FINDING WAS INTERPRETED AS EVIDENCE THAT ANTIBODIES ARE ABSOLUTELY REQUIRED, it must be considered that B cell-deficient mice NOT ONLY LACK ANTIBODIES but also display abnormal lymphoid architecture (Kitamura et al., 1991). Therefore, WE SOUGHT TO RE-EVALUATE THE RELATIVE CONTRIBUTION OF ANTIBODY-DEPENDENT AND -INDEPENDENT FUNCTIONS OF B CELLS TO PROTECTIVE IMMUNITY AGAINST VSV. We took advantage of a recently generated mouse strain, DHLMP2A, in which the JH segment of the IgH locus was replaced by the Epstein-Barr virus-derived LMP2A protein (Casola et al., 2004). Because LMP2A provides tonic survival signals, B cells develop without a B cell receptor; therefore, DHLMP2A mice retain B cells and normal lymphoid tissue architecture, YET ARE DEVOID OF SURFACE-EXPRESSED AND SECRETED ANTIBODIES.

Consistent with previous studies (Bründler et al., 1996), B cell-deficient (μMT) mice died within 10 days of i.v. infection, whereas WT mice survived the viral challenge (Figure 1A). DHLMP2A mice were also susceptible to death after i.v. VSV infection, with a clinical course and mortality rate that were indistinguishable from those of μMT mice (Figure 1A). However, when mice were

challenged s.c. (the natural transmission route for arboviruses, such as VSV [Mead et al., 1999]), ~60% of μMT mice died after developing ascending paralysis. In contrast, DHLMP2A MICE, like WT mice, WERE PROTECTED (Figure 1B), EVEN THOUGH (as expected) THEY WERE INCAPABLE OF MOUNTING A NEUTRALIZING ANTIBODY RESPONSE TO VSV (Figure 1C)."

"Although our results after i.v. viral challenge support an antibody requirement for survival of acutely cytopathic viral infection (Bachmann et al., 1997; Bründler et al., 1996), OUR FINDINGS IN THE s.c. INFECTION MODEL ARE NOT COMPARABLE WITH THIS ANTIBODY-CENTRIC PARADIGM. Rather, our findings in DHLMP2A mice imply that B cells may have an additional innate role in antiviral immunity THAT MUST BE ANTIBODY INDEPENDENT."

"ADAPTIVE IMMUNITY IS DISPENSABLE during Primary SubcutaneousVSV Infection MOST MACROPHAGE-DEPLETED WT MICE DIED WHEN CHALLENGED s.c. WITH VSV EVEN THOUGH THEY POSSESSED NEUTRALIZING ANTIBODY TITERS THAT WERE MUCH HIGHER THAN IN MACROPHAGE-SUFFICIENT ANIMALS (Iannacone et al., 2010). In fact, ANTIBODY TITERS WERE INDISTINGUISHABLE between macrophage-depleted WT mice that succumbed to VSV infection and those that remained asymptomatic (Figure S4C). Although the mechanism by which CLL-induced macrophage depletion leads to increased antibody titers in this model remains to be determined, these data, together with our findings in DHLMP2A mice, FIRMLY ESTABLISH THAT ANTIBODIES ARE NEITHER REQUIRED NOR SUFFICIENT FOR SURVIVAL OF A PRIMARY s.c. INFECTION WITH VSV.

This observation raised the question whether adaptive immunity mediated by other lymphocytes is required for protection against peripheral VSV infection. Indeed, both T cells (Kündig et al., 1996; Zinkernagel et al., 1978) and a subset of Thy1+ natural killer cells (Paust et al., 2010) can mount virus-specific effector and memory responses against VSV. To address this question, DHLMP2A mice were depleted of these adaptive lymphocytes by administration of Thy1 antibodies, which resulted in greater than 95% loss of circulating T cells (Figures S4D and S4E). REMARKABLY, DESPITE THE COMPLETE LACK OF BOTH HUMORAL AND CELLULAR ADAPTIVE IMMUNITY, ALL anti-Thy1-treated DHLMP2A MICE SURVIVED VSV INFECTION (Figure 7B). This indicates that the innate immune system, particularly the presence of fully differentiated SCS macrophages, provides sufficient protection to clear this acutely cytopathic viral infection WITHOUT THE NEED FOR ADAPTIVE IMMUNITY.

DISCUSSION

THE RESULTS PRESENTED HERE CONTRADICT THE CURRENT VIEW THAT B CELL-DERIVED

NEUTRALIZING ANTIBODIES ARE ABSOLUTELY REQUIRED TO SURVIVE A PRIMARY CYTOPATHIC VIRAL INFECTION, such as that caused by VSV. This paradigm arose originally from experiments in B cell-deficient mice (Bachmann et al., 1994, 1997; Bründler et al., 1996; Gobet et al., 1988), which lack antibodies, but also have abnormal lymphoid tissue architecture and altered macrophage phenotype. Our experiments in mice that lack antibodies but possess B cells and normal lymphoid tissues confirm that both B cells and antibodies are critical to survive a systemic infection after i.v. bolus administration of VSV. However, ONLY B CELLS ARE ESSENTIAL WHEN VSV IS ENCOUNTERED VIA THE MORE “NATURAL” s.c. ROUTE, WHEREAS ANTIBODIES ARE NEITHER NEEDED NOR SUFFICIENT FOR PROTECTION."

"Although VSV infections are typically self-limiting in mammals, rabies virus, a close relative, is responsible for >55,000 human deaths every year. NEUTRALIZING ANTIBODIES ARE ALSO BELIEVED TO BE REQUIRED TO SURVIVE RABIES INFECTIONS, as shown by the fact that passive antibody transfer and active vaccination to elicit humoral immunity are standard of care. Although neutralizing antibodies are undoubtedly effective prophylaxis against rhabdoviruses, OUR FINDINGS INDICATE THAT ANTIBODY THERAPY MAY BE INSUFFICIENT TO TREAT EXISTING RHABDOVIRAL INFECTIONS IN NONIMMUNE SUBJECTS, at least in the case of VSV. It is unclear whether this caveat applies also to rabies virus infection, but FAILURES OF BOTH PASSIVE AND ACTIVE VACCINATION AFTER EXPOSURE TO RABIES ARE KNOWN TO OCCUR (Anonymous, 1988). Thus, it will be important to further dissect the role of antibodies and interferon in this disease. In addition, recent years have seen the emergence and/or spread of other arthropod-borne neurotropic viral infections, such as West Nile virus, Japanese encephalitis virus, and Eastern and Western equine encephalitis virus, to name a few (Weaver and Barrett, 2004). It remains to be determined whether the cellular and molecular immunological events that occur upon inoculation of these pathogens in the skin are similar to the ones identified here."

"IN CONTRAST TO THE LIKELY BENEFIT OF ADAPTIVE IMMUNITY DURING REINFECTION, our results demonstrate that during a primary s.c. infection, recognition of viral epitopes by either antibody or TCR is neither necessary nor sufficient to prevent fatal VSV neuroinvasion. THIS OBSERVATION RUNS COUNTER TO THE COMMONLY HELD VIEW THAT DURING VIRAL INFECTIONS, INNATE IMMUNITY MUST ORCHESTRATE THE INDUCTION OF ANTIVIRAL ADAPTIVE RESPONSES TO ACHIEVE STERILIZING IMMUNITY."

"In summary, WE DEMONSTRATE THAT NAIVE MICE CAN SURVIVE a s.c. VSV CHALLENGE WITHOUT REQUIRING ANTIGEN-SPECIFIC ADAPTIVE IMMUNITY. Efficient protection against VSV is provided by SCS macrophages in the draining LNs that rely on contact with follicular B cells expressing LTα1β2 on their surface. The constant exposure to LTα1β2 induces and maintains the protective SCS macrophage phenotype. Consequently, SCS macrophages in B cell-deficient mice or in mice that lack B cell-expressed LTα1β2 display an altered phenotype that resembles that of medullary macrophages, which are not protective in VSV infection. Like medullary macrophages, SCS macrophages that are deprived of LTα1β2 capture lymph-borne VSV but fail

to replicate it. Without replication, SCS macrophages do not produce IFN-I that is required to prevent VSV invasion of intranodal nerves. These findings establish a critical innate function for B cells in antiviral immunity. THIS SETTING REQUIRES B CELLS NOT AS A SOURCE OF ANTIBODIES, but as providers of an anatomically restricted maintenance signal and as the day-to-day custodians of macrophage differentiation."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3359130/

In Summary:

-neutralizing antibodies were THOUGHT to be needed for protection against cytopathic "viruses"

-the researchers used mice which lacked antibodies and showed survival from "viral" infection was independent of antibodies and the adaptive immune response

-they concluded that B Cells were essential for survival but not antibodies/adaptive immune mechanisms

-they state that adaptive immunity/antibodies are THOUGHT to play a protective role against "viruses"

-however, mice with high antibody titers still succumbed to fatal neuroinvasive outcomes when infected

-they state again that it is THOUGHT that neutralizing antibodies were required for survival

-both dying and surviving animals had similar neutralizing antibody titers thus humoral (antibody) immunity was not essential for survival

-through their experiments they found that NEITHER Humoral nor cell-mediated Adaptive immunity was required for survival

-they state antibodies are dispensable (i.e. able to be replaced or done without)

-mice incapable of producing an antibody response were protected against the "virus"

-their findings were incompatible with the antibody-specific paradigm

-most macrophage-depleted mice died from infection even though they had higher antibody titers than their macrophage-sufficient counterparts

-they state that the data firmly established that antibodies are neither required nor sufficient

for survival

-despite the complete lack of BOTH humoral and cellular immunity, all anti-Thy1-treated DHLMP2A mice survived

-the innate system can work without the adaptive/antibody system

-they state that their results contradict the belief that antibodies are required for survival of "viral" infections

-they state that it is BELIEVED that antibodies are required to survive rabies but this is unclear as failures of both passive and active vaccinations after exposure to rabies has occurred

-they claim that their observation runs counter to the commonly held view that during viral infections, innate immunity must orchestrate the induction of antiviral adaptive responses to achieve sterilizing immunity

-they conclude that mice can survive without adaptive (antibody) immunity and that only B cells, not as a source of antibodies, are required for immunity

The 2012 findings that antibodies are not required for immunity were later backed up in a different study from 2016 involving the Dengue "virus." Highlights from this paper below:

ANTIBODIES ARE NOT REQUIRED TO A PROTECTIVE IMMUNE RESPONSE AGAINST DENGUE VIRUS ELICITED IN A MOUSE ENCEPHALITIS MODEL

"GENERATING NEUTRALIZING ANTIBODIES HAVE BEEN CONSIDERED A PREREQUISITE TO CONTROL DENGUE VIRUS (DENV) INFECTION. However, T lymphocytes have also been shown to be important in a protective immune state. In order to investigate the contribution of both humoral and cellular immune responses in DENV immunity, we used an experimental model in which a non-lethal DENV2 strain (ACS46) is used to intracranially prime Balb/C mice which develop protective immunity against a lethal DENV2 strain (JHA1). Primed mice generated envelope-specific antibodies and CD8+ T cell responses targeting mainly non-structural proteins. IMMUNE SERA FROM PROTECTED MICE DID NOT CONFER PASSIVE PROTECTION TO NAÏVE MICE CHALLENGED WITH THE JHA1 STRAIN."

"Severity of symptoms displayed by a DENV infection is highly associated with viremia titers (Horstick et al., 2015, Murgue et al., 2000, Vaughn et al., 2000). Historically, ANTIBODIES CAPABLE OF PREVENTING VIRUS OF INFECTING SUSCEPTIBLE CELLS HAVE BEEN THOUGHT TO REPRESENT THE MAIN AND, PERHAPS, THE SOLE PROTECTION CORRELATE (Guzman and Harris,

2014, Guzman et al., 2010, SABIN, 1952, Whitehead et al., 2007). Such antibodies were extensively demonstrated to be capable of preventing infection of the host cells in vitro (Blaney et al., 2005, Chiang et al., 2012, Guzman et al., 2010, Roehrig et al., 2008, Whitehead et al., 2007, Zhang et al., 2007), either by blocking the binding step of viral particles or by preventing conformational changes in the protein required for membranes fusion in endosome (Teoh et al., 2012), or by inducing a structural disruption of the viral envelope (Cockburn et al., 2012, Lok et al., 2008, Pierson and Kuhn, 2012). This rational was reinforced by reports of in vivo protection mediated by neutralizing antibodies in non-human primate model (Guirakhoo et al., 2004, Guirakhoo et al., 2001, Guirakhoo et al., 2000, Guy et al., 2010), and was adopted in the development of presently tested anti-dengue vaccine formulations which are based on chimeric or live-attenuated viruses which induce neutralizing antibodies to the four serotypes of DENV (Whitehead et al., 2007). In particular, chimeric live attenuated viruses between DENV and yellow fever virus (YFV) were constructed with the aim of inducing high titers of neutralizing antibodies against DENV envelope proteins (Guirakhoo et al., 2001). IN CLINICAL TRIALS CONSIDERABLE TITERS OF NEUTRALIZING ANTIBODIES WERE INDUCED IN VACCINEES VOLUNTEERS (Sabchareon et al., 2012, Villar et al., 2014). UNFORTUNATELY, ESPECIALLY FOR DENV2, THE VACCINE FORMULATION BASED ON THOSE CHIMERIC VIRUSES DID NOT ACHIEVE THE EXPECTED PROTECTIVE EFFICACY IN A PHASE III CLINICAL TRIALS IN DIFFERENT PARTS OF THE WORLD (Sabchareon et al., 2012, Villar et al., 2014)."

"WE FOUND THAT ANTIBODIES GENERATED IN MICE INOCULATED WITH THE ACS46 STRAIN, although capable of neutralizing the virus in vitro, WERE NOT CAPABLE TO CONFER PASSIVE PROTECTION IN THE ENCEPHALITIS MODEL. In contrast, depletion of CD4+ and CD8+ T lymphocytes drastically reduced the protection of ACS46-inoculated mice challenged with the neurovirulent DENV2 strain. Collectively, our results clearly show that cellular immune responses, particularly those targeting non-structural proteins, are specifically involved in the control of DENV infection in the mouse encephalitis model."

"Aiming to access the specific contribution of the humoral immune response to the protection induced by the ACS46 strain, we carried out in vivo serum transfer experiments. First, envelope-specific IgG titers were determined by ELISA in both serum and brain macerate from mice which were protected from the lethal challenge with the JHA1 strain. We found that envelope-specific antibodies were not present in brain macerate but only in sera (Fig. 4A). Thus, sera from protected mice were administered to naive mice in one, two or three doses. The transfer regimen with three doses resulted in in vivo envelope-specific IgG titers statistically indistinguishable from those found in donor animals (Fig. 4B). ALTHOUGH SERA FROM PROTECTED MICE SHOWED A MINIMUM PRNT50 TITER OF 1:40, WE OBSERVED THAT ITS TRANSFER IN ONE, TWO OR THREE DOSES DID NOT CONFER NEITHER PROTECTION FROM NOR EXACERBATION OF INFECTION UNDER A LETHAL CHALLENGE WITH 100 PFU of the JHA1 strain

(Fig. 4C). The lethality curves of animals treated with antibodies from mock or ACS46-primed mice were indistinguishable.

On the other hand, IFN-γ-secreting CD8+ T lymphocytes specific to NS1, NS3H and NS5 DENV2 proteins were significantly increased in ACS46-primed mice (Fig. 5A and 😎. Moreover, when CD4+, CD8+ or both populations of T lymphocytes were depleted from ACS46-primed mice the protective immunity was significantly reduced or completely abrogated (Figs. 5C and 6). THESE RESULTS INDICATE THAT ANTI-ENVELOPE ANTIBODIES INDUCED IN THE MOUSE ENCEPHALITIS MODEL DO NOT CONTRIBUTE TO PROTECTION, but T lymphocytes specific responses, particularly those for non-structural proteins, play major roles in controlling virus replication and prevention of encephalitis."

"Discussion

A very large number of people are infected by any of the DENV serotypes per year and a significant portion of them develop severe forms of dengue. Despite the significant epidemiological effect of this disease, there is not an effective anti-DENV vaccine formulation approved for use in humans. Relevantly, AN IMPORTANT VACCINE FORMULATION DESIGNED TO INDUCE GENERATION OF NEUTRALIZING ANTIBODIES DID NOT ACHIEVE THE EXPECTED PROTECTIVE EFFICACY IN A PHASE III CLINICAL TRIAL, especially against serotype 2 (Sabchareon et al., 2012, Villar et al., 2014). In other words, THE CORRELATE OF PROTECTION PROPOSED SEVEN DECADES AGO FOR DENV INFECTION SEEMS TO BE INCOMPLETE, AND THE NEED TO REFORMULATE IT IS CLEAR. In this study we found ANTIBODIES GENERATED IN A PROTECTIVE IMMUNITY INDUCED IN A Balb/C ENCEPHALITIS MODEL ARE NOT CAPABLE TO CONTROL IN VIVO ENCEPHALITIS CAUSED BY DENV2. However, CD4+ and CD8+ T lymphocytes are crucial in our experimental model. In addition, CD8+/IFN+ T lymphocytes targeting non-structural proteins are present in significantly increased levels in animals capable of controlling infection and play major roles in controlling encephalitis. Together, RESULTS PRESENTED IN THIS STUDY SHOWED THAT ANTIBODIES ARE NOT CAPABLE ALONE TO ESTABLISH PROTECTIVE IMMUNITY TO DENV2 in the Balb/C mouse encephalitis model."

"Another study has investigated the role of humoral versus cellular responses induced by a protective anti-DENV vaccine (Zellweger et al., 2013). IN THIS STUDY THE HUMORAL COMPONENT BY ITSELF WAS SHOWN TO BE UNABLE TO CONTROL THE INFECTION OF IMMUNE DEFICIENT MICE (AG129) with a DENV2 strain after intravenous administration."

"Even though this experimental infection model does not resemble the context of the infection seen in humans, it represents another clear evidence that the control of DENV infection requires the involvement of T cell responses, particularly to non-structural proteins. RESULTS PRESENTED IN THIS STUDY FURTHER REINFORCE THE NEED TO REEVALUATE THE ROLE OF

ANTIBODIES AS THE UNIQUE PARAMETER INVOLVED IN DENV INFECTION."

"Notably, these two subsets of T lymphocytes were shown to control infection WITHOUT REQUIREMENT OF ANTIBODIES, which again recall data generated with i.m. immunization, in which ENVELOPE-SPECIFIC ANTIBODIES ARE NOT CAPABLE TO CONTROL ENCEPHALITIS. Thus, THE PROTECTIVE IMMUNE RESPONSE OBSERVED IN OUR MODEL AND HEADED BY T LYMPHOCYTES DOES NOT SEEM TO REQUIRE THE TRIGGERING OF VIRAL ENVELOPE ANTIGENS, AT LEAST WITH REGARD TO INDUCTION OF ANTIBODY RESPONSES."

https://www.sciencedirect.com/.../pii/S004268221500433X

In Summary:

-the generation of antibodies was CONSIDERED to be a prerequisite to control Dengue fever

-the immune sera from protected mice did not transfer passive (antibody) immunity to naive mice

-antibodies were THOUGHT to be the main, and sole, protective correlate

-in clinical trials, neutralizing antibodies were generated in vaccinated volunteers but this had no protective effect

-antibodies generated in mice were incapable of providing a protective effect

-even though antibodies were generated, its transfer in 1, 2, and 3 doses did not pass on protective effects nor exacerbation of infection under lethal challenge trials

-they state that their results show that antibodies did not contribute to protection

-a vaccine formulated to induce generation of neutralizing antibodies did not achieve protective efficacy

-they state that the correlate of protection proposed seven decades ago was incomplete and needed to be reformulated

-the antibodies generated in their study were unable to control encephalitis caused by DENV2

-a separate study also showed that humoral immunity alone was incapable of protecting immune-deficient mice

-they state that their results show that the role of antibodies needed to be reevaluated

-their results show that T lymphocytes can control infection without antibodies, which were not

capable of controlling encephalitis

-they conclude that the protective immune response observed does not seem to require the triggering of viral envelope antigens, at least with regard to induction of antibody responses

These two studies show that the theoretical particles claimed to be antibodies are not a requirement for immunity nor protection. The results completely contradict the main theories regarding the function and role of antibodies in supposed immunity. Maybe the fact that these studies were unable to find a protective effect from antibodies goes a ways towards explaining how one can be considered HIV positive if antibodies are detected. Maybe it explains how having too many antibodies can be associated with an overactive immune system while too few antibodies is associated with a weakened immune system. Maybe it helps to explain why a second infection with Dengue fever is worse than the first and that the resulting antibodies are blamed for triggering life-threatening infections. Maybe it just goes to show that they truly have no idea what these unseen hypothetical/theoretical particles are, how they function, or if they even exist to begin with. One thing that is certain is that, when it comes to the theories about antibodies, they are not certain about anything at all. When nothing is for certain, any contradicting hypothesis or theory will seem possible.

https://docs.google.com/document/d/e/2PACX-1vQ2ipqUmdXnH_5gRwwPuUSxxzHx8MJrvvMyWx-AUpOg5acEx3rDlt9QNJUI5DmKA6biMCa8ciuHpnu-/pub

It's not looking too good for the claim of antibody SPECIFICITY…

HEADLINE: More evidence suggests COVID-19 was in US by Christmas 2019

HIGHLIGHTS:

"Like the CDC study, THESE RESEARCHERS LOOKED FOR ANTIBODIES IN THE BLOOD that are taken as evidence of coronavirus infection, and can be detected as early as two weeks after a

person is first infected.

The researchers say seven study participants — three from Illinois, and one each from Massachusetts, Mississippi, Pennsylvania, and Wisconsin — were infected earlier than any COVID-19 case was originally reported in those states.

One of the Illinois cases was infected as early as Christmas Eve, said Keri Althoff, an associate professor at the Johns Hopkins Bloomberg School of Public Health and the study's lead author.

