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Lyme Borreliosis: Host Responses to *Borrelia burgdorferi*

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INTRODUCTION

Lyme disease became an important public health problem during the decade following its clinical characterization in the late 1970s (122, 123), discovery of the etiologic agent, *Borrelia burgdorferi* (31, 67), and the isolation of the spirochete from patients (2, 3, 19, 92, 94, 103, 119). This multisystemic and chronic tick-borne spirochetosis is of worldwide distribution and has been the subject of various monographs (18, 66, 113, 121) and recent reviews (5, 47, 114). In this article, we will focus on studies investigating interactions between the spirochetes and the host. In so doing, we will attempt to present current hypotheses of how the disease progresses and to indicate the directions for future investigations into the chronic nature of Lyme disease.

The clinical manifestations of Lyme borreliosis coexist with a specific and vigorous immune response to the causative agent. To understand the role of active immunity in Lyme disease we need to ask two basic questions concerning this type of condition. First, is the immune response in Lyme disease ineffective? Second, is the immune response itself contributing to the production of injury in this disease? Taking these questions further, answers need to be sought regarding the mechanisms of persistence of the spirochetes or their poorly degraded components in various tissues. How do spirochetes evade the immune response? Is the chronic condition in Lyme disease the result of antigenic variability such as is seen in the relapsing-fever borrelia (8, 9), or is the chronic condition associated with persistent antigen perpetuated by a specific immune response to the spirochete? Does an autoreactive condition arise as a result of molecular mimicry between the bacterium and host, leading to a continuous cycle of injury in the patient?

The histopathological evidence so far has shown that spirochetes are not abundant in lesions from patients, and while technical difficulties may prevent detection, it is generally agreed that organisms are rare in affected tissues. Furthermore, the difficulty in isolating *B. burgdorferi* from the blood suggests that the spirochetemic phase is very short. Since there is a paucity of organisms, it has been reasoned that the inflammatory response in Lyme disease is the result of amplification by potent host-derived mediators released as a result of contact with the spirochete or its products. There are also open questions regarding infectivity. Are all strains of spirochete infective? Evidence so far presented has shown that the spirochete's ability to persist in an experimental animal is lost with increasing in vitro cultivation. How are these observed changes related to the virulence of the spirochetes?

Answers to these questions are being sought in many laboratories. Evidence which may point to one particular mechanism as the culprit for the causation of injury in this disease is almost always balanced by evidence which points to another as the possible explanation for pathogenesis. Hence, the multiple factors which may be operational in this disease and their possible relatedness to other bacterial diseases have led to this review.

NONSPECIFIC HOST CELL RESPONSES

An important nonspecific host antimicrobial mechanism is the eradication of the infectious agent by professional phagocytes, namely polymorphonuclear leukocytes (PMN), monocytes, and macrophages. At several stages during the course of Lyme disease, phagocytic cells and *B. burgdorferi* are found in proximity. Macrophages are present in the erythema migrans lesion, where spirochetes have also been visualized and isolated (45). Circulating PMN and monocytes are exposed to the spirochetes during dissemination of the bacteria to secondary sites. Finally, in joints where chronic disease is apparent, the synovial fluid contains abundant PMN and cultivatable spirochetes (103). Although information about the interaction between spirochetes and phagocytes in vivo is lacking, in vitro studies have been described which address basic aspects of this host response.

Purified proteins from *B. burgdorferi* have been shown to be chemotactic for human PMN (20). The proteins examined in these experiments included outer surface protein A (OspA), a basic molecule (21) which has been cloned and sequenced (28) and is now known to be a lipoprotein (29), and the flagellin, which has also been sequenced (52). Both OspA and the 41-kDa flagellin proteins, purified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electroelution, elicited a measurable chemotactic response in modified Boyden chamber assays. The 41-kDa protein provoked a larger response than OspA, stimulating PMN migration to 78% of that seen with formyl-methionyl-leucyl-phenylalanine. In addition to spirochetal proteins, host-

