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Adherence of Lyme Disease Spirochetes to Rat Lymphocytes

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Summary

In the present work, the capacity of Lyme disease (LD) Spirochetes to spontaneously adhere to rat lymphocytes has been evaluated. *Borrelia* organisms adhere to thymus, spleen, Payer's patches and peripheral blood lymphocytes in a higher frequency than that observed with *Salmonella minnesota* R345 (Rb) used as reference strain. Employing enriched splenic T and B cell populations, Spirochete binding to B lymphocytes is more elevated than that obtained with *Salmonella*, while similar percentage of T cells forms rosettes with both microorganisms.

Taken together, these findings provide evidence for a novel mode of interaction between LD Spirochetes and host immune system.

Introduction

Lyme disease (LD) is a systemic illness whose etiological agent is a Spirochete harbored by the ixodid tick, *Ixodes dammini* (5, 15). LD is characterized by inflammatory lesions in different organs such as skin, joints, nervous system and heart and either lymphomonocytes or neutrophils (3, 14, 16) have been found in the dermal infiltrates or synovial fluid from patients.

With regard to LD pathogenesis very few studies have been carried out. *Benach* et al. (2) and *Peterson* et al. (13) have provided evidence that murine, rabbit and human neutrophils and monocyte-macrophages are able to ingest and kill LD Spirochetes and phagocytosis is mediated by the Fc receptor (2). In addition, LD Spirochetes possess a lipopolysaccharide (LPS) (1, 6) which induces release of interleukin 1 from monocytes (7) and may play an important role in the pathogenesis of the disease. On the other hand, less investigations have been performed on LD Spirochete interaction with host lymphocytes. Since we have developed a system which enables to study *Salmonella* binding to human (8) or murine (10) lymphocytes, in the present study LD Spirochete cytoadherence to rat lymphoid cells has been evaluated.

Materials and Methods

Spirochetes: Heat-killed *Ixodes dammini*-associated *Borrelia* were kindly provided by Dr. *A. C. Steere* (Yale University, New Haven, Ct, USA).

Animals: 12 week old Wistar rats, maintained in the animal house of the Institute of Genetics (Bari, Italy), were used in this study.

Isolation of lymphoid cells: Rat thymus, spleen, and Peyer's patches (PP) lymphocytes were obtained by squeezing organs through wire screens (60 mesh) into RPMI 1640 (Eurobio, Paris, France) supplemented with penicillin (100 IU/ml), streptomycin (100 μg/ml), 2 mM glutamine and 5% heat-inactivated foetal calf serum (FCS) (complete medium). Peripheral blood mononuclear cells were obtained by hypotonic lysis of erythrocytes and gradient density centrifugation on Ficoll-Hysopaque (Pharmacia, Uppsala, Sweden) according to *Böyum* (4). All different cell suspensions were washed three times with Hanks' balanced salt solution and resuspended in complete medium before use.

Enrichment of T and B splenic lymphocytes by nylon wool column (18): Briefly, 2×10^8 cells/ml were run into a nylon wool(LP-1 Leuko-pak, Fenwall Laboratories, Morton Grove, Ill., USA) column and let to incubate in RPMI plus 2% FCS for 45 min at 37 °C –5% CO_2. Afterwards, T cell fraction was obtained by adding to the column 20 ml of warm RPMI plus 2% FCS, dropwise. B cell fraction was isolated by pouring 10 ml of warm RPMI plus 2% FCS on column and pressing with a sterile plunger. Cells were resuspended in complete medium and viability determined by trypan blue. T lymphocyte fraction contained 97 ± 2% T lymphocytes as assessed by immunofluorescence using an anti-T monoclonal antibody (R1-3B3) (11) in the presence of fluorescein isothiocyanate (FITC)-conjugated goat antibody to mouse IgG as the second reagent (Ortho Diagnostic Systems, Milan, Italy). B fraction contained 82 ± 3% of B cells when examined for surface immunoglobulins and 15 ± 4% T cells (10).

Binding of Spirochetes to rat lymphocytes from different anatomical sites: Salmonella minnesota R345 (Rb) was used as reference strain in these studies (8, 10). Briefly, Spirochetes were incubated with different lymphocyte suspensions at 50:1 ratio for 15 min at 4 °C. Cells were centrifuged 3 min at 2000 rpm, resuspended and washed three times 10 min at 1100 rpm. Cytoadherence was read by phase contrast microscopy in the case of *Salmonella* Rb, while cytocentrifuge preparations of Spirochete/lymphocyte mixtures were stained by Giemsa for the visualization of these organisms. At least 200 cells were counted in each test (8).