IT CAN BE DIFFICULT TO DISTINGUISH ANTIBODIES THAT NEUTRALIZE SARS-CoV-2, THE VIRUS THAT CAUSES COVID-19, FROM ANTIBODIES THAT FIGHT OTHER CORONAVIRUSES, INCLUDING SOME THAT CAUSE THE COMMON COLD. Researchers in both the NIH and CDC studies used multiple types of tests to minimize false positive results, BUT SOME EXPERTS SAY IT STILL IS POSSIBLE THEIR 2019 POSITIVES WERE INFECTIONS BY OTHER CORONAVIRUSES AND NOT THE PANDEMIC STRAIN."

https://www.yahoo.com/news/more-evidence-suggests-covid-19-133445284.html

So antibodies are "specific"...except they are not. If they can't tell one "coronavirus" from another one, how are these tests being used today to claim one either had the "virus" or that the vaccines are inducing the right antibodies for the right "virus?"

When you realize both "viruses" and "antibodies" have never been properly purified/isolated nor proven to exist, you realize the results of these tests and the data coming from them are absolutely MEANINGLESS.

https://docs.google.com/document/d/e/2PACX-1vTWyCcuRdv-WP7g4c31FUaCN8EavnKqnezaymD9Mcfn-_CmXrJlJygDfsipRFwh1pcblvb_iKe-YeF5/pub

ANTIBODY SPECIFICITY?:

"NO, THERE IS NO SUCH THING AS A MONOCLONAL ANTIBODY THAT, because it is monoclonal, RECOGNIZES ONLY ONE PROTEIN OR ONLY ONE VIRUS. It will bind to ANY PROTEIN having the same (or a very similar) sequence."

- Clifford Saper, one of the world’s leading authorities on monoclonal antibodies, Harvard Medical School professor

https://off-guardian.org/2021/03/06/the-antibody-deception/

In 2005, Clifford Saper wrote an open letter to the readers of the Journal of Comparative Neurology. In it, he described the problems facing antibody research: mainly the lack of specificity and the inability to reproduce results. He laid forth some criteria that needed to be met in his letter as well as in future papers he published. A few highlights from the letter:

"The Journal has repeatedly, over the last few years, RECEIVED DISTRESSED COMMUNICATIONS FROM AUTHORS, WHO HAVE HAD TO WITHDRAW PAPERS BECAUSE AN ANTIBODY AGAINST A NOVEL MARKER WAS FOUND TO STAIN TISSUE IN KNOCKOUT ANIMALS, WHO LACK THAT TARGET PROTEIN. In many cases these papers contained careful characterization of the antibodies and immunocytochemical controls. THIS ISSUE HAS SENSITIZED THE EDITORS TO THE PROBLEM OF ANTIBODY SPECIFICITY, and we soon realized that many of the papers we were publishing had VERY LIMITED CHARACTERIZATION OR CONTROLS FOR ANTIBODIES THAT WERE USED."

"It is important to recognize that ANTIBODIES ARE NOT SIMPLE REAGENTS THAT ALWAYS IDENTIFY THE SAME THING."

doi: 10.1002/cne.20839.

A little more background info on Saper's findings from the Nature article ANTIBODY ANARCHY: A CALL TO ORDER:

"A MOUSE FIRST ALERTED CLIFFORD SAPER TO THE FACT THAT ANTIBODIES WERE MISLEADING THE SCIENTIFIC COMMUNITY. As editor-in-chief of the Journal of Comparative Neurology between 1994 and 2011, he handled scores of papers in which scientists relied on antibodies to flag the locations of neurotransmitters and their receptors. Around the turn of the century, related investigations began to roll in from researchers using knockout mice, animals genetically engineered to not express a target gene. THE RESULTS WERE UNSETTLING. Antibody staining in knockout animals SHOULD HAVE SHOWN RADICALLY DIFFERENT PATTERNS from those in unmodified animals. BUT ALL TOO OFTEN THE IMAGES WERE IDENTICAL. “As we saw MORE AND MORE RETRACTIONS DUE TO THIS, I began to realize that we had NO SYSTEMATIC WAY TO EVALUATE PAPERS THAT USED ANTIBODIES,” recalls Saper, now chair of neurology at Beth Israel Deaconess Medical Center in Boston, Massachusetts."

"Today, biomedical researchers still collect tales of antibody woe faster than country-music labels spin out sad songs. The most common grumble is the cheating reagent: THE ANTIBODY PURCHASED TO DETECT PROTEIN X SURREPTITIOUSLY BINDS PROTEIN Y (AND PERHAPS IGNORES X ALTOGETHER). Another complaint is 'lost treasure': a run of promising experiments that stalls when a new batch of antibodies FAILS TO REPRODUCE PREVIOUS FINDINGS (see 'A market in a bind')."

"It is alarming, then, to discover that ANTIBODIES CAN BE UNRELIABLE REAGENTS. INSUFFICIENT SPECIFICITY, SENSITIVITY AND LOT-TO-LOT CONSISTENCY have resulted in false findings and wasted efforts."

https://www.nature.com/articles/527545a?proof=t

Below are highlights from two separate reviews Saper published regarding the antibody specificity and reproducibility problems:

A GUIDE TO THE PERPLEXED ON THE SPECIFICITY OF ANTIBODIES

"MANY INVESTIGATORS ARE UNAWARE OF THE POTENTIAL PROBLEMS WITH SPECIFICITY OF ANTIBODIES and the need to document antibody characterization meticulously for each antibody that is used."

"Since the description of indirect immunohistochemical (IHC) staining by Coons (1958), IHC staining has become a standard method used in most laboratories doing cellular or systems level localization of proteins and other cellular constituents. In fact, the methods have become so mundane that many current practitioners take for granted that an antibody that is sold to localize a particular molecular target will be both sensitive and specific. In the current era of very accurate DNA analyses by in situ hybridization, DNA chip analyses, and deep sequencing, IT IS OFTEN ASSUMED THAT IHC HAS AN ANALOGOUS ABILITY TO IDENTIFY MOLECULAR TARGETS ACCURATELY.

NOTHING COULD BE FURTHER FROM THE TRUTH.

In fact, IHC METHODS REMAIN AS PRIMITIVE, IN TERMS OF BOTH SENSITIVITY AND SPECIFICITY, as they were in the days when DNA sequencing was done by hand using sequencing gels. THE

FUNDAMENTAL PRINCIPLES on which antibody localization is based HAVE NOT IMPROVED AT ALL IN THE LAST TWO DECADES, and if anything, THE SLOPE OCCUPIED BY IHC HAS BECOME MORE SLIPPERY THAN EVER."

"By fusing individual antibody-producing cells with antibody-producing myeloma cells, individual cells can be immortalized, so that they divide into colonies of “hybridoma” cells, all of which produce the same, identical immune globulin, with the same variable region. These monoclonal antibodies have the property that they will only bind to molecules that bind that single variable site. Although this relationship imparts specificity to the interaction, IT IS POSSIBLE THAT THE VARIABLE SITE MAY BIND TO A VARIETY OF DIFFERENT TARGETS, particularly when tested in different tissues, and that THESE MAY BE QUITE DIFFERENT FROM THE MOLECULE AGAINST WHICH THE ANTIBODY WAS RAISED."

"Monoclonal Antibodies vs Polyclonal Antisera

As indicated above, access to monoclonal antibodies has provided us with antibodies that are pure reagents. The monoclonal antibodies are derived from hybridoma cells, WHICH ARE GROWN EITHER IN CULTURE OR BY INJECTING THEM INTRAPERITONEALLY IN A HOST ANIMAL. When the hybridoma cells are grown intraperitoneally, the host animals build up fluid, which is called ascites and which can be drawn off from the abdomen and contains high concentrations of the monoclonal antibodies. Either the culture fluid or ascites fluid containing the antibodies can be subjected to purification by precipitating the antibodies with protein A. The resulting RELATIVELY PURE antibody preparations are quantified based on the micrograms of protein.

Polyclonal antisera, in contrast, are derived by bleeding animals a few weeks after they have been immunized. Usually several “booster immunizations” are given, and several bleeds are taken. Blood volume in a mammal is usually ∼7% of body weight, and typically ∼10–15% of total blood volume may be exsanguinated at any one time without injury to the animal. Hence, a single bleed from a 3-kg rabbit may be 25 ml, whereas a bleed from a 30-kg goat can be 250 ml. When the red blood cells are spun down from the clotted blood, the remaining serum is usually about one half this volume. As a result, a single bleed from a larger animal can be used for a much larger number of IHC reactions than a bleed from a smaller animal. The advantage of having the larger amount of serum per bleed is that each bleed is essentially a unique combination of antibody clones. EVEN WHEN BOOSTING THE SAME ANIMAL WITH REPEATED IMMUNIZATIONS WITH THE SAME ANTIGEN, THE ANTIBODY CONTENT IN SEQUENTIAL BLEEDS MAY DIFFER MARKEDLY. Hence, the lot for a polyclonal antiserum is critical, AND EVEN ANOTHER BATCH FROM THE SAME ANIMAL MAY HAVE ENTIRELY DIFFERENT STAINING PROPERTIES. For this reason, experienced immunohistochemistry write down the lot numbers for each vial of antiserum and, when they have a good lot, buy up as much of that lot as they are likely to need in the foreseeable future to avoid inability to finish a project.``

"Synthetic Peptide Antigens and Antigen Mapping

THE ABILITY TO CREATE SYNTHETIC PROTEINS AND PEPTIDES HAS REVOLUTIONIZED THE WAY IN WHICH ANTIBODIES MAY BE MADE and how they can be characterized. Synthetic peptides are usually from a few amino acids up to ~25 or 30 in length. The current peptide synthesis technology results in decreasing yields as the peptide lengthens, so that synthetic peptides much longer than this, while possible, are not practical. On the other hand, much longer amino acid sequences can be prepared by recombinant technology, in which a corresponding nucleic acid sequence is expressed either in a cellular or cell-free protein expression system. It seems obvious that the exact sequence used to create the antibody is critical to its properties, and hence, we will return to this issue in the criteria for antibody suitability.

At the same time, the availability of amino acid sequences from different parts of the parent target molecule has allowed us to identify the target sites in the native molecule to which the antibody binds. When the antibody binds to a partial sequence or a partial sequence competes against binding to the native molecule, THE EPITOPE, or structural features that the antibody recognizes, IS PRESUMED TO BE LOCATED IN THAT SEQUENCE. This method is used to map the epitope that the antibody binds. HOWEVER, THIS DOES NOT INDICATE WHAT THE SEQUENCE WAS OF THE ORIGINAL IMMUNOGEN, BECAUSE THE ANTIBODY MAY HAVE BEEN MADE AGAINST AN OVERLAPPING SEQUENCE."

"Antibodies Against Different Portions of the Same Molecule

A related topic is the ability to generate antibodies against synthetic peptides that are derived from different components of the same molecule. Thus, it is possible, for example, to have antibodies against a large protein target that specifically bind to the N- or the C-terminal portions of the protein. This possibility gives us a powerful potential tool to use in determining antibody specificity. When the two different antibodies stain exactly the same pattern, IT IS HIGHLY LIKELY THAT THEY ARE STAINING THE CORRECT TARGET.

Antibodies Against Phosphorylated or Glycosylated Epitopes

Another possibility provided by the use of synthetic antigens is to prepare immunogens that are specifically altered, for example, with phosphorylation, glycosylation, or some other post-translational modification. Antibodies prepared in this way MAY BE ABLE to distinguish between different modified forms of the same molecule with great accuracy. However, SHOWING THIS

SPECIFICITY REQUIRES APPROPRIATE CONTROLS (such as staining after dephosphorylation)."

"Rules for Judging Whether an Antibody Is Showing What Is Expected in Tissue

MOST INVESTIGATORS WANT TO USE ANTIBODIES TO LOCALIZE CELLULAR COMPONENTS AND DO NOT WANT TO HAVE TO BECOME EXPERTS IN IMMUNOLOGY OR IHC to do so. Hence, it is useful to have a set of criteria for what constitutes A REASONABLE DEGREE OF ASSURANCE THAT THE ANTIBODY BEING USED IS ACTUALLY TARGETING ITS CORRECT ANTIGEN. The answers to the questions that follow are ones that investigators should ask for each antibody they are acquiring, before they ever use it in an experiment (why waste time on an invalid antibody?). IF ALL INVESTIGATORS FOLLOWED THESE RULES, THE LITERATURE WOULD BE MUCH MORE ACCURATE, AND INVESTIGATORS WOULD AVOID WASTING A LOT OF TIME ON INVALID ANTIBODIES.

What Immunogen Is Used to Raise the Antibody?

The first critical criterion in locating a valid antibody is that the immunogen against which the antibody was raised must be known. A KEY PRINCIPLE OF SCIENCE IS THAT THE WORK MUST BE REPEATABLE. Hence, IF THE ANTIBODY IS RAISED AGAINST A “PROPRIETARY” ANTIGEN (USUALLY A SECRET AMINO ACID SEQUENCE, to avoid competitors from copying the product), IT SIMPLY IS NOT VALID FOR SERIOUS SCIENTIFIC WORK. Some manufacturers have claimed that their “intellectual property” must be protected if they are to provide antibodies in the future, but in fact, this has become a routine process, and for most antibodies there are multiple manufacturers who do provide the sequence for their antigens. More importantly, if protecting their profits interferes with science, it is the use of their product that must be eliminated. Other manufacturers have claimed that they will provide their proprietary product to other laboratories in the future, so that the result of the experiment is repeatable. However, there is tremendous turnover in this field, and COMPANIES FRANKLY ARE IN BUSINESS TO MAKE PROFITS AND NOT TO PROTECT SCIENTIFIC INTEGRITY. If they find tomorrow morning that they can make more profit selling shoes than antibodies, that is exactly what they will do, AND NO ONE WILL BE ABLE TO REPEAT THE WORK. Hence, a key issue in buying any new antibody is to avoid products for which the identity of the immunogen is not provided at the time it is purchased.

What is the Evidence That the Antibody Binds Specifically to the Expected Target Molecule in the Tissue of Interest?

The second key criterion for using an antibody in a scientific project should be TO OBTAIN AT LEAST REASONABLE EVIDENCE THAT THE ANTIBODY DOES BIND TO ITS EXPECTED TARGET IN THE TISSUE IN WHICH IT WILL BE STUDIED AND NOT TO SOMETHING ELSE. This is often provided by a Western blot, which should show that the antibody stains a single band (or a set of bands) of appropriate molecular mass for that target. Note that IF EXTRANEOUS BANDS ARE STAINED, THIS INDICATES THAT THE ANTIBODY HAS OTHER ADDITIONAL TARGETS in the tissue and should raise red flags against using that antibody for IHC, unless you have taken additional precautions."

"Note the importance of doing the Western blot in the same tissue and species as the antibody will be applied for IHC. IT IS QUITE POSSIBLE FOR THE ANTIBODY TO SEE ONLY ONE BAND IN SOME TISSUES BUT TO SEE MULTIPLE EXTRANEOUS BANDS IN OTHER TISSUES FROM THE SAME ANIMAL. Similarly, manufacturers often try to “PROVE” specificity by running the antibody against a gel preparation of purified or recombinant protein. This may show that the antibody can bind to its target BUT DOES NOT TELL ANYTHING ABOUT WHAT ELSE IT MAY BIND TO IN TISSUE."

"What Controls Can Be Done to Insure That the Antibody Binds in Fixed Tissue Only to Its Target Molecule?

Despite our best attempts to insure specificity of the antibody against native proteins in the aqueous phase, ultimately we have to apply it to fixed tissue. IN THE FIXED STATE, IT IS POSSIBLE THAT THE ANTIBODY THAT WORKS WELL IN A WESTERN BLOT WILL FIND THAT ITS TARGET ANTIGEN IS DISTORTED BY THE FIXATION PROCESS AND NO LONGER RECOGNIZABLE. In fact, THIS OCCURS SO OFTEN THAT MOST MANUFACTURERS MARK ANTISERA AS USABLE for Western blotting or IHC, AND THE LATTER ARE BY FAR THE RARER.

When polyclonal antisera are raised against a peptide antigen, it is common that most of the antisera that are produced will stain fixed tissue poorly or not at all. In one case in which the author screened antisera, WE FOUND ONLY 2 OF 31 AGAINST A COMMON PEPTIDE HORMONE THAT COULD BE USED TO STAIN BRAIN TISSUE. If one applies the mathematics of a Poisson distribution to this problem (i.e., ASSUME THAT THE PROBABILITY OF STIMULATING A SINGLE ANTIBODY CLONE THAT RECOGNIZES THE FIXED MOLECULE IS AN INDEPENDENT EVENT), it is likely that, in most polyclonal sera, the antiserum is staining the tissue with only one or at most a small number of antibody clones (i.e., that the polyclonal, WHICH MAY CONTAIN THOUSANDS OF CLONES AGAINST OTHER ANTIGENS the host animal encountered in its lifetime, is functionally a monoclonal or oligoclonal for this purpose)."

"One of the best tests to show that the antibody can identify its target in fixed tissue is to transfect the DNA for the target protein into cells that normally do not make it in tissue culture. The transfected and untransfected controls can then both be fixed and stained, and the presence of staining in the transfected cells shows that the antibody really does stain its target. However, THIS CONTROL DOES NOT PROVE THAT THE ANTIBODY WILL ONLY STAIN ITS TARGET IN THE TISSUE OF INTEREST.

Another control for specific staining in tissue is the preadsorption test. Mixing the diluted antibody with an excess of the immunogen should completely block staining. This shows that the staining in the tissue is against something that is at least cross-reactive with the original protein (ALTHOUGH IT DOES NOT PROVE THAT THIS IS WHAT THE TARGET IN THE TISSUE ACTUALLY IS). In general, when the original immunogen is readily available, such as for a synthetic peptide, the preadsorption test should be run as a matter of course. This is less practical for large protein molecules and antibodies against partially purified tissue components. NOTE THAT THE PREADSORPTION CONTROL IS MEANINGLESS FOR A MONOCLONAL ANTIBODY (which is produced by screening for its binding to the target, and therefore will always bind it and always pass a pre adsorption test, by definition) and for antibodies that have already been affinity purified (for the same reason).

As a practical matter, the best controls for assuring that the staining in the tissue is the target molecule involve one of two approaches (Lorincz and Nusser 2008). First, if the staining is being evaluated in mice or a closely related rodent species, and there is a strain in which the target molecule is deleted, the absence of staining is a strong confirmation of specificity. UNFORTUNATELY, THIS IS NOT A PERFECT TEST, BECAUSE THE TARGET THAT IS STAINED IN THE TISSUE MAY BE A RELATED MOLECULE THAT IS DOWNREGULATED IN THE KNOCKOUT ANIMAL. In addition, this approach only applies to situations where there is a knockout strategy available, WHICH LIMITS IT TO A FEW MODELS SPECIES. Finally, in many so-called knockout mice, the original protein is not entirely eliminated. If only a portion of that protein is still expressed, IT MAY HAVE NO FUNCTIONAL PRESENCE BUT STILL STAIN WITH YOUR ANTIBODY. Hence, it is critical in a knockout control to make sure what the actual gene construct is and what is actually expressed.

The second molecular approach to confirming identity of the staining was alluded to above in the section on making antibodies against different components of the same target molecule. When the two antibodies are made in the same species, SHOWING THAT THE STAINING PATTERNS ARE VERY SIMILAR is a strong control. When the two antibodies are made in different species, simultaneous staining and showing colocalization is an even more satisfying and persuasive control."

"At the same time, we are always uncovering new ways that nature can fool us. Thus, NO ANTIBODY LOCALIZATION IS REALLY PERFECT, although following the practical guide provided

here should help investigators, especially those who are new to the mysteries of IHC, to insure the scientific integrity of their work."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2605712/

In Summary (Part 1):

-many investigators are unaware of the problems regarding antibody specificity

-it is ASSUMED that immunohistochemical (IHC) staining can identify molecular targets accurately yet he states nothing could be further from the truth

-IHC remains primitive in regards to both sensitivity and specificity

-IHC has not improved in the last two decades

-with monoclonal antibodies, it is possible that the variable site may bind to a variety of different targets

-these may be quite different from the molecule against which the antibody was raised.

-monoclonal antibodies are either grown in culture or grown in animals injected intraperitoneally

-with polyclonal antibodies, even when boosting the same animal with repeated immunizations with the same antigen, the antibody content in sequential bleeds may differ markedly

-batches from the same animal may have entirely different staining patterns

-SYNTHETIC peptides and proteins have "revolutionized" the way antibodies are MADE

-the epitope, or structural features that the antibody recognizes, is PRESUMED to be located in the sequence

-this method does not indicate what sequence was of the original immunogen, because the antibody may have been made against an overlapping sequence

-most investigators want to use antibodies to localize cellular components and do not want to have to become experts in Immunology or IHC

-it is useful to have a set of criteria for what constitutes a reasonable degree of assurance that

the ANTIBODY BEING USED IS ACTUALLY TARGETING ITS CORRECT ANTIGEN

-if investigators followed the rules, the literature would be much more accurate and time would not be wasted on INVALID antibodies

-a key principle in science is REPRODUCIBILITY of the work

-if the antibody is raised against a secretive "proprietary" antigen (to avoid competitors from copying the product), it is not valid for serious scientific work

-most companies only care for profits rather than scientific integrity

-keeping these "proprietary" products secretive means no one can reproduce the work

-a second criterion is to obtain AT LEAST REASONABLE EVIDENCE that the antibody does bind to its expected target in the tissue in which it will be studied AND NOT TO SOMETHING ELSE

-if extraneous bands are stained, this indicates that the antibody HAS OTHER ADDITIONAL TARGETS

-it is possible for the antibody to see only one band in a tissue yet see multiple extraneous bands in other tissues from the same animal

-manufacturers try to "prove" specificity by testing against gel or recombinant proteins but this will not tell what other bands an antibody might bind to

-in a fixed state, the antibody may find the antigen is distorted by the fixation process and becomes unrecognizable

-when polyclonal antisera are raised against a peptide antigen, it is common that most of the antisera that are produced will stain fixed tissue POORLY OR NOT AT ALL

-only 2 of 31 polyclonal antisera used against a common peptide could stain brain tissue

-polyclonal antibodies may contain thousands of clones against other antigens

-transfected and untransfected controls do not prove that the antibody will only stain its target in the tissue of interest

-the preadsorption control test does not prove that this is what the target in the tissue actually is

-the preadsorption control is MEANINGLESS for a monoclonal antibody

-the best control is the absence of staining yet this is not a perfect test because the target that is stained in the tissue may be a related molecule that is downregulated in the knockout animal

-he concludes that no antibody localization is perfect

ANTIBODY VALIDATION FOR PROTEIN EXPRESSION ON TISSUE SLIDES: A PROTOCOL FOR IMMUNOHISTOCHEMISTRY

"Antibodies play a crucial role in basic research and clinical decision-making. However, THERE ARE NO STANDARDIZED ALGORITHMS OR GUIDELINES TO ENSURE THEIR ACCURACY AND VALIDITY. There have been efforts to generate consensus, but, with the exception of clinical labs, ANTIBODY VALIDATION REMAINS VARIABLE IN THE LITERATURE AND SOMETIMES IN CLINICAL PRACTICE."