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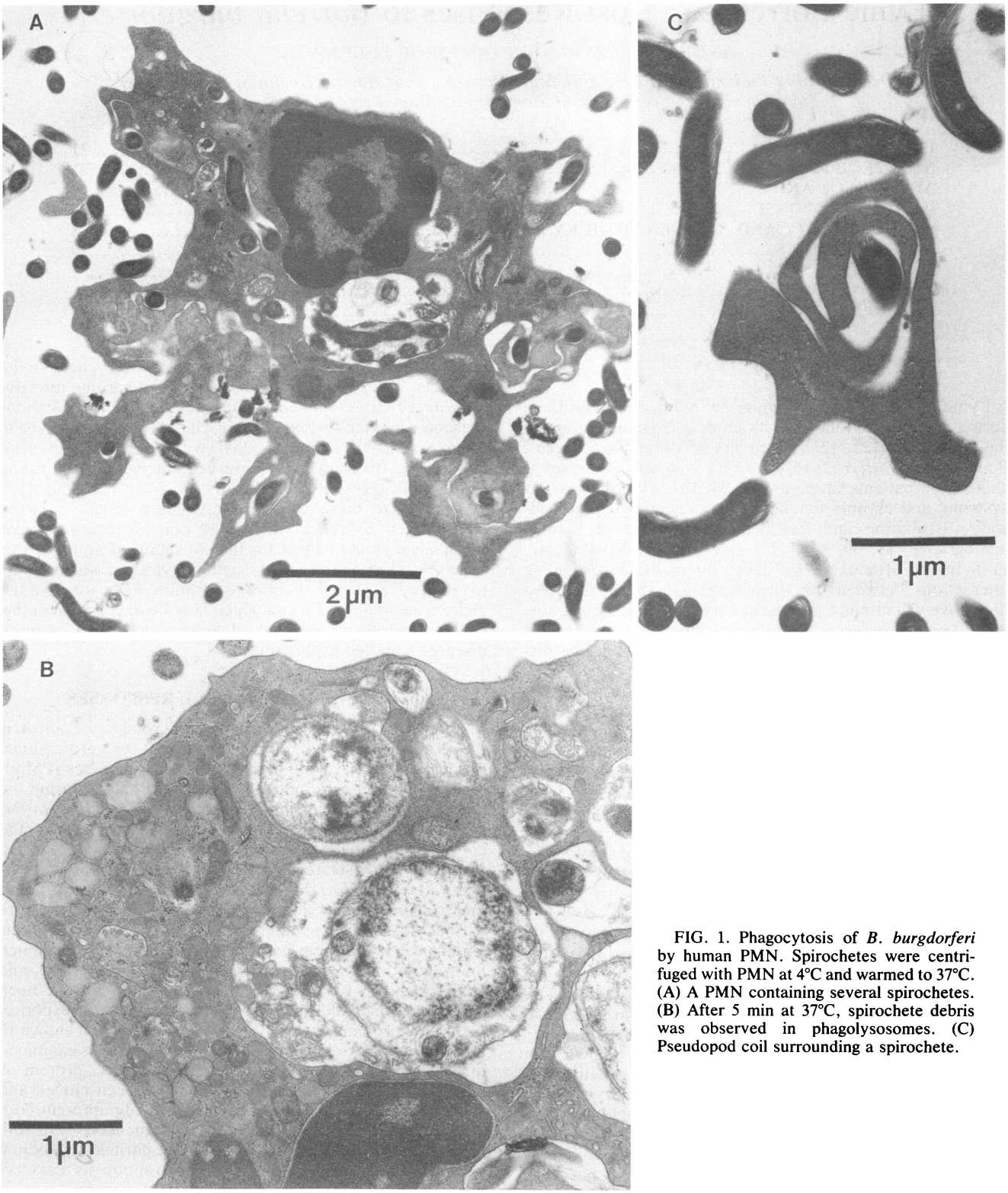


FIG. 1. Phagocytosis of *B. burgdorferi* by human PMN. Spirochetes were centrifuged with PMN at 4°C and warmed to 37°C. (A) A PMN containing several spirochetes. (B) After 5 min at 37°C, spirochete debris was observed in phagolysosomes. (C) Pseudopod coil surrounding a spirochete.

derived chemotactic proteins may be involved in recruitment of PMN to sites of persistent spirochetes. *B. burgdorferi* has been shown to activate complement in vitro (75). Complement activation at the surface of the organism would produce C5a, which is a potent chemoattractant for PMN. Immune complexes were detected in the blood and synovial fluid of Lyme disease patients (59, 60). The generation of C5a as a result of complement activation by immune complexes could also provide PMN chemoattractant activity. Alone or in combination, host- and spirochete-derived chemoattractants may contribute to the large influx of PMN found in the synovial fluid of patients with Lyme arthritis.