Results

Our previous studies in humans (8) and mice (10) have demonstrated that *Salmonella minnesota* Rb exhibits maximum degree of adherence to lymphocytes among all the *Salmonella* strains tested. Therefore, in this study we have compared LD Spirochete binding to lymphocytes with that of *Salmonella* Rb. In preliminary studies, it was necessary to demonstrate that rat lymphocytes could undergo binding to *Salmonella* Rb and, actually, cytoadherence occurred at higher frequency in animals with age similar to that of individuals selected for the present investigation (data not shown).

Table 1 shows that Spirochetes were able to bind lymphoid cells in a more elevated percentage than that observed with *Salmonella* Rb. Basically, lymphocytes from all organs considered (thymus, spleen, PP and blood) underwent a similar binding to Spirochetes. Additionally, it was of interest to determine the cell type(s) involved in the adherence to *Borrelia* organisms. In fact, *Salmonella* adherence is restricted to T cells only, and a few B cells form rosettes with these bacteria (8, 10). When enriched splenic T and B lymphocytes were used to evaluate cytoadherence, *Spirochetes* and *Salmonella* Rb bound to T cells with a more elevated frequency than that obtained with B-enriched lymphocytes. However, while *Salmonella* exhibited a negligible cytoadherence to the B-enriched fraction, *Borrelia* displayed a significant binding to those cells (results are illustrated in table 2). It is also worthwhile mentioning that both Spirochete and *Salmonella* binding increased in a significant manner by using enriched lymphocyte suspensions (table 2 versus table 1).

Table 1. Spirochete cytoadherence to rat lymphocytes in comparison with *Salmonella minnesota* R345 (Rb) binding

Lymphoid cell Source	% binding of Spirochetes[a]	% binding of *S. minnesota* Rb
Thymus	58.1 ± 2.9[b]	13.4 ± 2.1
Peyer's patches	51.4 ± 4.7	9.0 ± 0.4
Peripheral blood	50.3 ± 3.3	20.1 ± 1.9
Spleen	42.7 ± 3.7	28.1 ± 3.2

[a] Values are expressed as mean ± SD of three separate experiments.
[b] Binding was evaluated by counting at least 200 cells for each experiment.

Discussion

LD is a disease of recent identification and its pathogenetic grounds are not yet completely understood. On the other hand, spontaneous binding of bacteria to lymphoid cells is a novel biological phenomenon which might have importance in the modulation of host immune response. In this respect, since bacterial adherence has been demonstrated to occur *in vivo* (9), this may represent an alternative defence mechanism of the host against invading microorganisms.

Our data clearly indicate that Spirochetes bind to rat lymphocytes and this seems to be a common feature of other gram-negative or -positive bacteria (12, 17). However, Spirochetes display a higher frequency of binding to lymphocytes recovered from different sources in comparison with the reference strain *Salmonella minnesota* Rb. Interestingly, in spleen more B cells are involved in cytoadherence to Spirochetes, while very few B lymphocytes undergo binding to *Salmonella*. On the other hand, both *Borrelia* and *Salmonella* exhibit a similar percentage of adherence to enriched splenic T lymphocytes (table 2). Therefore, differences observed with whole splenic lymphocytes between the two organisms (table 1) may depend on the more elevated binding of Spirochetes to B lymphocytes. In our system, different strains of smooth or rough

Table 2. Spirochete and *Salmonella minnesota* R345 (Rb) cytoadherence to rat splenic T- and B-enriched lymphocytes

Cell type[a]	% binding of Spirochetes[b]	% binding of *S. minnesota* Rb
T-enriched lymphocytes	67.2 ± 6.3[c]	63.5 ± 4.3
B-enriched lymphocytes	39.2 ± 8.2	6.4 ± 0.3

[a] T and B lymphocytes were separated on nylon wool column as described in Materials and Methods. In the unfractionated population percentage of T lymphocytes was 70 ± 7 versus 30 ± 5 of B lymphocytes.
[b] See footnote b of Table 1
[c] See footnote a of Table 1

Salmonella (8, 10) or other gram-negative bacteria (unpublished observations) fail to display a significant binding to human or murine B cells. The reason for which *Borrelia* are able to adhere to B lymphocytes in higher frequency is unknown. Tentatively, since *Salmonella* LPS has been shown to play a major role in the binding of bacteria mainly to human (8) or murine (10) T lymphocytes, one can speculate that Spirochete LPS for its peculiar chemical composition or location in the cell wall (1) may be responsible for attachment also to other cell types.

In the light of the above facts, experiments are in progress to study Spirochete adherence to lymphocytes during experimental infections and evaluate the fate of these microorganisms once they are attached to lymphoid cells.

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