"Data from the Human Protein Atlas indicate that AT LEAST 50% OF OVER 2500 COMMERCIALLY AVAILABLE ANTIBODIES DID NOT PERFORM AS EXPECTED IN THEIR INTENDED ASSAY [2]. Improperly validated antibodies, such as several which target estrogen receptor β, have led to non rigorous research in promising fields [3]."

"The FDA defines validation as 'the collection and evaluation of data, from the process design stage through commercial production, which establishes scientific evidence that a process is capable of consistently delivering quality product'. In 2014, Fitzgibbons et al. developed laboratory practice guidelines for analytical validation and revalidation of IHC assays used in anatomic pathology clinical services [6]. Still, IT IS ESTIMATED THAT ONLY 52% OF LABORATORIES HAVE ADOPTED SOME OR ALL OF THE RECOMMENDATIONS [7]. Sfanos et al. explained that much of the 'REPRODUCIBILITY CRISIS' INVOLVED WITH ANTIBODY USE in IHC stems from END USERS BEING UNAWARE OF THE NEED TO PROPERLY VALIDATE AN ANTIBODY."

"The best antibodies have a very high affinity and very low cross-reactivity. It is also beneficial for them to have a fast on-rate and slow off-rate. However, EVEN WITH RECOMBINANT METHODS, IT IS HARD AND EXPENSIVE TO MAKE A PERFECT ANTIBODY, especially for use in IHC; these assays in particular involve unique conditions for antigens, as tissue fixation can hide epitopes exposed in native or denatured forms and expose epitopes that are not exposed when

the protein is in its native form in vivo [10,11]."

"For long-term use in a diagnostic test, and even for reproducible scientific studies, monoclonal antibodies are the best choice. POLYCLONAL ANTIBODIES are produced by several B-cell clones and essentially REPRESENT A POOL OF DIFFERENT ANTIBODY CLONES, each binding to a distinct epitope on the target antigen. While they may be good for rapid proof-of-concept studies, THEY REPRESENT A BATCH-SPECIFIC MIX THAT WILL ULTIMATELY BE EXHAUSTED AND IS THEN IMPOSSIBLE TO REPRODUCE EXACTLY."

"As has been shown, however, MONOCLONAL ANTIBODIES DIRECTED AT A TARGET ARE NOT ALL DEVOID OF THEIR OWN SPECIFICITY AND REPRODUCIBILITY ISSUES, and care must be taken in selecting the best monoclonal antibody, which should always be validated before use [12,13]."

"The first step toward antibody validation consists of demonstrating the expected localization of signal (Figure 2A). While this step was not included in Uhlen et al., we have found it is a critical first step in the IHC validation process. Based on current literature, WE FORM A HYPOTHESIS FOR THE EXPECTED LOCALIZATION OF EXPRESSION OF THE TARGET OF INTEREST. This hypothesis describes the anticipated tissue type (e.g., non-small-cell lung cancer vs breast cancer), histologic subtype (e.g., squamous cell carcinoma vs adenocarcinoma), and cellular (tumor vs stroma) and subcellular (cytoplasmic, membranous or nuclear) localizations of the signal. These hypotheses are based on published literature related to the marker, or data on mRNA from the Cancer Genome Atlas or similar online resources [16]. Generally, more than one source is helpful because RNA does not always correspond to protein. For example, if we were interested in EGFR antibodies, we would typically expect the candidate molecules to show a membranous staining pattern of tumor cells or cell lines known to overexpress EGFR based on mRNA data [17,18]. Because the candidate antibody has not yet been titrated, we often just select the vendor's recommended concentration for a first screen. Investigators should be extra cautious at this step, as APPLICATION OF THE CANDIDATE ANTIBODY IN SUBSTANTIALLY INCREASED CONCENTRATION MAY RESULT IN NONSPECIFIC STAINING PATTERNS (see Step 2)."

"ANTIBODY REPRODUCIBILITY IS A CRITICAL STEP FOR VALIDATION of any antibody to standardize an IHC assay, but was not mentioned as a pillar by Uhlen et al."

"ANTIBODY REPRODUCIBILITY CAN SOMETIMES BE CHALLENGING TO PROVE if the target is heterogeneously expressed in the target tissue."

"While reporting sequences seems like a good idea from a scientific perspective, MANY COMMERCIAL VENDORS POINT OUT THAT THE EPITOPE IS THE ‘SECRET SAUCE’ THAT MAKES THEIR ANTIBODY BETTER THAN OTHER COMPETITORS. Rather than try to patent each epitope

(which is not economically feasible), they maintain confidentiality about their epitope and its sequence [44]."

"Because RESEARCH LABS in the academic sector ARE NOT SUBJECT TO ANY CERTIFICATION AND PEER REVIEW IS THE ONLY 'REGULATORY BODY' in that sphere, it is perhaps unrealistic to expect strict adherence to any standardization requirements."

"Another key concept likely to be important in IHC in the future is antibody biochemistry. In general, THE AFFINITY AND DISSOCIATION CONSTANTS FOR MOST MONOCLONAL ANTIBODIES ARE NOT KNOWN OR NOT EASILY AVAILABLE. Optimal antibodies for IHC have a fast on-rate and slow off-rate, but the kinetics of antibody binding for IHC are essentially NEVER PUBLISHED."

https://www.future-science.com/doi/10.2144/btn-2020-0095

In Summary (Paet 2):

-there are no standardized algorithms or guidelines to ensure antibody accuracy and validity

-antibody validation remains variable in the literature and sometimes in clinical practice

-at least 50% of 2500 commercially available antibodies did not work as intended

-it is estimated that only 52% of laboratories have adopted some or all of the recommendations for IHC validation/revalidation

-reproducibility crisis in antibody research stems from end users being unaware of the need to properly validate an antibody

-even with recombinant methods, it is hard and expensive to MAKE a perfect antibody

-polyclonal antibodies represent a pool of different antibody clones

-polyclonal antibodies also represent a batch-specific mix that will ultimately be exhausted and is then IMPOSSIBLE TO REPRODUCE EXACTLY

-monoclonal antibodies directed at a target are not all devoid of their own SPECIFICITY and REPRODUCIBILITY issues

-their first step towards antibody validation is to FORM A HYPOTHESIS for the expected localization of expression of the target of interest

-application of the candidate antibody in substantially increased concentration may result in

NONSPECIFIC staining patterns

-antibody reproducibility is a critical step for validation of any antibody to standardize an IHC assay, but was not mentioned as a pillar by Uhlen et al

-antibody reproducibility can sometimes be challenging to prove if the target is heterogeneously expressed in the target tissue

-many commercial vendors point out that the epitope is the 'secret sauce' that makes their antibody better than other competitors

-research labs in the academic sector are not subject to any certification and peer review is the only 'regulatory body'

-the affinity and dissociation constants for most monoclonal antibodies are not known or not easily available

All of this is to say: ANTIBODIES ARE NOT SPECIFIC. Next time they try to claim that "specific" antibodies are targeting certain "viruses" or their "spike proteins," hopefully you will now know better and realize that this is never the case.

https://docs.google.com/document/d/e/2PACX-1vQbMvfH_zyuzbOFynD1PFEAdLYVCStkXeVld8PxJ6K0U7i2FMZ4hAx0qgdB2VITeTQeyAaKeeVR0YOM/pub

posts on the Reproducibility Crisis:

Science:

https://docs.google.com/document/d/e/2PACX-1vQnl8aBMGbDsNfRW1S9CH-s7bKatf9AQ_Ff9CvgqGpbiR_lcZ_zxf5tJdvDuSkvx6dUPJy5cfiJy9Ej/pub

Cell Cultures:

https://docs.google.com/document/d/e/2PACX-1vQfoMdQAMeYP0jJv6vHPPQtYleDrj1bsso67SQC3Q9iDHCjcoq1I_Yzj2vvbRaRTIUXHQjYD0HCLnoy/pub

More info on Antibody deception:

https://docs.google.com/document/d/e/2PACX-1vS5jTh_Jo-

hTt7mbMhQRF57rClqbiKBTWGuLKRBhzPt2QD77SYpMIGzTLi9fMcJKw7Kxg5lrssdGcwD/pub

essay on

The Misinterpretation of the Antibodies

Dr. Steffan Lanka

A closer look at antibodies is more important today than ever. After showing in my other articles that there is no proof of the existence of a pathogenic virus, because none of the claimed pathogenic viruses have fulfilled Koch's postulates, the "antibody" card has now

been played by the vaccination advocates. Their claim (which has been drilled into heads for decades) that they are the indirect proof of a pathogen, or offer protection against a pathogen X, is based on an error. This assertion has been repeatedly exposed as false. Since being asked again and again what these antibodies are, I would like to show in this article that antibodies are no proof of protection, nor that they work specifically as in the key/lock theory.

What is a titer increase?

Quote: Dr. Stefan Lanka: (backup available)

"The increase is nothing more than the body's reaction to poisoning [adjuvants], when the body is poisoned, holes are torn in the cells by these poisons and the cells are destroyed. The body's reaction when cells break down is to form sealing substances (globulins), small protein bodies that immediately expand in acidic environments, become flat and cross-link with their hydrogen sulphide groups (in which energy is stored) with other proteins and other things.

These cause blood to clot and wounds to heal and they seal our cells when toxins are injected into the body. Even if you get a blow on a muscle, (forming a bruise) or a blow on the kidney (especially sensitive), or the liver, there is an immediate increase in titer. The body reacts to this by sealing the damaged cells and sealing growing cells.

It's like a house that leaks until the windows are in and sealed. They called this an antibody and even a specific antibody, which is not true. The binding property of these hydrogen sulfidetype proteins is non specific, they bind to all sorts of things. You can manipulate this in the laboratory by changing the acid level, adding detergents that change the mineral concentration to achieve a binding or not.

The blood of a pregnant woman is full of globulins to seal the placenta, which is constantly

growing, to accomodate the baby. The blood of a pregnant woman has to be diluted 40 times to avoid a massive positive result in tests, such as an HIV test."

The approval of vaccines is limited to so-called seroconversion.

All vaccines for Europe are approved by the EMA (European Medicines Agency) in London. Their demand for proof of efficacy is limited solely to so-called seroconversion. Seroconversion shows the formation of measurable antibodies in the blood of vaccinated persons, which are equated to a protective effect.

However, when assessing immunity or the effectiveness of vaccinations, this decisive limitation is again put into perspective by the fact that (almost) all current vaccinations are developed primarily to form antibodies: "Although mucosal and cellular immune responses

are clearly important to protection by some vaccines, most vaccines licensed today depend for their efficacy on serum antibodies." (Plotkin 2010 [5] and 2001 [6]).This is not least of all important for the development and approval of vaccines, as they have to prove their efficacy in this context - which is done without exception (and in many cases exclusively!) by determination of provoked antibodies.

Even long-standing STIKO members do not always seem to be aware of this correlation when they question the usefulness of titres after vaccinations - after all, the proof of efficacy of the respective vaccinations is based on the detection of precisely these antibodies.

According to Prof. Heininger:

"For none of the generally recommended so-called basic vaccinations is a routine control

of the vaccination success planned or even advisable". (Heininger 2017) [7] or the blanket statement regarding the measles vaccination, "that a positive laboratory result does not certify protection" (Heininger 2016) [8] - If the latter were the case, the vaccination could not have been certified as effective and therefore approved...

However, in medicine we have known for decades that circulating antibodies are not synonymous with protection against a disease, a fact that can be understood even by laypeople using short examples.

If antibodies do indicate protection, how do the following statements of the RKI, STIKO and Arzneitelegram fit in?

1. The April 2001 telegram of medicines states: [1]

"Vaccine-induced titre increases are also unreliable substitutes for efficacy. What benefit or harm the vaccinated person can expect cannot be deduced from such findings."

2. The RKI (Robert Koch Institute) writes: [2]

"For some vaccine-preventable diseases (e.g. pertussis) there is no reliable serological correlate that could be used as a surrogate marker for existing immunity. Furthermore, the antibody concentration does not allow any conclusion to be drawn about a possible existing cellular immunity."

3. Prof. Heininger, a long-standing member of the STIKO (permanent vaccination commission) writes: [3]

"It is neither necessary nor useful to determine efficacy by blood sampling and antibody determination after a vaccination has been carried out. On the one hand, even an antibody determination does not provide a reliable statement about the presence or absence of vaccination protection, and on the other hand, it is simply too expensive."

4. Sick despite vaccination? [4]

An example of this was a 14-year-old boy who had received sufficient basic immunisation in childhood and a booster against tetanus six months earlier when he developed tetanus. Laboratory tests revealed antibodies so high that, according to the definition of antibody titres, he should have been protected. But he was not! This example shows that the theory

of antibodies as "protective magic bullets" is wrong. The RKI then coined the term "non-protective" antibodies.

5. Prof. Heininger - STIKO (2017) [7]

"The most important thing right from the start: For none of the generally recommended so-called basic vaccinations is a routine control of the vaccination success planned or even advisable".

6. Prof. Heininger - STIKO (2016) [8]

"...there are not only false-negative IgG antibody results (which would not bother us if the child received an MMR vaccination as a consequence), but unfortunately also false-positive results. This must be put to parents so that they understand that a positive laboratory result does not certify protection and that they are much better advised to give their child a second dose of MMR".

Remark: So again confirmation that a positive laboratory result is insignificant. The question arises again and again as to how you know that antibodies offer circulating protection when the highest authorities themselves say that a titer increase cannot prove protection exists. When people have high antibody levels, do they still fall ill? If no one can say exactly at what titer level there is real protection, why is the approval of a vaccine

based on that exact reading? Personally, this makes me more than a little suspicious. The following points are of crucial importance in this discussion:

•Firstly, that we cannot always be sure that the question of immunity can be clarified by means of an antibody determination for each vaccine (see below)

•Secondly, the antibodies that show up in routine tests are not automatically those that provide protection (immunity), but sometimes only those that indicate that (apart from the measured protective antibodies that are not decisive for immunity, and which are certainly not measured) protective antibodies have been produced. The measured ones are then a so-called surrogate parameter of immunity. This complicated hypothesis is based, on one hand, on the fact that the immune response produces numerous different antibodies with different functions and, on the other hand, that the determination of the actually decisive antibodies in some vaccinations would be too time-consuming for routine diagnostics. (Or to put it simply, the connection between antibodies and immunity is a myth)

•Thirdly, each 'immunity' is based on statistics and therefore relative whether it protects in the individual case or not. The true reasons for the state of the body being "symptom-free" lie buried in other justifications. "Thus protection is a statistical concept. When we say that a particular titer of antibodies is protective, we mean under the usual circumstances of exposure, with an average challenge dose and in the absence of negative host factors." [6

•Fourthly, the question of protection from what exactly is meant from the point of view of orthodox medicine is also crucial. For example, it is claimed that in the case

of HiB and measles, much lower antibody levels protect against contracting the disease oneself (protection from disease) than is necessary to prevent transmission to others (protection from infection).

Note: As there is still no scientific proof of the measles virus, the question naturally arises as to how the claim of protection from measles by antibodies can be claimed when the pathogen has not yet been proven. A fallacy. So the horse is being put before the cart here. I'm measuring some "antibodies", so I'm indirectly claiming to have a pathogen.

The measurable antibody titers after vaccination only shows the conflict of the immune system with the antigens, which are mostly coupled to adjuvants. Without these adjuvants there would be no antibody formation. Here it becomes clear that the immune system is much more complex and does not function exclusively through antibody formation.

Herpes sufferers develop circulating antibodies against the herpes virus. Nevertheless, herpes can flare up again and again by weakening the immune system, for many people disgust is enough. And this occurs even when herpes antibodies are detectable. Someone who is HIV-positive is also not happy about having circulating antibodies against HIV.

The hypothesis of antibodies does not work from start to finish. If they can offer protection, how is it that people who have a sufficient titer still fall ill? How is it possible that the logic of antibodies in HIV was turned 180 degrees, where high antibodies are deemed counterproductive?

No antibodies are required, protection by vaccination is always assumed without evidence. The phantom is always assumed, they don't even want to think in other directions! It's not science.

Source RKI

[1] Medication Telegram

[2] Epidemiological Bulletin (EpiBull) No. 30 / 2012 p.299

[3] U.Heininger "Handbuch Kinderimpfung Handbuch Kinderimpfung: Die kompetente Entscheidungshilfe für Eltern 2004

[4] Epidemiological Bulletin 2008; 24:193-195

[5] Plotkin SA. 2010. C linical and V accine I mmunology. July 2010, p. 1055-1065

[6] Plotkin SA. 2001. The Pediatric Infectious Disease Journal. 20(1):63–75

[7] Heininger U. 2017. Ars medici. 2017(4):172-75

[8] Heininger U. 2016. Pediatrician. 47(4):227

To claim an "antibody" you need a "body"

As I have already pointed out in my other articles, there is still no evidence of [measles virus] [SARS] alleged pathogenic viruses. So if I don't have any evidence for a body, how can I claim to have defined specific antibodies and above all, how in God's name can I test for them? You know the answer, it is simply not possible.

What does all this mean for the vaccinated person?

Since there is no scientific research on how often this phenomenon occurs where vaccinated individuals develop 'non protective antibodies', the possibility of disease still remains for each vaccinated individual. A complete vaccination record and also the detection of antibody titres, as is often done for example with rubella or hepatitis B, is no guarantee.

Could the non-protective antibodies, invented off the cuff, explain the situation where after vaccination (e.g. against measles, mumps, rubella or whooping cough etc.) the vaccinated individual may have antibodies, but still fall ill (with measles, mumps, rubella or whooping cough etc.)? Could they be the reason (apart from the alleged mutations that undermine

vaccination protection) for the epidemics despite high vaccination rates, in which, more often than not, a large percent of the sick were sufficiently vaccinated?

Circulating antibodies alone therefore do not provide reliable protection; this has been orthodox medical knowledge for many decades. On the other hand, the proof of efficacy in the approval of vaccines is based solely on the proof of the allegedly (sometimes?) protective antibody titres.

DIMDI, the German Institute for Medical Documentation and Information: Antibody titre is only a supplementary measurement.

A half truth from orthodox medicine - but still! ''Antibodies are surrogate endpoints, i.e. substitute measurement quantities invented on the basis of random correlations'', says DIMDI, the German Institute for Medical Documentation and Information:

"The use of surrogate endpoints is [...] not unproblematic. In the past, there have been many situations in which relying on surrogate endpoints was misleading or had fatal consequences

despite strong correlation with the clinical endpoint. This problem has been known for more than 30 years. [...] Some products that were approved on the basis of surrogate endpoints had to be withdrawn from the market at a later date because the benefit-risk balance was reversed in studies with mortality or morbidity endpoints."

Source: DIMDI, Cologne 2009

Remark: So we have been dealing with problematic "substitute markers" for decades, which have repeatedly led to completely wrong results and assumptions. Despite strong correlation (correlation is no scientific proof, only an indication) these were misleading and had fatal consequences! It is time to correct this false hypothesis about antibodies.

Working aid on the topic of antibodies: Stefan Lanka and Veronika Widmer from MACHT

IMPFEN SINN?

An excerpt from: "Does inoculating make sense? Disease-causing viruses? Isolated viruses? The Basic Law. How are new viruses identified? Commentary on viruses claimed to be isolated (German) Brochure - July 1, 2005":

Comment on the (wrong) question: What are antibodies?

Correct question: What is measured when antibodies are claimed to be present?

According to Pschyrembel, antibodies are "a possible reaction of the immune system. "Antibodies do not occur naturally." Was this formulation chosen because it is known that people with a high "antibody titre" can fall ill in the same way as people without "titre" remain healthy? Today's school of medicine distinguishes between the formation of foreign antibodies (pathogenic bacteria, toxins from viruses) and the body's own antibodies (tumour cells).

While we are told that after a vaccination the organism is protected by the formation of antibodies, conventional medicine also describes cases in which the presence of antibodies has adverse effects on the organism. For example, conventional medicine refers to allergies, AIDS, transplant rejection and autoimmune diseases. The Robert Koch Institute explains that: An increased total immunoglobulin concentration in the serum indicates in the majority of cases an allergic disease.

However, elevated levels can also occur in cases of parasite infestation or malignant tumours, for example. In the case of inhalation allergies, IgE levels are moderately to greatly increased, depending on the symptoms and the number of allergens causing the allergy. A normal IgE does not rule out an allergy.

If antibodies are diagnosed after a vaccination, conventional medicine tells us that the person concerned is now protected. However, it is concealed that people are ill despite the

presence of antibodies and people without antibodies remain healthy. HIV-antibodies detected by a test produce a diagnosis of fatally ill - or at least - will become fatally ill. Rubella antibodies detected by a test provide a diagnosis of - protected - to the affected person. A contradiction in terms. "Anti" bodies have never been detected.

Bodies, the immunoglobulins, which among other things play a role in the coagulation and cross-linking of proteins, have, however, been proven. The word "anti" assumes that the immunoglobulins can only bind to certain proteins. All experiments ever performed, however, rule this out. Whether or not binding takes place depends on the environment and state of the proteins: Whether acidic or basic, i.e. oxidised or reduced. Every scientist who has carried out such experiments or studied them knows this.

Antibody tests: The procedure in the laboratory

First, the blood is separated from its cells and the larger proteins. This is done, for example, by a centrifuge. 99% of all tests performed are carried out with the patient's serum, the remaining blood liquid. Now the laboratory technician is told what is to be detected by the antibody test. For this purpose, the so-called supernatant is then filled with corresponding, pharmaceutically produced, patented substances whose composition is kept secret (the government and the Paul-Ehrlich-Institute under its supervision keep strict secrecy). If there is a measurable reaction, the test is evaluated as "positive". Up to now, it has been claimed that if antibodies were detected, immune protection has been proven. The indirectly and not quantitatively determined amount of "antibodies" is then called a titer. In the case of AIDS, however, a death sentence is pronounced, if necessary, because it was claimed that the antibodies are now indicative of the presence of the AIDS virus. So it is not surprising that there is no scientific standard for titres and that the measurements are never comparable.