Studies have shown that *B. burgdorferi* were internalized by human PMN (Fig. 1) and peripheral blood monocytes, murine macrophages, and rabbit peritoneal exudate macrophages (4, 23, 26, 91, 125). Uptake of the organism by PMN and mononuclear phagocytes occurred in the presence or absence of serum-derived opsonins. The uptake of unopsonized radiolabeled spirochetes (added in suspension above adherent mononuclear phagocytes or in a suspension assay with PMN) was inefficient at low bacteria-to-phagocyte ratios (23). This in vitro system may represent the conditions present during spirochete dissemination. With few organisms present in the circulation, inefficient phagocytosis would allow spread of the organism to secondary sites. In a separate study, much greater uptake of unopsonized spirochetes was observed when the phagocytes were added to plastic surfaces coated with spirochetes, a process referred to as surface phagocytosis (91). Surface phagocytosis may more closely resemble events occurring in the connective tissue of the skin or secondary sites following spirochete dissemination.

Studies have shown that subculture of the spirochetes leads to a loss in infectivity in rodent models of disease (71, 107). However, phagocytosis of spirochetes freshly isolated from the spleens of infected Syrian hamsters was indistinguishable from the uptake of subcultured spirochetes (91), indicating that in vitro cultivation did not alter the uptake of spirochetes by phagocytic cells.

Opsonization of spirochetes facilitated their uptake by monocytes and PMN in an Fc receptor (FcR)-mediated process (23). Uptake of the bacterium was increased when the spirochetes were incubated in the presence of fluids containing antibodies to the organism, including sera from several sources and human synovial fluid (4, 23, 91). In contrast to unopsonized organisms, there was increased uptake of opsonized spirochetes following short incubation periods and at low spirochete-to-phagocyte ratios (4, 23, 91). Prior opsonization of the spirochetes with immune sera increased the number of cells involved in phagocytosis (23) as well as the number of organisms ingested per phagocyte (4). The role of the FcR in the uptake of opsonized spirochetes was clearly demonstrated following the downmodulation of these receptors with monomeric immunoglobulin G (IgG) or a monoclonal antibody (MAb) to FcγR III, to decrease the number of available receptors on monocytes and PMN, respectively (23). Moreover, downmodulation of these receptors led to a 90% decrease in the uptake of radiolabeled spirochetes.

The role of complement receptors in the phagocytosis of *B. burgdorferi* has not been examined directly. A cerebrospinal fluid (CSF) isolate of *B. burgdorferi* activated complement in normal human serum (75). The demonstration of complement activation and the deposition of C3 on the surface of the spirochete indicates that complement receptors may be important in the uptake of the organism in the absence of immune antibody. The increase in phagocytosis of *B. burgdorferi* incubated with PMN in whole blood compared with that of purified PMN suggests that complement may be involved in spirochetal uptake (4).

Results from transmission electron microscopic analysis demonstrated conclusively that spirochetes were internalized by PMN (23, 91, 125) and monocytes (91). Uptake of the bacteria occurred with pseudopods extending circumferentially around the diameter of the bacteria (125) (Fig. 1). Pseudopod coiling, a process noted in the uptake of *Legionella pneumophila*, has occasionally been seen in the uptake of *B. burgdorferi* (125) (Fig. 1). However, the relevance of this phenomenon is presently unknown.

The induction of the respiratory burst in PMN following exposure to *B. burgdorferi* has been described in two studies (91, 125) and was found to be lacking in a third (4). Opsonized and unopsonized spirochetes induced a respiratory burst in a dose-dependent manner when exposed to PMN (91, 125). The initial rate of oxygen radical production and the overall magnitude of the response was increased when spirochetes were first opsonized with immune rabbit serum (91). In one study, no detectable respiratory burst was measured in PMN stimulated with spirochetes (4). In fact, hydrogen peroxide production was reduced in the presence of Borreliae. It has been suggested that the spirochetes may possess a scavenging molecule for superoxide anion. However, no detectable superoxide dismutase activity was observed (4).