It is even less surprising then that there are no scientific criteria whatsoever as to when a titer can, should, may etc. be called "immune protection". The laboratory technician is told that the test kit contains one or more proteins exactly corresponding to the shape of the microbe. If the laboratory technician would think about it, he would realise that under the appropriate conditions the form of the proteins could not correspond to that of the claimed microbe, because the proteins are no longer in their natural environment. This is called denaturation of the proteins.

According to the delusional logic of compulsion, these unknown proteins are then named "antigens" by which the antibodies can be detected. The test kit also contains: e.g. dyes and substances that serve to produce a "positive" signal for reproduction. The apparatus, into which the whole thing is then placed, is calibrated again with substances whose composition is kept secret and which are monitored by the aforementioned Paul Ehrlich Institute. The fact that there are about 5% people in the entire population in whose blood, under laboratory conditions, little or no immunoglobulins can be detected, is not discussed and not investigated.

These people are then called "non-responders" after vaccination and are poisoned with more and more vaccines according to delusional logical compulsion. Blood group AB was invented for these 5% and according to compulsive logic, blood groups A and B, in addition to blood group 0 (40% of the population), for which little or no proteins that could clump in the test tube are found under the appropriate laboratory conditions.

The contradictions that arose from the dogma of blood groups were first dismissed by the discovery of a rhesus factor and later by the continuous introduction of thousands of sub blood groups.

Stefan Lanka: Facts that refute the claims about "antibodies" and a specific immune system.

• Because there are so-called autoimmune diseases and so-called allergies that occur at lightning speed. In psycho-neuro-immunology this is called a so-called "track''. Comment: It cannot be that "specific" antibodies react against "foreign" and then suddenly against "own" proteins.

• Alternating "foreign" intestinal bacteria exist side by side with immune cells which are supposed to carry out a specific defence against them. Comment: If there were specific antibodies, the intestinal colonisation should not be able to change.

• Humans, mammals, bony fish and sharks exist. They produce immunoglobulins. Comment: If there were specific antibodies, the offspring would be destroyed and breast milk would be toxic.

• In the development of humans and animals, under shock and in old age new proteins appear. Comment: Since, according to the never verified but only falsified immune hypotheses, "foreign" and "own" proteins are recognized in the thymus in earliest childhood and "antibodies" or the immune cells forming them are sorted out against "own" proteins. Proteins occurring later, such as hormones in puberty etc., would automatically lead to allergy, autoimmune diseases, destruction and death. This is not the case.

"Anti" bodies against viruses which do not exist at all cannot exist in principle either. Here the claim of the existence of specific antibodies and specific tests clearly turns out to be a crime and consequently a genocide. Comment: But since immunoglobulins are detected that are capable of binding other proteins, there is "body but not "anti". But globulins that first complete themselves in an oxidized, i.e. acidic environment (via reduced S-H groups, which in the oxidized state combine to form disulfite groups (-S-S-) and thus bind the protein chains to each other, which first makes up the complete immunoglobulin) and are

then able to bind proteins that are intended for transport, conversion or recycling.

Comment by Karl Krafeld: An antibody can only be claimed if the body has been detected. It is claimed that many viral antibodies can be detected (e.g. by tests) without the virus being able to be verified scientifically. Orthodox medicine knows its own nonsense which it habitually spreads: "Antibodies are formed in infectious diseases and the detection of antibodies is a proof of protection against the disease". According to orthodox medicine, HIV-positivity should be the best protection against AIDS. Every test measures what the test measures, only nobody knows exactly what the test measures. The tests react quite unspecifically to proteins, according to the coffee grounds reading principle: Is Eduscho or Tschibo better for reading coffee grounds? In any case, no test can detect antibodies if the underlying body has never been detected.

Antibodies in reality/religion

Antibody fraud of the vaccination religion: Vaccination = Antibody = Protection = Long life and health (I have shown in detail that this assumption (belief) is not true and has been disproved by several studies.

The reality:

Small proteins are called globulins. These globulins are always produced by the body when cells need to be multiplied, repaired or newly formed. From the vaccination religion, globulins are called antibodies against better judgement, because these proteins bind very easily with other proteins and molecules. The whole vaccination business is based on the globulins' ability to bind with other proteins and molecules.

The so-called "antibodies" today were "healing bodies" in Emil von Behring's case in 1892 and "magic spheres" in Paul Ehrlich's case. The globulins formed by vaccination poisoning are claimed to be a protection against freely invented pathogens and the combination of

globulins with proteins from chicken embryos or artificial cells (laboratory artefacts), which are claimed to be components of viruses, is claimed to be vaccination protection against diseases (alleged "immunity"), which in turn are claimed to be against better judgement then caused by pathogens, but which in reality do not exist at all. Antibodies are the blood's response to infiltrated (inoculated) foreign proteins and foreign substances as in allergy.

The term "immunity" would have to be replaced by a term like "healing ability". Healing ability cannot be produced by any kind of vaccination, it is an ability of the whole being (body-mind-spirit-unity) and depends on many factors.

The "more toxic" the adjuvant, the stronger the "antibody reaction"

The antibody titer measurement only indicates poisoning/damage to the body.

The powerful aluminium adjuvant from Gardasil- The three Merck lawyers who gave presentations were Dino Sangiamo, Sally Bryan and Christina Gaarder. Jo Lyn Valoff represented Kaiser.

"Among vaccinologists, it's axiomatic that the duration of immunity correlates directly to the toxicity of the adjuvant; the more toxic the adjuvant, the longer the duration of immunity."

That's perfectly put. The toxins are supposed to measurably boost the antibody blood levels so that something can be measured and "proved" and which a vaccine cannot produce without these adjuvants.

The deception starts where the measured value is pretended to be immunity, because in reality it only indicates the degree of poisoning, completely independent of the effectiveness of a vaccine according to the key-lock theory and the fairy tales of viral load, antigens etc.

The interesting finding with multiple personalities

In the book The Vaccinated Nation by Andreas Moritz, a fact is described that also causes the belief in the antibody doctrine to collapse. Quote on the fraud of antibodies as proof for the alleged functioning of uselessly harmful vaccines:

"Having produced antibodies against a certain substance, for example against a food or a vaccine, does not really determine whether a disease such as an infection or allergy will actually occur.

For example, people with a multiple personality disorder in the role of one personality can be highly allergic to orange juice (allergen), while the same allergen, once they have switched to a different personality, suddenly no longer causes an allergic reaction

One may also show symptoms of diabetes in one personality and be free of diabetes a few minutes later. Women may even have completely different menstrual cycles.

Another example- In a normal person who is allergic to cat hair, when they come into contact with the proteins of cat hair, the formation of antibodies and inflammatory reactions are triggered. However, it is not uncommon for someone to be allergic only to white or red cats, but not to black cats (or vice versa). Usually there was a previous traumatic experience with a white cat - for example its death - which was related to the formation of antibodies.

As soon as the person touches a white cat, the body reproduces the reaction, based on the memory of the previous emotional trauma. Since black cats were not part of this experience, touching black cats does not cause allergic reactions.

Similarly, someone who is allergic to gluten may have a problem when eating bread, but not when eating pasta, even though it contains gluten."

In other words: one cannot know whether the mere presence of antibodies formed by vaccination actually protects against mumps or measles viruses. The entire vaccination

theory is based on the idea that the presence of specific antibodies in the blood confers immunity to the disease in question.

Feli Popescu: Rhesus factor, blood groups, blood plasma, anti-D prophylaxis

Feli Popescu has written an incredibly interesting article on the subject of rhesus factor, blood groups, blood plasma, anti-D prophylaxis. This article shows extreme inconsistencies and discrepancies in how science works. You can see in the article, how the antibody thesis is defeated in this respect as well. Very interesting.

High vaccination rates cannot prevent measles outbreaks - antibodies failed

We need "information not fear" and "facts not expert opinions".

In the following link Libertas & Sanitas has compiled more than 50 well-known studies by the CDC, Oxford and others that clearly show that vaccination does not protect. The results of the first 10 studies have been summarised directly in the PDF. This is a practical example showing claimed antibodies do not reflect the protection attributed to them. Since this is not an article about vaccines, I will not mention all the other studies, they will become part of another article. Source: Libertas & Sanitas e.V. [PDF]

Correspondence between Hans Tolzin and Robert Koch Institute (RKI) on the topic of antibodies

This correspondence shows that the RKI does not consider the antibody level (titer) the sole criterion for protection.

Thus the RKI writes on 01.02.2005

"Neither the RKI nor the STIKO consider the level of the AK concentration as the sole criterion for immunity and do not define it as such. Cellular immunity (immunological memory), which is particularly important for long-term immunity, is not dependent on the detectable AK titres and therefore AK titres often only serve as "surrogate markers" for

immunity". .... "However, undetectable or low AK titers are no proof of non-existent immunity.”

So we see, no matter if antibodies are measured or not, according to the RKI there is a protection in case of non-existing as well as existing antibodies. Since we know that these "antibodies" are created when cells are poisoned/ destroyed, it cannot be claimed that a virus is the cause, but rather e.g. poisoning by a vaccination and your harmful adjuvants.

To the question of Hans Tolzin:

"If, as you write, the level of the AK concentration does not allow a reliable statement about immunity, how can it be the sole criterion for the proof of benefit in the vaccine approval? I don't understand."

Answered by the RKI:

"Dear Mr. Tolzin, we have replied at length. For capacity reasons we cannot continue the discussion. Yours sincerely"

Note: No further comment is required. In the construction of lies full of unscientific claims and consensus without scientific basis, even the best liar loses the plot when confronted with reality.

You can read further excuses of the RKI on the site.

Correspondence between Hans Tolzin and Paul Ehrlich Institute (PEI) on the topic of antibodies.

On 13 May 2006, [Hans Tolzin] also submitted a request to the Paul Ehrlich Institute (PEI), the German licensing authority for vaccines:

"Please name the basic scientific studies or publications relevant to the PEI that prove the connection between AK levels and immunity (in the sense of actual non-disease over a longer period of time)".

Answer of the PEI:

""There is no general statement of the PEI that a sufficiently highly regarded specific antibody titer is a guarantee of non-disease. This statement is undifferentiated and does not correspond to scientific standards. The European Pharmacopoeia specifies exactly how efficacy is to be tested for the various vaccines."

Thus, the PEI staff have no scientific documentation that a high titre means no disease. Instead, the responsibility is shifted to the EU level. However, the regulations there contain both mandatory and optional provisions regarding proof of efficacy, so that the reference to them does not say anything about which criteria the PEI considers binding for itself. A corresponding question from me [Hans Tolzin] has not yet been answered. Every little piece of information has to be prized from authority's nose Source: Email

Spiess, "Impfkompendium", 5th edition 1999, p. 180 (in the chapter on pertussis)

"A conclusion from the level of the measured titre on the immune status regarding protection against recurrence of the disease is currently not possible".

Another study published in the J ournal Immunity (a scientific journal) shows that antibodies are not necessary to fight them.

"Our results contradict the current view that antibodies are absolutely necessary to survive infections with viruses such as VSV (vesicular stomatitis virus). They represent an unexpected function of B cells as guardians of macrophages for antiviral immunity," said Dr. H. Uldrich of Andrian of Harvard Medical School. "There is a need for further research into the role of antibodies and interferons in immune defence against similar viruses that attack the nervous system, such as rabies, West Nile virus and encephalitis."

Note: Even though it is already assumed by these researchers that there are viruses that cause illness, it shows once again that even among "believers" of the same faith, different

results emerge and that antibodies are not at all synonymous with protection.

With HIV, the complete logic of antibodies was finally overturned

Der Spiegel writes: "In HIV-infected persons, on the other hand, the scientists were able to detect above-average numbers of antibodies against various viruses. This could be explained by the fact that the HI virus can weaken the immune system and make the affected persons more susceptible to further infections."

In HIV, therefore, antibodies are more likely to indicate that the person is weakened, even though he or she has extremely high antibody levels. In principle, he should be the most protected person of all. But we see no difference from "pseudo" medicine. If something doesn't fit, the film is shot until it supposedly does. The basic thesis is not even questioned, although especially with HIV the dissenting voices were extremely strong. The topic HIV is one of its own and would go beyond the scope of this.

WHO: no evidence that SARS-CoV-2 antibodies mean immunity to COVID-19 – 04/18/2020

In the course of the study of COVID-19 patients, who again showed positive smear results after surviving the disease, the WHO reported on 17.04.that there is no evidence that the presence of antibodies against SARS-CoV-2. Antibodies in the serum means immunity to COVID-19 (CNN 18.04.2020) If this fear is confirmed, this calls into question a whole range of concepts that have been put forward as saviors - from herd immunity to messianically transfigured vaccine...

The conclusion from the whole situation is frightening...

Obviously, the responsible federal authorities are not aware of any scientific evidence of protection by antibodies. As a substitute, the "state of the art" and the "general acceptance" of such substitute measured variables ("surrogate parameters") are invoked without obligation. The employees of the authorities therefore assume a protective titer

without ever having seen the proof! This is exactly the problem we see all the time. It is always assumed without question. We have the same problem with the claim of the pathogenic measles virus, which has never been proven. We also have the same problem with SARS-CoV-1 and SARS-CoV-2, again and again the proof is missing, each time it is assumed that it is so. We are at a point where we must finally uncover the misguided development in medicine and introduce a paradigm shift.

• We claim disease-causing viruses, without proof

• We use surrogates like antibodies for protection, which also have no scientific basis and the reality has not at any time confirmed this claim.

• We use a DNA test (PCR) which cannot produce proof of a virus but is a manipulation tool and has never been validated.

• We use leading consultants who have already been convicted of fraud

I could continue on, but you can already see the huge problems we have because we looked away too long, because we believed everything without question, because we just wanted to trust. Today reality is catching up with us and we must act now, not later, otherwise these false claims will become even worse and the situation will be irreparable.

My appeal to you: "Write to the politicians, write to the RKI and PEI, confront them with the facts. Do not allow any excuses. The authorities have known about this information for a long time, yet they have not bothered or dared to correct it. What did Horst Seehofer say to ZDF about the power of the pharmaceutical lobby? (Backup of the video available)

Meaning: "The pharmaceutical lobby is too strong, this has been the case for 30 years, up to the hour it is not possible to introduce meaningful changes because these structures are so powerful that the politicians cannot influence them".

Seehofer says: "I can only tell you that this is the case and this is working very effectively"

In response to the reporter's question: "How is it possible that the pharmaceutical lobby is stronger than the politicians of a country?

Says Seehofer: "I can't disagree with you there..."

So we see that we are dealing with very powerful commercial enterprises (lobby), where not even politicians can/may make their own decisions. Do we really want to continue to walk blindly into this world?

..... THE REST OF THIS ARTICLE HERE

Stefan-Lanka-The-Misinterpretation-of-the-Antibodies-English-Translation.pdf (truthseeker.se) Https://truthseeker.se/wp-content/uploads/Stefan-Lanka-The-Misinterpretation-of-the-Antibodies-English-Translation.pdf

It became clear to me durring 2020 when I had spent most of the 12 months learning the problems with "pandemic" narrative, the issues with Virology and finally being introduced to Terrain Model, that reports on "reinfection" of "Covid" (or showing symptoms twice) was also pointing to Terrain Model. Although Virology, being so loosely formed around hypothosis always has some excuse as to why its failing to show true evidence for their explainations. Its almost comical to hear Fauci or Galo talk about antibodies.

All Roads Lead to Terrain (preamble)

"De Jong, 22, had tested positive on 17 April and suffered mild symptoms for about 2 weeks. She tested negative on 2 May—just in time to say farewell to her dying grandmother—and returned to work as a nursing intern in a hospital in Rotterdam, the Netherlands.

But when her symptoms re-emerged, her doctor suggested she get tested again. “A reinfection

this soon would be peculiar, but not impossible," she told De Jong, who by then had again lost her sense of smell and had abdominal pains and diarrhea."

https://www.sciencemag.org/news/2020/11/more-people-are-getting-covid-19-twice-suggesting-immunity-wanes-quickly-some?fbclid=IwAR3KQs7c5lBlIem0aQ0ibbZr2kjCrWxZSjCU2_bP8MS69pcx15f6jJJ6BPc

" The woman was first diagnosed with Covid-19 in late January, according to a statement released by Osaka's prefectural government Wednesday. She was discharged shortly after, once her symptoms had improved. A subsequent test came back negative for the virus. Three weeks later she returned with a sore throat and chest pain and tested again.

You may have given blood and been told you have antibodies or had COVID-19 and trusted your body to fight off the infection. However, health officials say there is no hard evidence showing the antibodies built up can protect against reinfection.

.... someone testing positive for antibodies doesn't mean they are truly resistant to the disease, and it does not tell us they are resistant to infection," said Taylor."

https://www.news9.com/story/6008cfce6d58690bbf3a86bf/state-epidemiologist-says-no-clear-evidence-to-show-antibodies-protects-against-covid19-reinfection?fbclid=IwAR1hcOcKzFC3xqu_SYsTgMcLSc-2PSthPlLr3qV2dabu7t7wAPAjbkSQsGs

(THIS GOES FOR A VACCINE ALSO, TERRAIN model...)

At least 285 individuals in Mexico appear to have contracted the novel coronavirus twice, according to a preprint posted October 18 on medRxiv. ...

"If we find that our immunity is poor, or nonexistent . . . this will be a big problem for vaccination policies," study coauthor Carlos Hernandez-Suarez, a researcher at Universidad de Colima in San Sebastian, Mexico....

https://www.the-scientist.com/news-opinion/more-sars-cov-2-reinfections-reported-but-still-a-rare-event-68089?fbclid=IwAR3kwZFYr2jbwx9u89ejvfXCHPP_hAs4MfhMEnRGIto52kejpQOTlsjKR_M

(The whole tale of antibodies is how we are sold vaccines - yet they dont know really know much about them. This puts a giant holw in the idea we have an immunty system per say, putting the idea of vaccines in question. Looks like more fraud. Antibodies are clearly a poor measure for "infection" or "protection". So the immune system is another over thought and funded theory because it makes a fortune.)

Medcrave

Underlying the birth of virology was the doctrine of monomorphism--that all microorganisms are fixed species, unchangeable; that each pathological type produces only one specific disease; that microforms never arise endogenously, i.e., have absolute origin within the host; and that blood and tissues are sterile under healthy conditions.

Theoretically, under ideal health conditions, the blood might be sterile, though it has the inherent potential to develop morbid microforms, as discussed earlier. Long and repeated observation of live blood in the phase-contrast, dark-field microscope, however, shows that the blood can contain various microforms in an otherwise asymptomatic host, or in a condition, or in a condition defined as normal or healthy in orthodox terms.

Monomorphism was the cornerstone of developments in 20th-century medical research and treatments. Refusal by the mainstream to examine fairly, much less accept, the demonstrated facts of pleomorphism--that viruses and bacteria, yeast, fungi and mold, are evolutions from microzyma; that microforms can rapidly change their form (evolve and devolve) in vivo, one becoming another, dependent upon conditions in the biological terrain (environment); that blood and tissues are not necessarily sterile; and that there are no specific diseases, but only specific disease conditions--was the foundation of the debate. It is so called because those who wore the "robes" of scientific authority would not be swayed from folly when resented with its contrary proofs.

These proofs began in earnest with Antoine Béchamp in the nineteenth-century.

For a plesant detour this link here, and learn about more the more clearly observable biological functions.

https://medcraveonline.com/IJVV/IJVV-02-00032?fbclid=IwAR1wdBWcsnB84P9esKu5Zb5gdXQnAfdMUeDmawz6MSIEPs0Dhc6ctOswfmI

WE HAVE BEEN SOLD ON THIS ASSUMPTION

A piece of a live or dead virus created immunity and taught our body to create protecting antibodies.

Here is part of that history.

HISTROY OF ANTIBODY THEORY

_____

LINUS PAULING'S 1940 ANTIBODY STRUCTURE THEORY:

Linus Pauling was an American Chemist who published a vast library of work with well over 1200 papers and books. He is the only person to win two unshared Nobel Prizes and is considered one of the 20 greatest scientists of all time. Pauling also gets the distinction of "proving" correct Paul Ehrlich's lock and key mechanism for how antibodies/antigens work:

"Ehrlich proposed a ‘lock and key’ mechanism of antibody-antigen interaction but this WAS NOT CONFIRMED UNTIL THE 1940s BY LINUS PAULING."

https://www.rcpath.org/uploads/assets/077f9015-91d1-41a4-ba3fd408b884967b/17-

Structure-of-an-Antibody.pdf

"THE NEXT MAJOR ADVANCE WAS IN THE 1940s, WHEN LINUS PAULING CONFIRMED THE LOCK-AND-KEY THEORY PROPOSED BY EHRLICH by showing that the interactions between antibodies and antigens depend more on their shape than their chemical composition."

https://en.m.wikipedia.org/wiki/Antibody

Pauling was able to prove Ehrlich's theoretical explanations and drawings correct by...providing his own theoretical explanations and drawings. And depending on what source you look to, he was either correct in his theory or entirely wrong:

"Pauling was intrigued by Landsteiner's work, and began reading about antibodies; he was interested and puzzled by what he found. While the scientific community knew that antibodies worked, HOW EXACTLY THEY WORKED AND HOW EXACTLY THEY WERE FORMED WERE STILL UNKNOWN.

At the time, there were FOUR MAIN SCHOOLS OF THOUGHT REGARDING THE CREATION OF ANTIBODIES: the Antigen-Incorporation theory, the Side-Chain theory, the Instruction theory, and the Selection theory."

"The Instruction theory states that the body uses antigens as a template, then manufactures antibodies to specifically combat the antigen that the antibody is based off of. PAULING EVENTUALLY BELONGED TO THIS SCHOOL OF THOUGHT, as did Landsteiner, Michael Heidelberger, Felix Haurowitz, and Jerome Alexander. THIS GROUP WAS FAR FROM UNIFIED HOWEVER; the only point on which adherents to this school agreed was that antigens acted as templates. HOW ANTIBODIES WORKED, AND HOW THEY WERE PRODUCED, WAS STILL A HIGHLY CONTENTIOUS QUESTION."

"Another argument developed in the 1940 paper was that antibodies are bivalent – that is, they have two sites which can bind to antigens. In addition to being bivalent, PAULING HYPOTHESIZED THAT EACH OF THE "ARMS" OF AN ANTIBODY COULD LATCH ONTO DIFFERENT KINDS OF ANTIGENS. While PAULING WAS INCORRECT ON THE LATTER PART – antibodies can only grab onto one type of antigen – he was correct that they are bivalent.

Pauling had gotten off to a strong and noticeable start in the field of immunology. WHETHER CORRECT OR INCORRECT, he was making progress towards a greater understanding of how the body protects itself."

https://paulingblog.wordpress.com/2013/08/01/thinking-about-the-creation-of-antibodies/

"THE MODEL FIT A GREAT DEAL OF DATA. It was simple and clean. Pauling wrote it up and SENT

WHAT HE HOPED WOULD BE A LANDMARK PAPER ON ANTIBODY FORMATION to the Journal of the American Chemical Society in the summer of 1940. "What is the simplest structure which can be suggested . . . for a molecule with the properties observed for antibodies, and what is the simplest reasonable process of formation for such a molecule?" he asked at the beginning. Then he elegantly and persuasively put forward his ideas.

HIS PAPER MADE A GREAT STIR. It was another demonstration of the power of applying chemical ideas to biology. It was a new vindication of Weaver's approach, which he was now calling "molecular biology."

IT WAS ONLY LATER THAT IT WAS PROVEN COMPLETELY WRONG."

http://scarc.library.oregonstate.edu/.../narr.../page17.html

Below are some highlights from Pauling's 15-page Theory:

A THEORY OF THE STRUCTURE AND PROCESS OF FORMATION OF ANTIBODIES

"I. Introduction

During the past four years I have been making

an effort to understand and interpret serological phenomena in terms of molecular structure and molecular interactions. The field of immunology is so extensive and the experimental observations are so complex (AND OCCASIONALLY CONTRADICTORY) that NO ONE HAS FOUND IT POSSIBLE TO INDUCE A THEORY OF THE STRUCTURE OF ANTIBODIES FROM THE OBSERVATIONAL MATERIAL. As an alternative method of attack we may propound and attempt to answer the following questions: WHAT IS THE SIMPLEST STRUCTURE WHICH CAN BE SUGGESTED, on the basis of the extensive information now available about intramolecular and intermolecular forces, for a molecule with the properties observed for antibodies, AND WHAT IS THE SIMPLEST REASONABLE PROCESS OF FORMATION OF SUCH A MOLECULE? Proceeding in this way, I HAVE DEVELOPED A DETAILED THEORY OF THE STRUCTURE AND PROCESS OF FORMATION OF ANTIBODIES and the nature of serological reactions which is more definite and more widely applicable than earlier theories, and which is compatible with our present knowledge of the structure and properties of simple molecules as well as with most of the

direct empirical information about antibodies. This theory is described and discussed below.

11. The Proposed Theory of the Structure and Process of Formation of Antibodies

When an antigen is injected into an animal some of its molecules are captured and held in the region of antibody production.2 An antibody to this antigen is a molecule with a configuration which is complementary to that of a portion of the antigen molecule. This complementariness gives rise to specific forces of appreciable strength between the antibody molecule and the antigen molecule; we may describe this as a bond between the two molecules. I ASSUME, with Marrack, Heidelberger, and other investigators, THAT THE PRECIPITATE OBTAINED IN THE PRECIPITIN REACTION IS A FRAMEWORK, AND THAT TO BE EFFECTIVE IN FORMING THE FRAMEWORK AN ANTIBODY MOLECULE MUST HAVE TWO OR MORE DISTINCT REGIONS WITH SURFACE CONFIGURATION COMPLEMENTARY TO THAT OF THE ANTIGEN. THE RULE OF PARSIMONY (the use of the minimum effort to achieve the result) suggests that there are only two such regions, that is, that the antibody molecules are at the most bivalent. THE PROPOSED THEORY IS BASED ON THIS REASONABLE ASSUMPTION. It would, of course, be possible to expand the theory in such a way as to provide a mechanism for the formation of antibody molecules with valence higher than two ; but this would make the theory considerably more complex, and it is likely that antibodies with valence higher than two occur only rarely, if at all."

"The effect of an antigen in determining the structure of an antibody molecule might involve the ordering of the amino-acid residues in the polypeptide chains in a way different from that in the normal globulin, as suggested by Breinl and Haurowitz" and Mudd. I ASSUME, HOWEVER, THAT THIS IS NOT SO, BUT THAT ALL ANTIBODY MOLECULES CONTAIN THE SAME POLYPEPTIDE CHAINS AS NORMAL GLOBULIN, AND DIFFER FROM NORMAL GLOBULIN ONLY IN THE CONFIGURATION OF THE CHAIN; that is, in the way that the chain is coiled in the molecule. THERE IS AT PRESENT NO DIRECT EVIDENCE SUPPORTING THIS ASSUMPTION. The assumption is made because, although I HAVE FOUND IT IMPOSSIBLE TO FORMULATE IN DETAIL A REASONABLE MECHANISM WHEREBY THE ORDER OF AMINO-ACID RESIDUES IN THE CHAIN WOULD BE DETERMINED BY THE ANTIGEN, a simple and reasonable mechanism, described below, can be advanced whereby the antigen causes the polypeptide chain to assume a configuration complementary to the antigen. The number of configurations accessible to the polypeptide chain is so great as to provide an explanation of the ability of an animal to form antibodies with considerable specificity for an apparently unlimited number of different antigens,8 without the necessity of invoking also a variation in the amino-acid composition or

amino-acid order.

The Postulated Process of Formation of Antibodies.

LET US ASSUME THAT THE GLOBULIN MOLECULE CONSISTS OF A SINGLE POLYPEPTIDE CHAIN, containing several hundred amino-acid residues, and that the order of amino-acid residues is such that for the center of the chain one of the accessible configurations is much more stable than any other, whereas the two end parts of the chain are of such a nature that there exist for them many configurations with nearly the same energy. (This point is discussed in detail in Section IV.) Four steps in our postulated process of formation of a normal globulin molecule are illustrated on the left side of Fig. 1."

"III. Some Points Of with Experiment

a. The Heterogeneity Of Immune Sera

THE THEORY REQUIRES THAT THE SERUM HOMOLOGOUS TO A GIVEN ANTIGEN BE NOT HOMOGENEOUS, BUT HETEROGENEOUS, CONTAINING ANTIBODY MOLECULES OF GREATLY VARIED CONFIGURATIONS. Many of the antibody molecules will be bivalent, with two active ends with configuration complementary to portions of the surface of an antigen molecule. Great variety in this complementary configuration would be expected to result from the accidental approximation to one or another surface region, and further variety from variation in position of the antigen molecule relative to the point of liberation of the globulin chain end and from accidental coiling and linking of the chain end before it comes under the influence of the antigen. Some of the antibody molecules would be univalent, one of the chain ends having, because of its too great distance from the antigen, folded into a normal globulin configuration."

"b. The Bivalence of Antibodies and the

Multivalence of Antigens.

OUR THEORY IS BASED ON THE IDEA THAT THE PRECIPITATE FORMED IN THE PRECIPITIN REACTION IS A NETWORK OF ANTIBODY AND ANTIGEN MOLECULES IN WHICH MANY OR ALL OF THE ANTIBODY MOLECULES GRASP TWO ANTIGEN MOLECULES APIECE AND THE ANTIGEN MOLECULES ARE GRASPED BY SEVERAL ANTIBODY MOLECULES. The direct experimental evidence for this picture of the precipitate has been ably discussed by its propounders and supporters, Marrack and Heidelberger and Kendall, and need not be reviewed here. TO THE STRUCTURAL CHEMIST IT IS CLEAR THAT THIS PICTURE OF THE PRECIPITATE MUST BE CORRECT.

The great specificity of antibody-antigen interactions requires that a definite bond be formed between an antibody molecule and an antigen molecule. If antibodies or antigens were univalent, this would lead to complexes of one antigen molecule and one or more antibody molecules (or of one antibody molecule and one or more antigen molecules), and we know from experience with proteins that these aggregates would in general remain in solution. If both antibody and antigen are multivalent, however, the complex will grow to an aggregate of indefinite size, which is the precipitate."

"IT SEEMS PROBABLE THAT ALL ANTIBODIES HAVE THIS STRUCTURE-THAT THEY ARE BIVALENT, WITH THEIR TWO ACTIVE REGIONS OPPOSITELY DIRECTED. Heidelberger and his collaborators and Marrack have emphasized the multivalence of antibodies and antigens,14 but limitation of the valence of antibodies to the maximum value two (ignoring the exceptional case of the attachment of two or more antigens or haptens to the same end region of an antibody) has not previously been made."

"c. The Antibody-Antigen Molecular Ratio in Precipitates.

OUR THEORY PROVIDES AN IMMEDIATE SIMPLE EXPLANATION OF THE OBSERVED ANTIBODY-ANTIGEN MOLECULAR RATIOS IN PRECIPITATES. Under OPTIMUM CONDITIONS a precipitate will be formed in which all the valences of the antibody and antigen molecules are satisfied. AN IDEALIZED REPRESENTATION OF A PORTION OF SUCH A PRECIPITATE IS GIVEN IN Fig. 3. The figure shows a part of a layer with each antigen molecule bonded to six surrounding antibody molecules ; this structure represents the value N = 12 for the valence of the antigen, each antigen molecule being attached also to three antibody molecules above the layer represented and to three below. Each antibody molecule is bonded to two antigen molecules, one at each end. An ideal structure of the antibody-antigen precipitate for N = 12 may be described as having antigen molecules at the positions corresponding to closest packing, with the twelve antibody molecules which surround each antigen molecule lying along the lines connecting it with the twelve nearest antigen neighbors.

SIMILAR IDEAL STRUCTURES CAN BE SUGGESTED FOR OTHER VALUES OF THE ANTIGEN VALENCE. The antigen molecules might be arranged for N = 8 at the points of a body-centered cubic lattice, and for N = 6 at the points of a simple cubic lattice, with antibody molecules along the connecting lines. For N = 4 the antigen molecules, connected by antibody molecules, might lie at the points occupied by carbon atoms in diamond; or two such frameworks might interpenetrate, as in the cuprous oxide arrangement (copper and oxygen atoms being replaced by antibody and antigen molecules, respectively).

IT IS NOT TO BE INFERRED THAT THE ACTUAL PRECIPITATES HAVE THE REGULARITY OF STRUCTURE OF THESE IDEAL ARRANGEMENTS. The nature of the process of antibody formation,

INVOLVING THE USE OF A PORTION OF THE ANTIGEN SURFACE SELECTED AT RANDOM AS THE TEMPLATE FOR THE MOLDING OF AN ACTIVE END OF AN ANTIBODY MOLECULE, INTRODUCES SO MUCH IRREGULARITY IN THE FRAMEWORK THAT A REGULAR STRUCTURE ANALOGOUS TO THAT OF A CRYSTAL IS PROBABLY NEVER FORMED. The precipitate is to be compared rather with a glass such as silica glass, in which each silicon atom is surrounded tetrahedrally by four oxygen atoms and each oxygen atom is bonded to two silicon atoms, but which lacks further orderliness of arrangement. Additional disorder is introduced in the precipitate by variation in the effective valence of the antigen molecules and by the inclusion of antibody molecules with only one active end."

"IT IS SEEN THAT OUR THEORY PROVIDES A SIMPLE EXPLANATION OF THE FACT THAT FOR ANTIGENS OF MOLECULAR WEIGHT EQUAL TO OR LESS THAN THAT OF THE ANTIBODY THE PRECIPITATE CONTAINS CONSIDERABLY MORE ANTIBODY THAN ANTIGEN. The values given in Table I are not to be considered as having rigorous quantitative significance. The calculated maximum molecular ratio would be larger for elongated antibody molecules than for spherical antibody molecules, and larger for non-spherical than for spherical antigen molecules, and, moreover, in many sera antibodies might be complementary in the main only to certain surface regions of the antigen, the number of these determining the valence of the antigen. That this is so is indicated by the observation that after long immunization of a rabbit with egg albumin serum was obtained giving a precipitate with a considerably larger molecular ratio than that for earlier bleedings."

"d. The Use of a Single Antigen Molecule as the Template for an Antibody Molecule.

THERE ARE TWO WAYS IN WHICH AN ANTIBODY MOLECULE WITH TWO OPPOSED ACTIVE REGIONS COMPLEMENTARY TO THE ANTIGEN MIGHT BE PRODUCED. One is the way described in Section 11. The other would involve the which (with an occasional exception) both antigen manufacture of the antibody molecule in its and antibody are bivalent, the molecular ratio final configuration between two antigen molecules, one of which would serve as the pattern for one antibody end and the other for the second. NO ATTEMPTS TO DECIDE BETWEEN THESE ALTERNATIVES SEEMS TO HAVE BEEN MADE BEFORE; evidence, however, some of which is mentioned below, to indicate that the first method of antibody production, involving only one antigen molecule, occurs predominantly. IT IS FOR THIS REASON THAT I HAVE DEVELOPED THE RATHER COMPLICATED THEORY DESCRIBED ABOVE, with the two end portions of the antibody forming first, one (or both) then separating from the antigen, and the central part of the antibody then assuming its shape and holding the active ends in position for attachment to two antigen molecules.

THIS THEORY REQUIRES THAT THE FORMATION OF ANTIBODY BE A REACTION OF THE FIRST ORDER WITH RESPECT TO THE ANTIGEN, whereas the other alternative would require it to be of

the second order. THERE EXISTS VERY LITTLE EVIDENCE AS TO WHETHER ON IMMUNIZATION WITH SMALL AMOUNTS OF ANTIGEN THE ANTIBODY PRODUCTION IS PROPORTIONAL TO THE AMOUNT OF ANTIGEN INJECTED OR TO ITS SQUARE."

"e. Criteria for Antigenic Power.

THERE HAS BEEN EXTENSIVE DISCUSSION OF THE QUESTION OF WHAT MAKES A SUBSTANCE AN ANTIGEN, BUT NO GENERALLY ACCEPTED CONCLUSIONS HAVE BEEN REACHED. OUR THEORY PERMITS THE FORMULATION OF THE FOLLOWING REASONABLE CRITERIA FOR ANTIGENIC ACTIVITY:

1. The antigen molecule must contain active groups, capable of sufficiently strong interaction with the globulin chain to influence its configuration.

2. The configuration of the antigen molecule must be well-defined over surface regions large enough to give rise to an integrated antibody antigen force sufficient to hold the molecules together.

3. The antigen molecule must be large enough to have two or more such surface regions, and in case that the antigenic activity depends upon a particular group the molecule must contain at least two of these groups. (This criterion applies to antibodies effective in the precipitin and agglutinin reactions and in anaphylaxis.)"

"IV. A More Detailed Discussion of the Structure of Antibodies and Other Proteins

THERE HAS BEEN GATHERED SO FAR VERY LITTLE DIRECT EVIDENCE REGARDING THE DETAILED STRUCTURE OF PROTEIN MOLECULES. Chemical information is compatible with the polypeptide-chain theory of protein structure, and this theory is also supported by the rather small amount of pertinent X-ray evidence.25"

"LAYER STRUCTURES OTHER THAN THIS ONE MIGHT ALSO BE ASSUMED, in which the chains are not extended. Some fibrous proteins, such as a-keratin, are known to have structures of this general type, BUT THE NATURE OF THE FOLDING OF THE CHAINS HAS NOT YET BEEN

DETERMINED.

WE HAVE POSTULATED THE EXISTENCE OF AN EXTREMELY LARGE NUMBER OF ACCESSIBLE CONFIGURATIONS with nearly the same energy for the end parts of the globulin polypeptide chain. A layer structure, with variety in the type of folding in the layer, would not, it seems to me, give enough configurational possibilities to explain the great observed versatility of the antibody precursor in adjusting itself to the antigen, and I think that skew configurations must be invoked.``

V. Further Comparison of the Theory with Experiment. Possible Experimental Tests of Predictions

a. Methods of Determining the Valence of Antibodies.

"The bivalence of antibodies and our postulate that only one antigen molecule is involved in the formation of an antibody molecule require that a precipitin-effective antihapten be produced only if the injected antigen contains at least two hapten groups. PERTINENT DATA HAVE BEEN OBTAINED ON THIS POINT BY HAUROWITZ and his collaborators,"

FROM THE FOOTNOTES:

"From the data discussed above, WHICH IN OUR OPINION indicate that two hapten groups combine with a bivalent antibody molecule, HAUROWITZ DREW THE DIFFERENT CONCLUSIONS that one arsenic-containing group in the antigen combines with one antibody molecule."

"IF WE ASSUME THAT THE CONDITIONS OF EACH PRECIPITATION WERE SUCH THAT ONLY SUITABLE BIVALENT ANTIBODY MOLECULES WERE INCORPORATED IN THE PRECIPITATE, the equations above would require the third precipitate to weigh about 24 mg. ; the experiment accordingly provides some support for the theory."

"THE QUALITATIVE EXPERIMENTAL RESULTS which have been reported ARE IN PART COMPATIBLE AND IN PART INCOMPATIBLE WITH THE THEORY."

VI. Processes Auxiliary to Antibody Formation

"It seems not unlikely that certain processes auxiliary to antibody formation occur. The reported increase in globulin (aside from the antibody fraction) after immunization suggests the

operation of a mechanism whereby the presence of antigen molecules accelerates the synthesis of the globulin polypeptide chains. THERE IS LITTLE BASIS FOR SUGGESTING POSSIBLE MECHANISMS FOR THIS PROCESS AT PRESENT."

"Summary

IT IS ASSUMED THAT ANTIBODIES DIFFER FROM NORMAL SERUM GLOBULIN ONLY IN THE WAY IN WHICH THE TWO END PARTS OF THE GLOBULIN POLYPEPTIDE CHAIN ARE COILED, these parts, as a result of their amino-acid composition and order, having accessible a very great many configurations with nearly the same stability; under the influence of an antigen molecule they assume configurations complementary to surface regions of the antigen, thus forming two active ends. After the freeing of one end and the liberation of the central part of the chain this part of the chain folds up to form the central part of the antibody molecule, with two oppositely-directed ends able to attach themselves to two antigen molecules.

Among the points of comparison of the theory and experiment are the following: the heterogeneity of sera, the bivalence of antibodies and multivalence of antigens, the framework structure and molecular ratio of antibody-antigen precipitates, the use of a single antigen molecule as template for an antibody molecule, criteria for antigenic activity, the behavior of antigens containing two different haptens, the antigenic activity of antibodies, factors affecting the rate of antibody production and the specificity of antibodies, and the effect of denaturing agents. It is shown that MOST OF THE REPORTED EXPERIMENTAL RESULTS ARE COMPATIBLE WITH THE THEORY. Some new experiments suggested by the theory are mentioned."

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In Summary:

-the science and findings of Immunology were so vast, complex, and contradictory that no one had attempted to describe the structure and formation of antibodies

-Pauling based his theories on what could be the simplest explanation for the structure and formation of an antibody

-he ASSUMED that the precipitate in the precipitin reactions was the framework for an antibody and that there must be 2 or more regions complementary to an antigen

-he based his assumptions of the form of an antibody on the rule of parsimony (the use of

minimum effort to achieve a result)

-he ASSUMED that all antibodies are similar to normal globulins and differ only in the configuration of their chains

-however. he admits there is no direct evidence supporting his assumptions

-Pauling based his assumption on the fact that he could not formulate a reasonable mechanism where the amino-acid chains were determined by the antigen

-he once again ASSUMES that the globulin molecule is a single polypeptide chain containing several hundred amino-acid residues

-his theory requires that the serum homogulous to the given antigen not be homogeneous but heterogeneous containing antibody molecules consisting of many configurations

-his theory is based on the idea that the precipitate is a network of antibody/antigen molecules in which antibody molecules grasp two antigen molecules a piece and the antigens are grasped by several antibody molecules

-he states the picture of the precipitate is clear to structural chemists so it must be correct

-he states it is PROBABLE that antibodies are bivalent

-he believed his theory provided a simple explanation for the antibody-antigen molecular ratios in precipitates under optimum conditions

-Pauling provided drawings of idealized representations of antibodies and antigens

-however, he states it should not be inferred that the precipitates have the regularity of structure as presented in the idealized versions

-he states the nature of antibody formation, using a portion of the antigen surface selected at random as the template for the molding of an active end of an antibody molecule, probably never forms the perfect crystalline structure represented

-Pauling states there are two ways in which antibodies with two-opposing active regions complementary to the antigen might be produced, yet no attempts have been made to decide between them, which is why he created his complicated theory

-his theory requires that the formation of the antibody be a reaction of the first order in regards to the antigen

-Pauling says there have been extensive discussions of what makes a substance an antigen but

accepted conclusions have not been reached

-he states his theory allows for the formulation of reasonable criteria for antigenic activity

-he admits there has been very little direct evidence on the detailed structure of protein molecules

-there are potential layer structures which can be assumed but no determination has been made on the folding of the chains

-he postulated the existence of very large numbers of accessible configurations

-Pauling provides evidence from Haurowitz which he states supports his conclusion that two hapten groups combine with the bivalent antibody but admits in the footnotes that Haurowitz came to a different conclusion

-he ASSUMES that the conditions in the precipitation were such that only suitable bivalent antibody molecules were present in the precipitate

-he admits experimental results are somewhat compatible and also somewhat incompatible with his theory

-he admits there is little evidence to propose mechanisms for how globulin increases after immunization

-Pauling concludes by stating it is ASSUMED that antibodies differ from serum globulin only in the way the two globulin polypeptide chains are coiled

-he states most of the experimental evidence is compatible with his theory

If you enjoy your "proof" in the form of assumptions and guesswork based on cherry-picked experimental research, Pauling's paper will be right up your alley. The amount of times he admits to making assumptions is astonishing. Granted, it is admitted to be a THEORY on the structure and formation of antibodies. However, it is lauded by some as proof of the form of antibodies as well as confirmation of Ehrlich's own theory regarding the lock-and-key mechanism of action. One unproven theoretical explanation does not get to confirm another one.