Morphological evidence provided by electron microscopy and fluorescence microscopy has indicated that once internalized, spirochetes do not survive inside phagocytic cells. Electron microscopic studies revealed the presence of disrupted spirochetes in the phagosomes of PMN (23, 124a) (Fig. 1). Also, the presence of fluorescent bodies stained with antispirochete antisera in adherent mononuclear phagocytes demonstrated the presence of spirochetal debris within the phagocytes (23). Acridine orange, a vital dye, was used to demonstrate the presence of nonviable spirochetes internalized by PMN and monocytes (91). Interestingly, PMN from a patient with chronic granulomatous disease, which are defective in their ability to induce a respiratory burst, efficiently killed *B. burgdorferi*, indicating that oxygen-independent bactericidal compounds may be functioning in the killing of these spirochetes (91).

A lipooligosaccharide moiety which demonstrated properties similar to those of endotoxin was found in *B. burgdorferi* (16). Phenol-chloroform-petroleum-ether treatment of the B31 strain produced an extract which possessed biologic activities characteristic of lipopolysaccharide (LPS). This extract was pyrogenic in rabbits, mitogenic for human mononuclear cells and murine splenocytes, cytotoxic for murine macrophages, and positive in the *Limulus* amebocyte lysate assay for endotoxin. Analysis of the extract by polyacrylamide gel electrophoresis revealed a fast-migrating form characteristic of rough-form LPS. Although the biologic activities of this extract were similar to those of LPS, lipid A, the hydrophobic backbone characteristic of classic LPS, was not detected in another study (127).

A molecule with LPS-like activity has been suggested to play a role in Lyme disease by stimulating an inflammatory reaction in the host which is amplified by host-derived cytokines (55, 56). Interleukin-1 (IL-1) is a potent immunoregulatory cytokine with a diverse range of effects on host cells (41). Studies have shown that this cytokine was produced primarily by mononuclear phagocytes but also by several other cell types (41). IL-1 has been detected in the

synovial fluid of patients with Lyme arthritis and was produced by cells following exposure to *B. burgdorferi*. Human monocytes, murine macrophages, a human histiocytic cell line (U937), and cells isolated from human synovium produced IL-1 following exposure to *B. burgdorferi* (14, 55, 56). The IL-1 activity was neutralized with antisera to human monocyte IL-1 (14, 56). In addition to whole spirochetes, LPS and peptidoglycan fractions also elicited IL-1 production by cells isolated from synovial fluid and by mononuclear phagocytes (14, 15).

Several activities of IL-1 indicate that it may be an important mediator in the development of inflammatory responses in the joint. Intradermal injection of IL-1 caused PMN extravasation in vivo (17), and these cells have been found in the synovial fluid of Lyme arthritis patients (45). Also, IL-1 stimulated the release of prostaglandin E_2 (PGE_2) and collagenase from chondrocytes (41). Synovial tissue derived from a patient with Lyme arthritis produced elevated levels of PGE_2 and collagenase (116). Many of the destructive changes occurring in the joint space could be initiated by IL-1 production in response to localized infection. The influx of phagocytic cells and the stimulation of proteolytic activity of resident synovial cells would provide the joint space with a high oxidant and protease burden, which would undoubtedly contribute to erosive changes in this tissue.

ADHERENCE AND INVASION

After reaching the bloodstream, *B. burgdorferi* invades several different organ systems. The wide range of systems affected in humans with Lyme borreliosis (45) and the successful culture of spirochetes from several tissues in experimentally infected rodents (12, 71) indicate that *B. burgdorferi* is capable of adhering to cells at different tissue sites. In vitro studies have demonstrated the adherence of spirochetes to the surface of cells from invertebrate and vertebrate sources, including tick cells (80), human epithelial cells (20, 62), human umbilical vein endothelial cells (126, 128), and cells derived from neonatal rat brain (50). One study has described the adherence of spirochetes to extracellular matrices produced by endothelial cells in culture (126). This section will review these results, with specific attention paid to studies with endothelial cells and neonatal rat brain cultures.