PAUL EHRLICH'S 1900 SIDE-CHAIN THEORY PART 2:

THE COMPLEMENT SYSTEM

The last part of Ehrlich's presentation to the Royal Society formed around the idea of the complement system. In short, this is a part of the immune system that complements the ability of antibodies to clear out the damaged cells and the inhabiting microbes through inflammation in order to attack the pathogen and eliminate it from the body. A brief overview of Ehrlich's role in the creation of this theory:

"Paul Ehrlich described the side-chain theory of antibody formation, especially the mechanisms of antibody neutralisation by toxins that induced bacterial lysis with the help of complement (which has replaced the historical term alexin). According to his theory, the immune cells contained receptors that could recognise antigens, and following immunisation, these receptors multiplied and were shed into the circulation as ‘amboceptors’ (NOW CALLED ANTIBODIES). These antibodies attached not only to specific antigens but also to a heat-labile antimicrobial component called ‘complement’ [8, 9]. EHRLICH'S THEORY PROPOSED THAT THE ANTIBODY AND COMPLEMENT COMBINED TO FORM A COMPLEX ENZYME CAPABLE OF ATTACKING AND KILLING CELLS AND MICROORGANISMS. In the ensuing years, this concept had a protagonist in the form of Bordet who argued that the antigen-antibody union was reversible, CONTRADICTING EHRLICH'S VIEW THAT THE ANTIGEN-ANTIBODY UNION WAS A FIRM AND BASED ON STEREO CHEMICAL SPECIFICITY [10]. Ehrlich’s concept EMPHASIZED THE PRESENCE OF MULTIPLE ANTIGENS AND COMPLEMENTS IN THE SERUM, while Bordet’s view revolved around a ‘single complement’ component that bound non-specifically to the antigen."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3956958/

Below are some highlights from the remainder of Ehrlich's report on his theoretical investigation into immunity which details the complement system. I provided some additional insight afterwards regarding how this theory was utilized:

"Bordet showed shortly thereafter, that in the case quoted there was present in the serum a specific hsemolysine which dissolved the blood corpuscles of the rabbit. He also proved that these hsemolysines as had already been shown by Buchner and Daremberg in the case of similarly acting bodies which are present in normal blood lost their solvent property on being maintained during half an hour at a temperature of 55° C. Bordet added, further, the new fact, that the blood-solvent property of these sera which had been deprived of solvent power by heat, the solvent action could be restored if certain normal sera were added to them.

By this important observation an exact analogy was established with the facts of bacteriolysis as elicited by the work of Pfeiffer, Metchnikoff, and Bordet. In the work on the Pfeiffer phenomenon of bacteriolysis, it had already been ascertained that the solution of bacteria by specific bacteriolysines WAS BROUGHT ABOUT BY THE COMBINED ACTION OF TWO DIFFERENT BODIES: ONE WHICH WAS SPECIFIC, AROSE DURING THE IMMUNISATION AND WAS STABLE; AND ANOTHER, A VERY UNSTABLE BODY, WHICH WAS PRESENT IN NORMAL SERUM.

In collaboration with Dr. Morgenroth, I have sought in regard to this question, for which haemolysis offered prospects favourable to experimentation, to make clear the mechanism concerned in the action of these two components—THE STABLE, WHICH MAY BE DESIGNATED "IMMUNE BODY," AND THE UNSTABLE, WHICH MAY BE DESIGNATED "COMPLEMENT"—which,' acting together, effect the solution of the red blood corpuscles. For this purpose, in the first place, solutions containing either only the " immune body " or only the " complement " were brought in contact with suitable blood corpuscles, and after separation of the fluid and the corpuscles by centrifugalising, we investigated whether these substances had been taken up by the red blood corpuscles or remained behind in the fluid. The proof of its location in the one position or in the other was readily forthcoming, since to restore to the hsemolysine its former activity, IT WAS ONLY NECESSARY TO ADD TO THE "IMMUNE BODY" A FRESH SUPPLY OF "COMPLEMENT," OR TO THE "COMPLEMENT" A FRESH SUPPLY OF "IMMUNE BODY," in order that the presence of the hsemolysine in its integrity might be shown by the occurrence of solution of the blood-cells.

The experiments proved that, after centrifugalising, THE "IMMUNE BODY" IS QUANTITATIVELY BOUND TO THE RED BLOOD CORPUSCLES, AND THAT THE "COMPLEMENT," ON THE CONTRARY, REMAINS ENTIRELY BEHIND IN THE FLUID.

The presence of the two components in contact with blood corpuscles only occasions the

solution of these at higher temperatures, and not at 0° C. And an active haemolytic serum (with “immune body” and "complement” both present) having been placed in contact with red blood corpuscles and maintained for a while at 0° C., it was found after centrifugalising that, under these circumstances also, the “immune body” had united with the red blood corpuscles, but that the “complement” remained in the serum. This experiment showed that both components must, at a temperature of 0° C., have existed alongside one another in a free condition.

But when analogous experiments were undertaken at a higher temperature it was found that both components were retained in the sediment.

THESE FACTS CAN ONLY BE EXPLAINED BY MAKING CERTAIN ASSUMPTIONS REGARDING THE CONSTITUTION OF THE TWO COMPONENTS, i.e., OF THE “IMMUNE BODY” AND THE “COMPLEMENT.” In the first place, two haptophore groups must be ascribed to the “immune body,” one having a great affinity for a corresponding haptophore group of the red blood corpuscles and with which at lower temperatures it quickly unites, and another haptophore group of a lesser chemical affinity, which at a higher temperature becomes united with the “complement” present in the serum. Therefore, at the higher temperature, the red blood corpuscles will draw to themselves those molecules of the “immune body” which in the fluid have previously become united with the “complement.”

In this case the “immune body” represents in a measure the connecting chain which binds the complement to the red blood corpuscles, and so brings them under its deleterious influence. Since under the influence of the “complement”—at least, in the case of the bacteria—appearances are to be observed (for example, in the Pfeiffer phenomenon) which must be regarded as analogous to digestion, we shall not seriously err if we ascribe to this “complement” a ferment-like character.

IT IS OBVIOUS THAT WHEN THE NORMAL SERUM OF ONE ANIMAL POSSESSES HAEMOLYTIC ACTION ON THE BLOOD OF ANOTHER, the component of the hsemolysine which here unites with the red blood corpuscle and forms the connecting link between it and the “complement” which is essential to the occurrence of solution, CANNOT, IN THE ABSENCE OF ANY PRECEDING PROCESS OF IMMUNISATION, BE DESIGNATED “IMMUNE BODY.” In its characteristics and action, however, IT ONLY DIFFERS FROM THIS IN OCCURRING NATURALLY, AND MAY WELL BE DESIGNATED “INTERMEDIATE BODY”

(Zwischenkorper). It may here be stated that the constitution of a haemolysine is graphically represented in fig. 7, Plate 7.

Very important for the conclusion that only with the assistance of the "intermediate body" or of the "immune body" can the "complement," which leads to the solution, become united with the blood corpuscle, is the following experiment. The serum of the dog has very considerable solvent action upon guinea-pig's blood, but loses this property if warmed. If dog's serum, thus rendered inactive by warming, is brought into contact with suspended corpuscles of guinea-pig's blood, these are not dissolved; but, if to such a mixture there are also added guinea-pig serum, i.e., the serum normal to these red blood corpuscles, the erythrocytes are at once dissolved. HERE THE ONLY EXPLANATION IS THAT THE "INTERMEDIATE BODY," which possesses a specific affinity for guinea-pig erythrocytes, and is present in the inactive dog's serum, IS ABLE TO SEIZE ON ONE OF THE MANY "COMPLEMENTS" PRESENT IN GUINEA-PIG'S SERUM, with the result that the "complement" which cannot normally attach itself to the corpuscles, comes now to exercise its destructive influence.

We see at the same time from this experiment that the HSEMOLYSINES OCCURRING NATURALLY, OBEY THE SAME LAWS AS THOSE PRODUCED THROUGH THE PROCESS OF IMMUNISING. In fact, for them also, in a great number of instances, precisely similar behaviour has been demonstrated.

The character of the specific union made it possible to find solutions for a number of important questions. In the first place, regarding the multiplicity of the heemolysines, which occur normally in serum, it is well known that numerous sera are able to dissolve blood corpuscles of different species. For example, serum of the dog dissolves blood corpuscles of the rabbit, guinea-pig, rat, goat, sheep, &c. The complex nature of these haemolysines has been already indicated.

Another question arises whether in a serum that is capable of such manifold action there is present one single haemolysine that destroys different red blood-cells, or whether a whole series of hsemolysines come into action, of which one is adapted to guinea-pig blood, another to rabbit blood, &c. The solution of this question may be approached in another way. The serum may be rendered inactive by heat, and then placed in contact with red blood corpuscles of a given kind. Then, supposing, for example, that rabbit blood has been employed, it is found that if the fluid is freed from the erythrocytes by centrifugalisation and the "complement" afterwards added, it is no longer in a position to dissolve rabbit blood, but has not suffered any impairment of its action on other kinds.

By this method of elective absorption it is proved that the normally occurring hsemolysines which chain the blood corpuscles of the rabbit to themselves, are specifically adapted to this purpose. IF WITH SUITABLE ADJUSTMENT OF CONDITIONS SIMILAR EXPERIMENTS BE CONDUCTED WITH OTHER KINDS OF BLOOD, RESULTS ARE OBTAINED WHICH FORCE US TO THE CONVICTION THAT IN SUCH A SERUM ACTING ON VARIOUS KINDS OF BLOOD THERE ARE PRESENT ABSOLUTELY DIFFERENT "INTERMEDIATE BODIES" (analogues of the "immune bodies"), OF WHICH EACH ONE IS SPECIFIC FOR ONE KIND OF BLOOD, i.e., one is adapted for rabbit's blood, a second for calf's blood, &c. Dr. Morgenroth and I have in some cases, indeed, SUCCEEDED IN PROVING THAT THE "COMPLEMENTS" WHICH ARE ADAPTED TO FIT THEMSELVES TO THESE "INTERMEDIATE BODIES," AND OCCUR IN NORMAL SERA, DIFFER AMONG THEMSELVES. If we reflect that in normal blood, in addition to these different hsemolysines, there are besides a long series of analogous bodies, agglutinines of very different kinds, bacteriolysines, enzymes, anti-enzymes, WE ARE BROUGHT MORE AND MORE TO THE CONVICTION THAT THE BLOOD SERUM IS THE CARRIER OF SUBSTANCES INNUMERABLE AS YET LITTLE KNOWN OR CONCEIVED OF.

Having obtained a precise conception of the method of action of the lysines of the serum—of the hsemolysines, and thereby also of the bacteriolysines—it becomes possible for us to attempt to solve the mystery of the origin of these bodies. I have in the beginning of this lecture fully developed the "side-chain theory," according to which the antitoxines are merely certain of the protoplasm "side-chains," which have been produced in excess and pushed off into the blood.

The toxines, as secretion products of cells, are in all likelihood still relatively uncomplicated bodies; at least, by comparison with the primary arid complex albumins of which the living cell is composed. If a cell of the organism has, with the assistance of an appropriate "side-chain," fixed to itself a giant molecule, as the proteid molecule really is, then, with the fixation of this molecule, there, is provided one of the conditions essential for the cell nourishment. Such giant molecules cannot at first be utilised by the cells, and are only made available when, by means of a ferment-like process, they are split into smaller fragments. THIS WILL BE VERY EFFECTUALLY ATTAINED IF, FIGURATIVELY SPEAKING, THE "TENTACLE" OR GRAPPLING ARM OF THE PROTOPLASM POSSESSES A SECOND HAPTOPHORE GROUP ADAPTED TO TAKE TO ITSELF FERMENT-LIKE MATERIAL OUT OF THE BLOOD FLUID. Through such complex organisation, by which the " tentacle" acts also as the bearer of a ferment-functioning group, this group is brought into close relation with the prey destined to be digested and assimilated.

For such appropriate arrangements, in which THE "TENTACULAR" APPARATUS ALSO EXERCISES A DIGESTIVE FUNCTION—IF IT BE PERMISSIBLE TO PASS FROM THE ABSTRACT TO THE CONCRETE—we find analogies in the different forms of insectivorous plants. Thus it has been

known since the famous researches of Darwin that the tentacles of Drosera secrete a proteid-digesting fluid.

If we now recognise that the different lysines only arise through absorption of highly complex cell material—such as red blood corpuscles or bacteria—then the explanation, in accordance with what I have said, is that there are present in the organism “side-chains” of a special nature, so constituted that they are endowed not only with an atomic group by virtue of the affinities of which they are enabled to pick up material, but also with a second atomic group, which, being ferment-loving in its nature, brings about the digestion of the material taken up. SHOULD THE PUSHING-OFF OF THESE “SIDE-CHAINS” BE FORCED, AS IT WERE, BY IMMUNISATION, THEN THE "SIDE-CHAINS" THUS SET FREE MUST POSSESS BOTH GROUPS, AND WILL THEREFORE IN THEIR CHARACTERISTICS ENTIRELY CORRESPOND TO WHAT WE HAVE PLACED BEYOND DOUBT AS REGARDS THE “IMMUNE-BODY” OF THE HSEMOLYSINE.

In this manner is simply and naturally explained the astonishingly specialised arrangement that, through the introduction of a definite bacterium into the body, SOMETHING IS PRODUCED WHICH IS ENDOWED WITH THE POWER OF DESTROYING BY SOLUTION THE BACTERIUM WHICH WAS ADMINISTERED AND NO OTHER. This contrivance of the organism is to be regarded as nothing more than a repetition of a process of normal cell-life, and the outcome of primitive wisdom on the part of the protoplasm."

https://doi.org/10.1098/rspl.1899.0121

In Summary:

-Ehrlich states that there are two different bodies acting in combination for bacteriolysines (the rupture of a bacterial cell by antibodies): a stable one brought about by immunization and an unstable one already present in the blood -he called the stable element the "immune body" and the unstable element the "complement"

-after centrifugation, he states the "immune body" stays with the red blood cells while the "complement" is left behind

-through his experiments, he determined that temperature had an effect on whether or not the substances were left behind after centrifugation

-in order to explain these facts, Ehrlich states that ASSUMPTIONS about the two bodies need to be made

-he states that when the blood of a non-immunized animal has haemolytic action on the blood

of another animal, this can not be considered the action of the "immune body"

-he termed this phenomena the "intermediate body" even though it had exactly the same action and characteristics of the "immune body" and the only difference was it occurred naturally

-he states that the process of hsemolysines (lysis of the red blood cells) is the same when it occurs naturally or through immunizations

-he states that if experiments are carried out on various blood types, and there are multiple "intermediate bodies" specific for each kind of blood (rabbits blood, calf blood, Guinea pig blood, etc.), then he succeeded in proving that there are different complements that fit these "intermediate bodies"

-he was convinced the blood contains innumerable substances for which little is known or conceived of

-he talks of a "tentacle" aiding in the process of digestion/fermentation to eliminate toxins

-he says his abstract "tentacular" apparatus is part of the digestive function

-he believes immunization forces the pushing off of "side-chains" (antibodies) into the bloodstream

This process of binding serum complement was yet another indirect method used in an attempt to prove antibodies exist. If a reaction occurs, it is assumed the antibody-antigen exists in the mixture. The complement system described by Erhlich formed the basis for the complement fixation test which has been used as an indirect method to discover "novel viruses" or to determine someone positive for a known "virus." Here is a brief description of what this test entails:

"The complement fixation test consists of two components.

The first component is an indicator system that USES COMBINATION OF SHEEP RED BLOOD CELLS, COMPLEMENT-FIXING ANTIBODY SUCH AS IMMUNOGLOBULIN G PRODUCED AGAINST THE SHEEP RED BLOOD CELLS AND AN EXOGENOUS SOURCE OF COMPLEMENT USUALLY GUINEA PIGS SERUM. When these elements are MIXED IN OPTIMUM CONDITIONS, the anti-sheep antibody binds on the surface of red blood cells. Complement subsequently binds to this antigen -antibody complex formed and will cause the red blood cells to lyse.

The second component is Test System (A KNOWN ANTIGEN AND PATIENT SERUM ADDED TO A

SUSPENSION OF SHEEP RED BLOOD CELLS IN ADDITION TO COMPLEMENT). These two components of the complement fixation method are tested in sequence. Patient serum is first added to the known antigen, and complement is added to the solution. If the serum contains antibodies to the antigen, the resulting antigen-antibody complexes will bind all of the complement. SHEEP RED BLOOD CELLS AND THE ANTI-SHEEP ANTIBODY ARE THEN ADDED. If complement has not been bound by an antigen-antibody complex formed from the patient serum and known antigens, it is available to bind to the indicator system of sheep cells and anti-sheep antibodies. Lysis of the indicator sheep red blood cells signifies both a lack of antibody in patient serum and a negative complement fixation test. If the patient's serum does contain a complement-fixing antibody, A POSITIVE RESULT WILL BE INDICATED BY THE LACK OF RED BLOOD CELL LYSIS."

https://bio.libretexts.org/.../12.2G%3A_Complement_Fixation

There are some noted drawbacks for the test beyond the combining of human blood with that from Sheep/Guinea pigs:

"DISADVANTAGES OF COMPLEMENT FIXATION TEST

NOT SENSITIVE – cannot be used for immunity screening.

Time-consuming.

OFTEN NON-SPECIFIC e.g. CROSS-REACTIVITY between Herpes Simplex Virus and Voricella Zoster Virus."

https://microbenotes.com/complement-fixation-test-steps-advantages-and-disadvantages

So the test, based on the theoretical theory proposed by Ehrlich, is not sensitive nor is it specific and often leads to cross-reactivity with other "viruses." This obviously poses a problem as in order to know which antibody reacts and is specific for a "virus" in order to detect it, one would have to have first purified/isolated an actual "virus" directly from a human sample. Without this, sensitivity and specificity can not be concluded nor can specific antibodies be known.

This is the problem with basing tests on theoretical concepts and experimental reactions. To date, the antibody-antigen relationship is still nothing but an unproven theory. This was brilliantly summarized in the paper EHRLICH'S "BEAUTIFUL PICTURES:"

"The term antibody today refers to discrete biochemical entities present in the blood.

THE BELIEF THAT ANTIBODIES ARE SUCH ENTITIES is held not only by scientists and physicians, but also by the lay public, who learns about "antibodies," if not in school, then at least in the course of routine medical practices such as vaccination and, increasingly, through the so-called popularization of science. In addition, some people will readily associate the term antibody with the characteristic Y-shaped structure found not only in textbooks and specialized scientific articles but also, more recently, in advertisements and even as the logos of pharmaceutical and biotechnology companies. In order to understand the beginnings of immunological imagery at the turn of the century, WE HAVE TO DISCARD OUR PRESENT-DAY NOTIONS ABOUT THE REALITY AND STRUCTURE OF ANTIBODIES."

"THE DEBATE OVER THE EXISTENCE, NATURE, AND-PROPERTIES OF "ANTIBODIES" LASTED FOR DECADES. Very few people shared the extreme position of Felix Le Dantec, who DENIED THE PHYSICAL EXISTENCE OF WHAT HE CALLED "PHENOMENINES"-that is, MYTHICAL SUBSTANCES that, like Moliere's "vertu dormitive," WERE BEING USED TAUTOLOGICALLY TO ACCOUNT FOR EMPIRICALLY OBSERVED PHENOMENA:

'While making fun of the physicians of his time, our great Moliere had foreseen Ehrlich's system. EHRLICH HAS ADDED NOTHING TO THE EXPLANATION OF THE IMAGINARY INVALID. Rather than saying that chloral causes sleep because it contains a soporific power ("vertu dormitive"), we would today say, according to the German scholar, that chloral contains a soporine ("dormitine"); well, it is the same thing! . . . Here then is a therapeutic serum which produces, when inoculated into the rabbit, a given phenomenon. How will you explain this particular activity? It is very simple: put on your glasses and your doctor's cap and gravely say: "THE SERUM PRODUCES THIS PHENOMENON BECAUSE IT CONTAINS A PHENOMENINE WHICH HAS THAT POWER." Nobody will laugh. Point out timidly that the same serum has a different action on the snake; that it produces a second phenomenon; that will be because it contains a second phenomenine. If the same substance produces a thousand different phenomena in a thousand different species, then it contains a thousand different phenomenines; and there you go! Why had we not thought of this earlier?

HENCEFORTH WE HAVE THE EXPLANATION FOR EVERYTHING!'

"Moreover, other researchers, while not sharing Le Dantec's extreme position, did share his

hostility to "terminology-based" explanations. Henry Dean, for instance, noted that "agglutinins, precipitins, amboceptors ARE MERE WORDS, and A PASSIVE BELIEF IN THE EXISTENCE OF SUCH BODIES TENDS TO IMPEDE RATHER THAN ADVANCE OUR UNDERSTANDING OF WHAT IS ACTUALLY TAKING PLACE"; he added that "IGNORANCE, HOWEVER APTLY VEILED IN AN ATTRACTIVE TERMINOLOGY, REMAINS IGNORANCE."

"So, DESPITE VARIOUS PROFESSIONS OF FAITH in the material nature of "antibodies," their ontological status remained uncertain, a situation ascribed by some scientists to THE FAILURE TO PURIFY CHEMICALLY THE ELUSIVE ENTITIES AND THUS TO ASCERTAIN WHETHER THEY WERE INDEED MATERIAL SUBSTANCES."

"In a 1902 letter nominating Ehrlich for the Nobel Prize, Bernhard Naunyn noted that the German researcher could be credited with the introduction of a stereochemical approach into biology and could thus be compared with the great scientists (August Kekule, Adolf von Baeyer) who had done the same in their own disciplines; BUT HE ADDED THAT EHRLICH'S CONTRIBUTION SHOULD STILL BE REGARDED AS TENTATIVE OR PREMATURE, SINCE THE ISOLATION AND PURIFICATION OF THE RELEVANT SUBSTANCES (namely, "ANTIBODIES") WOULD LONG REMAIN A CHIMERA. Those same substances were treated, in a 1910 German textbook, as didactic devices:

'In order to learn the nature of these antibodies attempts have been made to isolate them chemically. THUS FAR ALL SUCH TRIALS HAVE BEEN UNSUCCESSFUL. IT IS EVEN UNCERTAIN WHETHER THESE SO-CALLED ANTIBODIES ARE DEFINITE CHEMICAL ENTITIES. Only the effects of the serum as a whole are known, AND THE INGREDIENTS IN IT TO WHICH THESE ACTIVITIES ARE ATTRIBUTED ARE THOUGHT OF AS ANTIBODIES. For didactic purposes antibodies, as antitoxins, agglutinins, etc., will be spoken of in this book when the antitoxin or agglutinating properties, exclusively, are meant. 15'

Julius Citron's 1910 statement was echoed nineteen years later by Wells, according to whom:

We attribute this altered reactivity [of sera] to the presence of "antibodies," DESPITE THE

FACT THAT WE HAVE ABSOLUTELY NO KNOWLEDGE OF WHAT THESE ANTIBODIES MAY BE, OR EVEN THAT THEY EXIST AS MATERIAL OBJECTS. Like the enzymes, WE RECOGNIZE THEM BY WHAT THEY DO WITHOUT DISCOVERING JUST WHAT THEY ARE. We do not know whether they are specific molecular aggregates OR MERELY PHYSICAL FORCES DEPENDENT ON ALTERED SURFACE ENERGY OF THE SAME SUBSTANCES PRESENT IN THE BLOOD BEFORE THE PROCESS OF IMMUNIZATION WAS BEGUN."

https://www.jstor.org/stable/235104

Ehrlich provided a theory that weaved together various disparate elements like the best fictions writers do. He defined the concepts of antibody, antigen, the complement system, and provided a framework for how immunity works. This led to indirect non-specific and non-sensitive complement fixation tests used as evidence for the existence of "viruses" and/or as proof that the vaccines are effective against them. The problem is that Ehrlich's theories were just mere words with nothing physical backing them. He created concepts and ideas for that which he could not physically see. They belong to the realm of fantasy and fairy tales.

As Henry Dean stated: "ignorance, however aptly veiled in an attractive terminology, remains ignorance."

Part 1:

https://docs.google.com/document/d/e/2PACX-1vSWQ7pM49P09Qvdn1pj20KLoOCyX4Oyy5ShBJUWHGz5CQAqYrcLT4iGostVXFwA76qmKWp3ig2kh0uj/pub

ASTRID FAGRAEUS 1948 PLASMA CELLS PRODUCE ANTIBODIES CORRELATION:

A "breakthrough" in Immunology came in 1947/48 when Astrid Fagraeus published her doctoral dissertation "Antibody Production in relation to the Development of Plasma Cells." She is given the credit for showing that the plasma cells produce antibodies. Before her paper, their function was unknown. But did Astrd Fagraeus really prove the correlation between plasma cells and antibodies definitively?

According to the book "Advances in Immunology" by Frederick W. Alt (Image # 3 below):

(My paraphrasing)

In 1938, Fred Kolouch Jr. directly correlated the formation of plasma cells with the injection of

an antigen. From this correlation, he postulated that plasma cells produce antibodies even though he had no direct experimental evidence backing up his hypothesis. It wasn't until 1948, with the experiments of Astrid Fagraeus, where a breakthrough in Immunology occurred. She performed in vitro studies with plasma cell-enriched spleen biopsies from horse serum-injected rabbits and found a correlation between the numbers of plasma cells in the tissues and the amount of secreted antibodies in the culture medium. However, even with her findings, she was unable to provide clear evidence that the plasma cells were in fact the cells that secrete antibodies.

However, the lack of clear evidence did not stop the scientific community from recognizing Fagraeus and her findings as proof that plasma cells produce the still unseen antibodies:

"Fagraeus' doctoral dissertation, 'Antibody Production in relation to the Development of Plasma Cells', attracted international attention and was CONSIDERED A MILESTONE IN MODERN IMMUNOLOGY. In this work, SHE WAS THE FIRST TO SHOW THAT PLASMA CELLS PRODUCE ANTIBODIES (IgG)."

https://en.m.wikipedia.org/wiki/Astrid_Fagraeus

"It took another 60 years until ASTRID FAGREUS DISCOVERED THE CELLS SECRETING THOSE ANTIBODIES, THE “PLASMA CELLS”, describing them as “terminal stages of B cell differentiation”

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3337994/

"In 1943, Mogens Bjørneboe and Harald Gormsen were the first to experimentally show that repeated immunization of rabbits with polyvalent vaccines leads to massive proliferation of plasma cells in most organs and that THIS PROLIFERATION CORRELATES WITH ANTIBODY CONCENTRATION.

That finding was supported a few years later by Astrid Fagraeus, who reported that plasma cells produce antibodies in vitro. Tissue cultures of spleens from rabbits immunized with live bacteria showed abundant formation of plasma cells. FAGRAEUS CONCLUDED THAT PLASMA CELLS APPEAR IN CONNECTION WITH STRONG ANTIGEN STIMULATION."

https://www.nature.com/milestones/mileantibodies/full/mileantibodies03.html

The actual paper was difficult to find but I did manage to find and take an image of a Nature article in which she details her work (Image # 2 below) as well a source which provided a brief

summary:

THE PLASMA CELLULAR REACTION AND ITS RELATION TO THE FORMATION OF ANTIBODIES IN VITRO

The Journal of Immunology 58 (1), 1-13, 1948

"1.During secondary response, elicited in sensitized rabbits by means of intravenous injections of antigen, A GREAT INCREASE IN THE NUMBER OF PLASMA CELLS (pl.c.) IN THE SPLEEN WAS RECORDED SIMULTANEOUSLY WITH THE INCREASE OF CIRCULATING ANTIBODIES.

2.Pl.c. were confined almost exclusively to the red pulp, especially after the injection of living S. typhi. They originated apparently from reticulum cells, passing through a chain of development: transitional cell → immature pl.c. → mature pl.c.

3.Pieces of spleen were excised at different times during the period of antibody formation in rabbits, DIFFERENTIAL CELL COUNTS were made and the capacity of excised splenic tissue to form antibodies in vitro was investigated. The following observations were made:

a.THE AMOUNT OF ANTIBODY LIBERATED IN PLAIN TISSUE EXTRACTS, under conditions preventing growth or metabolism of cells, WAS VERY LOW, WHEREAS SIGNIFICANT YIELDS ERE OBTAINED IN TISSUE CULTURES.

b.The capacity of the red pulp abundant in pl.c. to produce antibodies in vitro was considerably superior to that of lymph follicles, rich in lymphocytes but devoid of pl.c.

c.Antibody production was comparatively poor in tissue containing only transitional cells, reached a maximum when numerous immature pl.c. were present and receded when predominantly mature pl.c. were found.

4.After intravenous injections the antigen accumulated in those places where pl.c. subsequently

developed.

The conclusion is drawn that ANTIBODIES UNDER THE CONDITIONS OF THE EXPERIMENTS, are formed by cells of the R.E.S., passing through a chain of development, the final link of which is the mature pl.c."

https://www.jimmunol.org/content/58/1/1.short

doi: 10.1038/159499a0.

It appears that the scientific community is very much of the mindset that correlation equals causation. They seem to forget this very basic premise:

"Can correlation ever equal causation?

Correlation tests for a relationship between two variables. HOWEVER, SEEING TWO VARIABLES MOVING TOGETHER DOES NOT NECESSARILY MEAN WE KNOW WHETHER ONE VARIABLE CAUSES THE OTHER TO OCCUR. This is why we commonly say “CORRELATION DOES NOT IMPLY CAUSATION.”

https://www.jmp.com/en_us/statistics-knowledge-portal/what-is-correlation/correlation-vs-causation.html

MICHAEL HEIDELBERGER 1929 ANTIBODY EQUATION:

Michael Heidelberger is considered the "Founder of Immunochemistry." He was the first to apply mathematics to the reaction of antibodies and their antigens. He is also known for "proving" that antibodies are proteins by showing that the antigens of pneumococcus bacteria are polysaccharides (or carbohydrates). Here is a brief overview of his work:

"Heidelberger and Avery's discovery came at a time when ANTIBODIES WERE REGARDED—BY THOSE WHO BELIEVED THEY EXISTED AT ALL—AS MYSTERIOUS SUBSTANCES THAT FLOATED AROUND IN SERUM. “It appeared to me that there was a crying need to determine the true

nature of antibodies," wrote Heidelberger in 1979, "and that until this was done there could be no end to the polemics and uncertainties that were plaguing immunology" (2). Heidelberger later purified the antibodies from his precipitin reactions and showed that they themselves were proteins. As a result, says friend and colleague Victor Nussenzweig (New York University), "THERE WERE NO MORE MYSTICAL IDEAS ABOUT WHAT ANTIBODIES WERE."

Heidelberger and his postdoctoral fellow Forrest Kendall later quantitated the precipitin reaction (5), BRINGING MUCH-NEEDED MATHEMATICS TO THE STUDY OF ANTIBODY–ANTIGEN INTERACTIONS AND LIFTING ANTIBODIES EVEN FURTHER OUT OF THE REALM OF THE MYSTERIOUS (see the next "From the Archive").

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2212983

Two of Heidelberger's papers are most often cited as the proof that antibodies are proteins. The first is a paper he did with Oswald Avery in 1923. Some highlights below:

THE SOLUBLE SPECIFIC SUBSTANCE OF PNEUMOCOCCUS.

"In 1917 Dochez and Avery (1) showed that WHENEVER PNEUMOCOCCI ARE GROWN IN FLUID MEDIA, THERE IS PRESENT IN THE CULTURAL FLUID A SUBSTANCE which precipitates specifically in antipneumococcus serum of the homologous type. This soluble substance is demonstrable in culture filtrates during the initial growth phase of the organisms; that is, during the period of their maximum rate of multiplication

when little or no cell death or disintegration is occurring. THE FORMATION OF THIS SOLUBLE SPECIFIC MATERIAL BY PNEUMOCOCCI ON GROWTH IN VITRO SUGGESTED THE PROBABILITY OF AN ANALOGOUS SUBSTANCE BEING FORMED ON GROWTH OF THE ORGANISM IN THE ANIMAL BODY. Examination of the blood and urine of experimentally infected animals gave proof of the presence of this substance in considerable quantities in the body fluids following intraperitoneal infection with pneumococcus. In other words, this soluble material elaborated at the focus of the disease readily diffuses throughout the body, is taken up in the blood, passes the kidney, and appears in the urine unchanged in specificity. Similarly, a study of the serum of patients suffering from lobar pneumonia has revealed a substance of like nature in the circulating blood during the course of the disease in man. Furthermore, EXAMINATION OF THE URINE OF PATIENTS having pneumonia due to pneumococci of Types I, II, and III HAS SHOWN THE PRESENCE OF THIS SUBSTANCE IN SOME STAGE OF THE DISEASE IN APPROXIMATELY TWO-THIRDS OF THE CASES. Recently from filtered alkaline extracts of pulverized bacteria of several varieties, including pneumococci, Zinsser and Parker (2) have prepared substances which appear free from coagulable protein. These substances, called "residue antigens," are

specifically predpitable by homologous antisera. THESE OBSERVERS CONSIDER THESE ACID- AND HEAT-RESISTANT ANTIGENIC MATERIALS ANALOGOUS TO THE SOLUBLE SPECIFIC SUBSTANCE OF PNEUMOCOCCUS described by Dochez and Avery (1). In spite of the fact that these "residue antigens" are predpitable by homologous sera produced by immunization with the whole bacteria, Zinsser and Parker HAVE SO FAR FAILED TO PRODUCE ANTIBODIES IN ANIMALS BY INJECTING THE RESIDUES."

"EXPERIMENTAL

The organism used in the present work was Pneumococcus Type II. The most abundant source of the soluble specific substance appeared to be an 8 day autolyzed broth culture; hence this material was used as the principal source of supply. For comparison dissolved pneumococci and lots of urine containing the specific substance were also worked up, with essentially the same results, as will be seen from Table I.

The process for the isolation of the soluble specific substance consisted in concentration of the broth, precipitation with alcohol, repeated re-solution and reprecipitation, followed by a careful series of fractional precipitations with alcohol or acetone after acidification of the solution with acetic acid, and, finally, repeated fractional precipitation with ammonium sulfate and dialysis of the aqueous solution of the active fractions."

"THE RESIDUAL MATERIAL (Preparation 17, in Table I), FOR WHICH NO CLAIM OF PURITY IS MADE, AS EFFORTS AT ITS FURTHER PURIFICATION ARE STILL UNDER WAY, contained, on the ash-free basis, 1.2 per cent of nitrogen. IT WAS ESSENTIALLY A POLYSACCHARIDE, as shown by the formation of 79 per cent of reducing sugars on hydrolysis, and by the isolation and identification of glucosazone from the products of hydrolysis."

"ATTEMPTS TO STIMULATE ANTIBODY PRODUCTION BY THE IMMUNIZATION OF ANIMALS WITH THE PURIFIED SUBSTANCE YIELDED NEGATIVE RESULTS."

"DISCUSSION.

While it has long been known that the capsular material of many microorganisms consists, at least in part, of carbohydrates (4), any connection between this carbohydrate material and the specificity relationships of bacteria appears to have remained unsuspected. WHILE IT CANNOT BE SAID THAT THE PRESENT WORK ESTABLISHES THIS RELATIONSHIP, it certainly points in this direction. Evidence in favor of the probable carbohydrate nature of the soluble specific substance is the increase in specific activity with reduction of the nitrogen content, the increase in optical rotation with increase in specific activity, the parallelism between the Molisch

reaction and specific activity, the high yield of reducing sugars on hydrolysis, and the actual isolation of glucosazone from a small quantity of the material. The small amounts of substance available up to the present have hindered the solution of the problem, and it is hoped that efforts at further purification of the soluble specific substance, now in progress with larger amounts of material, will definitely settle the question.

SUMMARY

1. A method is given for the concentration and purification of the soluble specific substance of the pneumococcus.

2. The material obtained by this method is shown to consist mainly of a carbohydrate which appears to be a polysaccharide built up of glucose molecules.

3. WHETHER THE SOLUBLE SPECIFIC SUBSTANCE IS ACTUALLY THE POLYSACCHARIDE, OR OCCURS MERELY ASSOCIATED WITH IT, IS STILL UNDECIDED, although the evidence points in the direction of the former possibility."

https://doi.org/10.1084/jem.38.1.73

In Summary:

-whenever pneumococci is grown in culture fluid media, a soluble specific substance is found

-they assume that this growth is culture suggests that this also same substance occurs inside the animal body

-examination of the urine of patients with pneumococci showed the substance in only 2/3rds of the samples

-some other researchers found similar substances with other bacteria and believe they are the same as that of the pneumococci

-injecting these substances in animals has FAILED to produce antibodies

-they make no claim as to the purity of the material in their experiments and state further

purification is being attempted

-they believe it is ESSENTIALLY a polysaccharide

-they were also UNABLE to stimulate antibodies by injecting their substance in animals

-they state that their work CAN NOT ESTABLISH a relationship between bacteria and carbohydrates

-whether the soluble specific substance is a polysaccharide or occurs merely associated with it was left undetermined

This paper doesn't seem to be the slam-dunk proof that bacteria antigens are carbohydrates so it seems rather odd to assume antibodies are proteins based off this work, but assume they did:

"Since the pneumococcal capsular antigen was a polysaccharide, AND ANTIBODIES WERE THOUGHT TO BE PROTEINS, Heidelberger realized that by MEASURING THE AMOUNT OF PROTEIN IN SPECIFIC PRECIPITATES MADE WITH THE CAPSULAR ANTIGEN HE COULD DETERMINE THEIR ANTIBODY CONTENT. Together with Forrest Kendall, who had joined the Heidelberger lab, THE PROTEIN CONTENT OF IMMUNE PRECIPITATES WAS DETERMINED BY MEASURING TOTAL NITROGEN, using the Kjeldahl procedure that came to be the hallmark of laboratories carrying out Heidelberger-type quantitative immunochemistry."

https://www.google.com/url?sa=t&source=web&rct=j...

Since they assumed the pneumococcal bacteria was a polysaccharide, that meant any nitrogen left over was the antibody content. This second paper shows how Heidelberger came to this conclusion using the precipitin test (images below) and mathematics as proof that antibodies exist. I edited out the long mathematical sections with his equations so if you are interested in Heidelberger showing his work, I recommend reading the full paper. Highlights below:

A QUANTITATIVE STUDY OF THE PRECIPITIN REACTION BETWEEN TYPE III PNEUMOCOCCUS POLYSACCHARIDE AND PURIFIED HOMOLOGOUS ANTIBODY

"Of all the reactions of immunity the precipitin test is perhaps the most dramatic and striking. While other immune reactions are more delicate, the precipitin test is among the most specific and least subject to errors and technical difficulties. ATTEMPTS AT ITS QUANTITATIVE INTERPRETATION AND EXPLANATION (1, 2) HAVE BEEN HAMPERED EITHER BY THE DIFFICULTY

OF FINDING SUITABLE ANALYTICAL METHODS OR BY THE FAILURE TO SEPARATE THE REACTING SUBSTANCES FROM CLOSELY RELATED, NON-SPECIFIC MATERIALS WITH WHICH THEY ARE NORMALLY ASSOCIATED.

With the aid of recent work IT HAS BEEN FOUND POSSIBLE TO AVOID THESE DIFFICULTIES TO SOME EXTENT. The isolation of bacterial polysaccharides which precipitate antisera specifically (3) and possess the properties of haptens (4) has not only afforded one of the components of a precipitin reaction IN A STATE OF COMPARATIVE PURITY, but has greatly simplified the analytical problem. Since many of these polysaccharides contain no nitrogen, AND ANTIBODIES PRESUMABLY ARE NITROGENOUS, the latter may be determined in the presence of any amount of the specific carbohydrate. Moreover, Felton's method for the separation of pneumococcus antibodies from horse serum (5) not only permits the isolation of a high proportion of the precipitin, FREED FROM AT LEAST 90 PERCENT OF THE SERUM PROTEINS AND MUCH OF THE SERUM LIQUID, but is also applicable on a sufficiently large scale to furnish the amounts of antibody solution needed to make quantitative work possible. IT IS REALIZED THAT ANTIBODY SOLUTIONS OF THIS TYPE DO NOT CONTAIN PURE ANTIBODIES--INDEED, ONLY 40 TO 50 PERCENT OF THE NITROGEN IS SPECIFICALLY PRECIPITABLE-- BUT SINCE SO SMALL A PROPORTION OF THE ORIGINAL SERUM PROTEIN REMAINS WITH THE ANTIBODY a far-reaching purification actually has been effected. It should thus be possible with the aid of antibodies purified by Felton's method to OBTAIN DATA OF A PRELIMINARY CHARACTER which should point toward the mechanism of the reaction. The present paper is concerned with such data obtained in a quantitative study of the precipitin reaction between the soluble specific substance of Type III pneumococcus and Type III pneumococcus antibody solution.

EXPERIMENTAL

1. Materials and Methods.--a. Solutions of Soluble Specific Substance, Type Ill Pneumococcus.--The soluble specific substance of Type III pneumococcus (6)* used was kinky supplied by Drs. O. T. Avery and W. F. Goebel of The Rockefeller Institute for Medical Research. It was ash-free, contained 0.04 per cent of nitrogen, and showed [aid = -32 °. A weighed amount of anhydrous substance was suspended in 0.9 per cent saline, dissolved with the aid of 0.1 normal sodium hydroxide, and the solution was diluted with saline, adjusted to pH 7.6 and made up to volume with saline to yield a 1 per cent solution. This was sterilized in the autoclave and used as a stock solution for making up other dilutions. These were prepared with sterile saline under aseptic precautions, and were kept in the ice-box.

b. Type III Pneumococcus Antibody Solution.--The antibody solutions used were prepared essentially according to Felton's procedure (loc. cir.) from Type III anti pneumococcal horse

serum containing no preservative and supplied by the New York State Department of Health through the courtesy of Dr. A. B. Wadsworth and Dr. Mary B. Kirkbride. 100 to 200 cc. of serum were stirred slowly into 20 volumes of ice-cold water containing 9.5 cc. of molar potassium dihydrogen phosphate and 0.5 cc. of molar dipotassium hydrogen phosPhate per liter. The final pH varied from 5.6 to 6.3. Mter standing overnight in the cold the supernatant was decanted and the precipitate was centrifuged off in the coldt and dissolved in a volume of chilled 0.9 percent saline equal to that of the serum taken. 0.1 normal hydrochloric acid was then added until a precipitate no longer formed on dilution of a test portion with two volumes of water, after which 0.1 normal sodium hydroxide solution was added until a slight precipitate again formed on dilution. In general, 5 cc. of acid and 1.5 cc. of alkali per 100 cc. of serum were satisfactory, although as Felton emphasizes, DIFFERENT LOTS VARY AND NO ABSOLUTELY DEFINITE PROCEDURE CAN BE GIVEN. In the present work the process of purification was followed either by testing the agglutinating power of the fractions against a heat-killed Type III pneumococcal vaccine, or by the precipitin reaction, or by both methods. After addition of the alkali the opalescent solution was diluted with 2 volumes of water and centrifuged in the cold. The almost inactive precipitate was discarded and the supernatant poured into 6.7 volumes of the chilled buffer solution previously used, (equivalent to 20 times the volume of saline employed), also adding enough 0.1 normal sodium hydroxide to neutralize the remaining acid. The resulting precipitate was collected and dissolved in a volume of 0.9 per cent saline equal to that of the serum taken, and the pH was adjusted to 7.6. The solution was sterilized by passage through a Berkefeld N grade filter which previously had been washed with saline containing a drop of normal sodium hydroxide, followed by saline alone.