Adherence of *B. burgdorferi* to monolayers of endothelial or rat primary brain cells occurred in both a dose- and time-dependent manner. Spirochete adherence to cells increased with inoculum size (50, 126, 128) and could not be saturated even at spirochete-to-endothelial cell ratios of 1,000:1. The inability to saturate binding may reflect two phenomena: adherence of the spirochetes to one another, which is unavoidable at high spirochete densities, and the ability of the spirochetes to penetrate the cell monolayers during the long time course of adherence assays (2 to 24 h). The presence of excess numbers of unlabeled spirochetes reduced the adherence of radiolabeled organisms, suggesting that the binding was specific (126, 128). Treatment of primary brain culture cells with vincristine and cytochalasin B, which destabilize microtubules and microfilaments, respectively, had no effect on spirochete adherence (50), indicating that these cells did not internalize the spirochetes.

Spirochetes treated by Formalin fixation or heat showed reduced association with primary rat brain cultures (50) and human umbilical vein endothelial cells (HUVEC) (128). These results suggest that spirochete viability is necessary for cell adherence. Alternatively, destruction of adhesion molecules by fixation or heat treatment may have occurred. When adherence assays were performed at 4°C, spirochete binding was greatly reduced (50). The reduction of spirochete adherence at low temperatures may reflect decreased metabolic activity and motility of the spirochetes or a decrease in the fluidity of the outer membrane, which would prohibit the aggregation of surface molecules involved in adherence. The aggregation of OspA, a surface lipoprotein of *B. burgdorferi*, has been described (10).

The adherence of several strains of *B. burgdorferi* to endothelial cells has been compared. The adherence of a high-passage strain, B31, was significantly reduced compared with that of isolates of lower passage from both human and tick sources (126, 128). Since low-passage strains tend to aggregate, the increase in adherence may be due at least in part to spirochete-spirochete association. Strain differences have also been described in the ability of *B. burgdorferi* to penetrate HUVEC monolayers (37, 126). Although it is apparent that the high-passage strain B31 adheres poorly to endothelial cells, this may only reflect the adherence capacity of this particular strain. Direct evidence relating a strain's pathogenicity and its ability to adhere to any cell type is lacking.

The molecular mechanism of spirochete adherence and the nature of the bacterial adhesin(s) have not been described. Reports have demonstrated the effects of human and rat immune antisera and MAbs to OspA, OspB, or flagellin (41 kDa) on spirochete adherence to cells (20, 50, 128). While human immune antisera greatly reduced the adherence of spirochetes to HUVEC (128), immune rat serum did not inhibit the adherence of spirochetes to rat primary brain culture cells (50). This rat immune serum contained a strong antibody response to both OspA and OspB, as detected by Western immunoblot. Pretreatment of spirochetes with MAbs directed against OspA reduced the adherence of the bacteria to HEp-2 cells (20) but not to human umbilical vein endothelial cells (128) or primary rat brain cultures (50). An MAb directed against OspB has been shown to reduce the association of spirochetes with HUVEC (128). Antibodies to the flagellin protein have only a slight inhibitory effect on cell adherence (20, 128). While these results suggest that spirochete adherence may involve the major outer surface proteins (OspA and OspB) of the organism, adherence to certain cells, namely rat brain cells, may occur independently of these proteins.

The nature of cell surface receptors for *B. burgdorferi* has not been elucidated. The role of cell surface carbohydrate has been investigated in studies with HEp-2 cells (20). Neuraminidase treatment of these cells reduced spirochete attachment by 43%. Growth of HEp-2 cells in the presence of tunicamycin, which inhibits N-linked glycosylation of proteins, did not significantly reduce spirochete binding. Soluble monosaccharides, including L-fucose, galactose, α-methylmannoside, *N*-acetylglucosamine, and *N*-acetylgalactosamine, did not inhibit spirochete adherence to HUVEC (126). Spirochete receptors in neonatal rat brain cultures may be partly protein, since trypsin treatment of the cells reduced subsequent adherence of the bacteria (50).