ANTIBODY SOLUTIONS PREPARED IN THIS WAY WERE FOUND TO BE RATHER UNSTABLE UNDER THE USUAL CONDITIONS OF THE PRECIPITIN TEST, AND IT THEREFORE WAS NECESSARY TO SUBJECT THEM TO A PRELIMINARY "AGEING" TREATMENT in order that control solutions might be relied upon to remain clear. This consisted in immersing the solution in a water bath at 37 ° for 2 hours, letting it stand in the ice-box overnight, centrifuging off the precipitate which usually formed, readjusting the pH if necessary, and filtering through a Berkefeld candle prepared as above. THIS TREATMENT WAS REPEATED AS MANY TIMES AS NECESSARY, but the solutions usually remained clear after the second incubation at 37 ° . MUCH TIME WAS LOST AND VERY INCONSISTENT RESULTS WERE OBTAINED UNTIL "AGEING" WAS RESORTED TO.*

THE RELATIVE ANTIBODY CONTENT OF THE RESULTING SOLUTIONS WAS ESTIMATED by determining the agglutination titer against a single heat-killed Type III pneumococcus suspension."

"DISCUSSION

For purposes of discussion IT WILL BE ASSUMED WITH FELTON (lot. cir.) THAT ANTIBODY IS MODIFIED PROTEIN, AND THAT, IN ORDER TO PROVIDE A UNIFORM METHOD OF MEASUREMENT, IT MAY BE EXPRESSED AS NITROGEN PRECIPITATE BY SPECIFIC POLYSACCHARIDE, MULTIPLIED BY 6.25. Since only relative values are under consideration, the actual magnitude of the factor used is of little significance so long as it be used throughout.

Moreover, Table I shows a correspondence between this measure of antibody content and the agglutination titer, so that its use as a relative measure is independent of the nature of Type III pneumococcal antibodies."

"WHETHER OR NOT THESE CONCEPTIONS ARE OF GENERAL APPLICATION REMAINS TO BE TESTED, and work along these lines is under way."

doi: 10.1084/jem.50.6.809.

In Summary:

-Heidelberger acknowledges that the precipitin test used during this experiment has 2 drawbacks:

a) quantitative interpretation/explanation is difficult due to lack of a suitable analytical method

b) failure to separate out the reacting substances from non-specific material which these substances are closely related to and associated with

-he states it is possible to avoid these failures TO SOME EXTENT

-he PRESUMES antibodies are nitrogenous

-he states at least 90% of the precipitin can be freed from serum proteins and liquid

-Heidelberger admits that these are NOT PURE ANTIBODIES and that only 40-50% of nitrogen is precipitable while small amounts of serum remain

-antibodies were found to be unstable during testing so they were put through preliminary "ageing" processes as many times as needed until they got the result they wanted

-much time was lost and results were inconsistent until the 'ageing" process was implemented

-the antibody content was then ESTIMATED

--since Heidelberger thought pneumococci antigens were carbohydrate, he ASSUMED that

meant the antibodies were protein and thus any nitrogen left over after the precipitin test contained the antibodies

-whether or not his conceptions were of general application remained to be tested

It seems rather obvious that many assumptions were made about a substance (antibodies) for which they could not see. The conclusions drawn were born out of chemistry experiments which have no bearing on reality and mathematical equations attempting to quantify the unquantifiable. Whether or not these indirect experiments and assumptions provide proof that antibodies exist and are proteins, I leave up to the reader. However, keep in mind that no antibodies had ever been seen nor proven to exist up to that time (and to date). This work is based off of theoretical explanations of immunity for which nothing could be observed.

One last interesting fact. Remember the pneumococci bacteria they based their "antigen" on and for which they could not produce antibodies with when testing on animals? It is regularly found in healthy people.

"Infection is acquired mainly through pneumococci contained in respiratory droplets. THERE ARE MANY HEALTHY, ASYMPTOMATIC CARRIERS OF THE BACTERIA but no animal reservoir or insect vector."

https://www.who.int/ith/diseases/pneumococcal/en/

If an antigen is a toxin or foreign substance which produces an immune response creating antibodies, the pneumococci bacteria doesn't seem to meet that definition at all.

FRANK MACFARLANE BURNET 1957 CLONAL SELECTION THEORY:

"A SCIENTIFIC THEORY is not the end result of the scientific method; THEORIES CAN BE PROVEN OR REJECTED, JUST LIKE HYPOTHESES. Theories can be improved OR MODIFIED as more information is gathered so that the accuracy of the prediction becomes greater over time."

https://www.livescience.com/21491-what-is-a-scientific-theory-definition-of-theory.html

At the time that Frank M. Burnet proposed the Clonal Selection Theory of antibody formation in 1957, there were a few different theories floating about. They were the:

1. Direct Template Theory

2. Indirect Template Theory

3. Side Chain Theory

4. Natural Selection Theory

Each of these theories proposed different ways antibodies formed based on the scientific evidence of the time. Naturally, Burnet saw the burning need for a new theory to be thrown into the mix which is why he utilized Jerne's Natural Selection Theory to rework and create another one of his own. Thus the Clonal Selection Theory was born and it has gone on to become the most widely accepted explanation for the formation of antibodies, which were still unseen particles assumed to be within the blood:

"Clonal selection theory is a scientific theory in immunology that explains the functions of cells of the immune system (lymphocytes) in response to specific antigens invading the body. The concept was introduced by Australian doctor Frank Macfarlane Burnet in 1957, in an attempt to explain the great diversity of antibodies formed during initiation of the immune response.[1][2] THE THEORY HAS BECOME THE WIDELY ACCEPTED MODEL FOR HOW THE HUMAN IMMUNE SYSTEM RESPONDS TO INFECTION and how certain types of B and T lymphocytes are selected for destruction of specific antigens.[3]"

https://en.m.wikipedia.org/wiki/Clonal_selection

"THE CLONAL SELECTION HYPOTHESIS HAS BECOME A WIDELY ACCEPTED MODEL for how the immune system responds to infection and how certain types of B and T lymphocytes are selected for destruction of specific antigens invading the body."

https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(Boundless)/11%3A_Immunology/11.07%3A_Antibodies/11.7C%3A_Clonal_Selection_of_Antibody-Producing_Cells

"In 1957, Burnet proposed that central tolerance to self-antigens occurred by clonal deletion of self-reactive lymphocytes. However, as we will discuss, THIS MODEL HAS BEEN 'UPDATED' ON SEVERAL OCCASIONS."

https://www.cell.com/trends/immunology/fulltext/S1471-4906(04)00311-4

Notice the MODEL word thrown about in each of those descriptions? What could that possibly be referring to?

"SCIENTIFIC MODELING, the generation of a physical, CONCEPTUAL, or mathematical REPRESENTATION of a real PHENOMENON THAT IS DIFFICULT TO OBSERVE DIRECTLY."

https://www.britannica.com/science/scientific-modeling

This Clonal Theory, which can be rejected just like any Hypothesis, is the current Model, which is a conceptual representation of a phenomenon that is DIFFICULT TO OBSERVE DIRECTLY. The previous theories/models of this unseen phenomenon were unceremoniously thrown out in favor of Burnet's Clonal Theory. However, even Clonal Theory is being challenged today, but I digress. Burnet's entire theory is presented below:

A MODIFICATION OF JERNE'S THEORY OF ANTIBODY PRODUCTION USING THE CONCEPT OF CLONAL SELECTION

"THERE ARE THREE CURRENT THEORETICAL INTERPRETATIONS OF ANTIBODY PRODUCTION which, following Talmage (1957), may be referred to as the DIRECT TEMPLATE THEORY in which the antigen serves as a template against which the specific pattern of the antibody is synthesized, the INDIRECT TEMPLATE THEORY which postulates a secondary template incorporated into the genetic-synthetic processes of the antibody producing cells (Burnet,1956), and the NATURAL SELECTION THEORY in which the antigen acts essentially by selection for excess production of natural antibody molecules of corresponding type (Jerne, 1955).

THE TWO LATTER THEORIES WERE DEVISED PRIMARILY TO ACCOUNT FOR TWO SETS OF PHENOMENA FOR WHICH THE DIRECT TEMPLATE THEORY SEEMS QUITE IRRELEVANT. The first is the absence of immunological response to "self" constituents and the related phenomena of immunological tolerance; the second is the evidence that antibody production can continue in the absence of antigen. Some means for the recognition and differentiation of potentially antigenic components of the body from foreign organic material must be provided in any acceptable formulation.

In Burnet and Fenner's (1949) account, a positive recognition of "self" material was ascribed to the presence of "self markers" in all potentially antigenic macromolecules, and corresponding recognition units in the scavenger cells of the body. AT THE TIME IT WAS REGARDED AS INCONCEIVABLE THAT A MECHANISM COULD EXIST WHICH WOULD RECOGNIZE IN POSITIVE FASHION ALL FOREIGN MATERIAL AND NO ATTEMPT WAS MADE TO DEVISE ONE, despite the fact that we have always recognised the clumsy character of the self-marker, self-recognition scheme.

IT IS THE GREAT VIRTUE OF JERNE'S HYPOTHESIS THAT IT PROVIDES AN APPROACH TO THIS ALTERNATIVE METHOD OF RECOGNIZING SELF FROM NONSELF. There is no doubt about the presence in all mammalian or avian sera of a wide range of reactive globulins which can legitimately be called "natural antibodies." JERNE ASSUMED THAT AMONGST THESE GLOBULIN MOLECULES WERE ALL THE POSSIBLE PATTERNS NEEDED FOR SPECIFIC IMMUNOLOGICAL TYPE REACTIONS WITH ANY ANTIGEN, except for those patterns corresponding to body antigens which would be eliminated by in vivo absorption. When a foreign antigen enters the blood it unites, according to Jerne's scheme, with one of the corresponding natural antibody molecules. The complex is taken up by a phagocytic cell in which the antigen plays no further part, but the antibody globulin provokes the production by the cell of a fresh crop of similar molecules which are liberated as antibodies. IF THIS BASIS IS ACCEPTED, MOST IMMUNOLOGICAL PHENOMENA CAN BE WELL DESCRIBED IN TERMS OF THE THEORY. Its major objection is the absence of any precedent for, and the intrinsic unlikelihood of, the suggestion that a molecule of partially denatured antibody could stimulate a cell, into which it had been taken, to produce a series of replicas of the molecule.

TALMAGE (1957) HAS SUGGESTED THAT JERNE'S VIEW IS BASICALLY AN EXTENSION OF EHRLICH' SIDE CHAIN THEORY of antitoxin production and that it would be more satisfactory if the replicating elements essential to any such theory were cellular in character ab initio rather than extracellular protein which can replicate only when taken into an appropriate cell. TALMAGE DOES NOT ELABORATE THIS POINT OF VIEW BUT CLEARLY ACCEPTS IT AS THE BEST BASIS FOR THE FUTURE DEVELOPMENT OF ANTIBODY THEORY. He stresses the multiplicity of the globulin types that can be present in the blood and is profoundly sceptical of any approach

which attempts to "unitarian" an interpretation of antibody. In his view properdin has as much right to be called an antibody as any other globulin.

Before receiving Talmage's review we had adopted virtually the same approach but had developed it from what might be called a "clonal" point of view. THIS IS SIMPLY A RECOGNITION THAT THE EXPENDABLE CELLS OF THE BODY CAN BE REGARDED AS BELONGING TO CLONES WHICH HAVE ARISEN AS A RESULT OF SOMATIC MUTATION OR CONCEIVABLY OTHER INHERITABLE

CHANGES. Each such clone will have some individual characteristic and in a special sense will be subject to an evolutionary process of selective survival within the internal environment of the body.

IT IS BELIEVED THAT THE ADVANTAGES OF JERNE'S THEORY CAN BE RETAINED AND ITS DIFFICULTIES OVERCOME IF THE RECOGNITION OF FOREIGN PATTERN IS ASCRIBED TO CLONES OF LYMPHOCYTIC CELLS AND NOT TO CIRCULATING NATURAL ANTIBODY. The resulting formulation may be stated as follows:

The plasma-globulins comprise a wide variety of individually patterned molecules AND PROBABLY several types of physically distinct structure. Amongst them are molecules with reactive sites WHICH CAN CORRESPOND PROBABLY with varying degrees of precision to all, or virtually all, the antigenic determinants that occur in biological material other than that characteristic of the body itself. Each type of pattern is a specific product of a clone of mesenchymal cells and IT IS THE ESSENCE OF THE HYPOTHESIS THAT EACH CELL AUTOMATICALLY HAS AVAILABLE ON ITS SURFACE REPRESENTATIVE REACTIVE SITES EQUIVALENT TO THOSE OF THE GLOBULIN THEY PRODUCE. For the sake of ease of exposition these cells will be referred to as lymphocytes, it being understood that other mesenchymal types may also be involved. Under appropriate conditions, cells of most clones can either liberate soluble antibodies or give rise to descendant cells which can.

IT IS ASSUMED THAT WHEN AN ANTIGEN ENTERS THE BLOOD OR TISSUE FLUIDS IT WILL ATTACH TO THE SURFACE OF ANY LYMPHOCYTE CARRYING REACTIVE SITES WHICH CORRESPOND TO ONE OF ITS ANTIGENIC DETERMINANTS. The capacity of a circulating lymphocyte to pass to tissue sites and there to initiate proliferation is now relatively well established (cf. Gowens, 1957; Simonsen 1957). IT IS POSTULATED THAT WHEN ANTIGEN-NATURAL ANTIBODY CONTACT TAKES PLACE ON THE SURFACE OF A LYMPHOCYTE THE CELL IS ACTIVATED to settle in an appropriate tissue, spleen, lymph node or local inflammatory accumulation, AND THERE UNDERGO PROLIFERATION TO PRODUCE A VARIETY OF DESCENDANTS. In this way preferential proliferation will be initiated of all those clones whose reactive sites correspond to the antigenic determinants on the antigen used. The descendants

will include plasmacytoid forms capable of active liberation of soluble antibody and lymphocytes which can fulfill the same functions as the parental forms. The net result will be a change in the composition of the globulin molecule population to give an excess of molecules capable of reacting with the antigen, in other words the serum will now take on the qualities of specific antibodies. The increase in the number of circulating lymphocytes of the clones concerned will also ensure that the response to a subsequent entry of the same antigen will be extensive and rapid, i.e. a secondary type immunological response will occur.

SUCH A POINT OF VIEW IS BASICALLY AN ATTEMPT TO APPLY THE CONCEPT OF POPULATION GENETICS TO THE CLONES OF MESENCHYMAL CELLS WITHIN THE BODY. It is clear that the internal environment involved is an exceedingly complex one and in all probability many factors will impinge on clones of antibody-producing cells from that environment. It is equally certain that inheritable changes (at the clonal level) will occur as a result of somatic mutation OR OF THE STILL OBSCURE PROCESSES RESPONSIBLE FOR DIFFERENTIATION DURING DEVELOPMENT OF REGENERATION AND REPAIR.

IT WOULD BE INAPPROPRIATE TO ELABORATE THIS VIEW MUCH FURTHER IN A PRELIMINARY COMMUNICATION, but it should be immediately evident that it has highly relevant implications for the general function of the lymphocyte, for the fact that sensitization and homograft immunity reactions seem to be mediated by lymphocytes or other mesenchymal cells without liberation of classical antibody, and for recent findings of extremely rapid liberation of antibody from normal cells. A PRELIMINARY SURVEY OF A VARIETY OF PATHOLOGICAL CONDITIONS WHICH INVOLVE ANOMALOUS IMMUNE REACTIONS ALSO SUGGESTS THAT THIS CELLULAR APPROACH HAS GREATER RELEVANCE TO THE PROBLEMS THAN ANY OF THE OTHER HYPOTHESES. These aspects will be elaborated in a more extensive contribution now in preparation.

One aspect, however, should be mentioned. THE THEORY REQUIRES AT SOME STAGE IN EARLY EMBRYONIC DEVELOPMENT A GENETIC PROCESS FOR WHICH THERE IS NO AVAILABLE PRECEDENT. In some way we have to picture a "randomization" of the coding responsible for part of the specification of gamma globulin molecules, so that after several cell generations in early mesenchymal cells there are specifications in the genomes for virtually every variant that can exist as a gamma globulin molecule. This must then be followed by a phase in which the randomly developed specification is stabilized and transferred as such to descendant cells. At this stage, again following Jerne, any clones of cells which carry reactive sites corresponding to body determinants will be eliminated. The necrotic effect of the tuberculin on sensitized

fibroblasts might be taken as a crude analogue of the process by which clones with unwanted reactivity can be eliminated in the late embryonic period with the concomitant development of immune tolerance.

THE HYPOTHESIS HAS MANY OF THE SAME IMPLICATIONS AS THE BURNET-FENNER AND THE JERNE THEORIES. Its chief advantage over the former is its relevance to the nature of normal antibodies including the red cell isoagglutinins and the SIMPLER INTERPRETATION of tolerance to potential antigens experienced in embryonic life. Its advantages over Jerne's theory are its capacity to cover homograft and related types of immunity as well as the production of classical antibody, AND TO ELIMINATE THE VERY UNLIKELY ASSUMPTION that entry of a globulin molecule into a cell will stimulate the cell to produce exact replicas of that globulin.

DESPITE THE SPECULATIVE CHARACTER OF MUCH OF THE DETAIL OF THIS MODIFICATION OF JERNE'S THEORY --WHICH MIGHT BE REFERRED TO AS THE "CLONAL SELECTION HYPOTHESIS" -- IT HAS SO MANY IMPLICATIONS CALLING FOR EXPERIMENTAL INQUIRY that it has been thought justifiable to submit this preliminary account for publication."

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In Summary:

-Burnet admits to 3 different theories on the formation of antibodies (excluding the Side Chain Theory for some reason)

-he states the Indirect and Natural Selection were devised to explain two phenomena that the Direct Theory could not explain

-it was considered inconceivable that there could be a mechanism within the body which could detect all foreign material and no explanation was ever devised in regards to this hypothesis

-Burnet credits Jerne's hypothesis for allowing him to create his own alternative take

-Jerne ASSUMED that among the globulin molecules were all the necessary patterns to attack any antigen

-Burnet states that if the basis of Jerne's theory is accepted, than most phenomena can be

explained by the theory

-Burnet states that Talmage believed Jerne's theory to be an extension of Ehrlich's Side Chain Theory

-Talmage accepts as the best basis for the continued future evolution of antibody theory that the replicating elements be of cellular rather than extracellular nature

-Burnet states that the Clonal Theory is simply a recognition that the expendable cells of the body can be regarded as belonging to clones which arise due to either somatic mutation OR CONCEIVABLY other inheritable changes

-Burnet believes he can keep Jerne's basic theory but improve on it by stating that the recognition of foreign material is ascribed to lymphatic cells rather than to natural antibodies

-it is the essence of his HYPOTHESIS that each cell automatically has available on its surface representative reactive sites equivalent to those of the globulin they produce

-Burnet ASSUMES that when an antigen enters the blood/tissue fluids that it will attach to any lymphocyte carrying reactive sites which correspond to one of its antigenic determinants

-he postulates that when a antigen attaches to a lymphocyte it activates the cell to produce descendants

-Burnet states that his point of view is basically attempting to apply population genetics to the clones of mesenchymal cells in the body

-he believes inheritable changes occur either at the somatic level or by the yet obscure process of differentiation of repair/regeneration

-he states it would be inappropriate to elaborate on his views any further in a preliminary communication

-he says preliminary survey of a variety of pathological conditions which involve anomalous immune reactions SUGGESTS his cellular hypothesis has more relevance than any of the other hypotheses

-he mentions that his theory requires at some stage in early embryonic development a genetic process for which there is NO AVAILABLE PRECEDENT

-Burnet states that his hypothesis has many of the same advantages as those of Burnet-Fenner (his previous theory) and Jerne

-Burnet admits that much of his theory is SPECULATIVE and requires experimental inquiry

Thus we have yet another in a long line of theories about the formation/function of antibodies. Realize that these theories are not based on any direct observations on antibodies themselves but are a collection of indirect observations of many unrelated and often contradictory experiments. These scientists then attempt to put together the results they like and create an explanation for them while disregarding evidence/theories which do not fit their criteria. But as can be seen by the previous antibody theories, there is always a new theory that seemingly comes along to wipe out the old ones.

This can be seen from these highlights from "The Clonal Selection Theory: what it really is and why modern challenges are misplaced" by Arthur M. Silverstein:

"THE CLONAL SELECTION THEORY (CST) 2 of Macfarlane Burnet and David Talmage SEEMS TO BE UNDER FIRE CURRENTLY FROM SEVERAL DIRECTIONS. Irun Cohen 3, in considering the role of autoimmunity in the economy of the body, suggested that, “PROGRESS IN IMMUNOLOGY APPEARS TO HAVE RENDERED THE CLONAL SELECTION PARADIGM INCOMPLETE, IF NOT OBSOLETE; true it accounts for the importance of clonal activation, but IT FAILS TO ENCOMPASS, REQUIRE, OR EXPLAIN MOST OF THE SUBJECTS BEING STUDIED BY IMMUNOLOGISTS TODAY ...”. Polly Matzinger4 has gone even further: based upon her view that the “DANGER THEORY” has negated Burnet’s classical view of self-nonself discrimination, SHE EXTENDS THIS TO SUGGEST THAT THE ENTIRE CLONAL SELECTION PARADIGM THAT HAS RULED IMMUNOLOGY FOR SOME 35 YEARS HAS BEEN OVERTHROWN."

"GIVEN THIS WIDE-OPEN THEORETICAL TERRAIN, IT IS NO WONDER THAT DEBATE CONTINUES on such questions as a “big bang” versus the continuous generation of diversity, the relative roles of central versus peripheral mechanisms of tolerance, the number and type of signals required for one or the other response 43, whether autoimmunity is dangerous or beneficial (Cohen3) and whether the immune apparatus evolved to recognize infectious pathogens (Cohn 44 and Janeway 45), “danger” (Matzinger 46) or, following Jerne 47, “self” (Coutinho 48 and Cohen 3)."

"In the end, however, we must not lose sight of the fact that the CST IS ONLY A THEORY OF HOW ANTIBODIES ARE FORMED, NOT A THEORY OF WHY THEY ARE FORMED."

DOI:10.1038/ni0902-793

More info on Antibody deception:

https://docs.google.com/document/d/e/2PACX-1vS5jTh_Jo-hTt7mbMhQRF57rClqbiKBTWGuLKRBhzPt2QD77SYpMIGzTLi9fMcJKw7Kxg5lrssdGcwD/pub

Vaccines & Vaccination https://medcraveonline.com/IJVV/IJVV-02-00032?fbclid=IwAR1wdBWcsnB84P9esKu5Zb5gdXQnAfdMUeDmawz6MSIEPs0Dhc6ctOswfmI