B. burgdorferi has been shown to adhere to the subendothelial matrix produced by HUVEC (126) (Fig. 2). This extracellular matrix contains basement membrane collagens and several glycoproteins, including fibronectin and von Willebrand factor. When the extracellular matrix was pretreated with a polyclonal antibody to human plasma fibronectin, spirochete adherence was reduced 48 to 63%

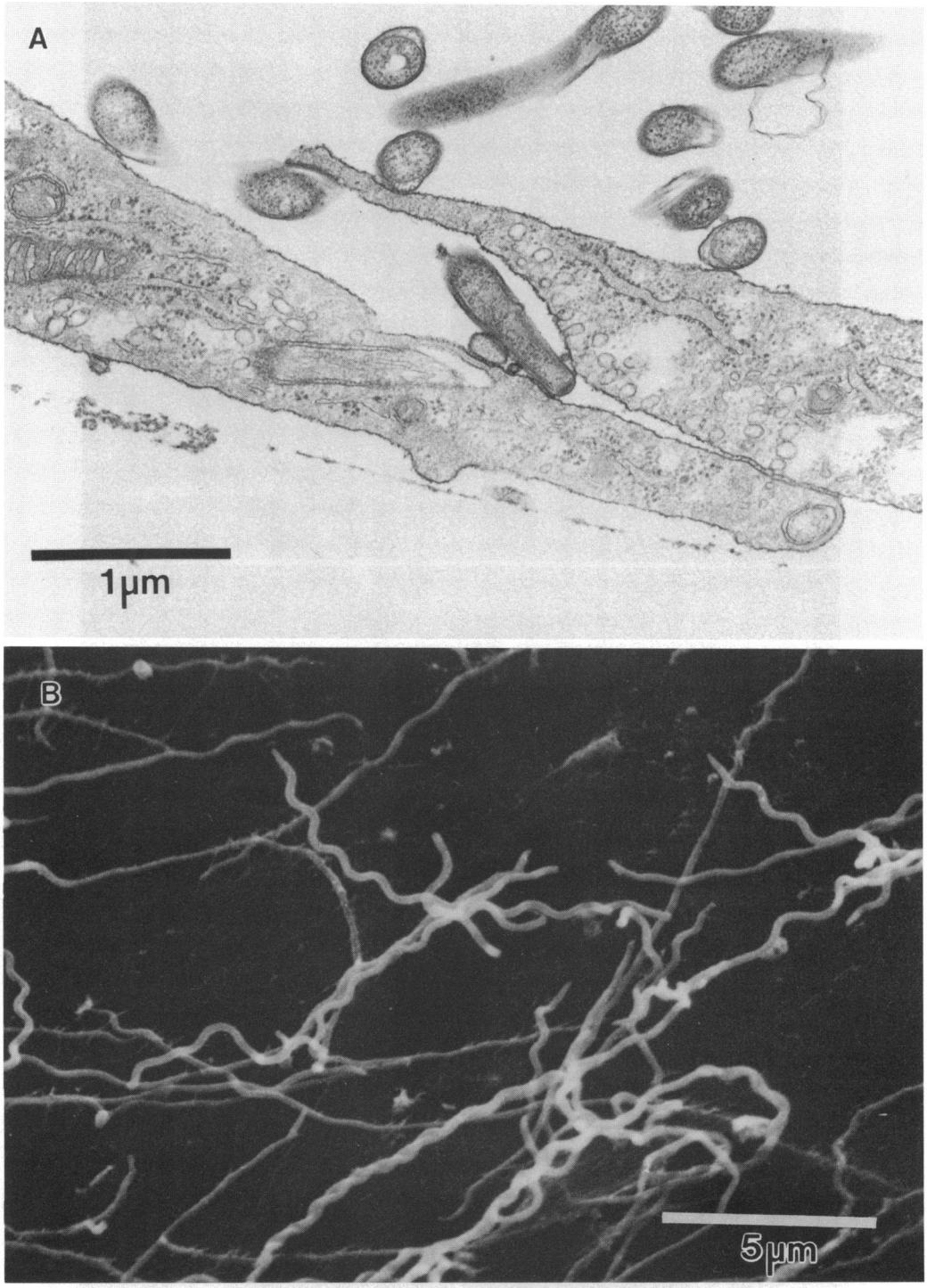
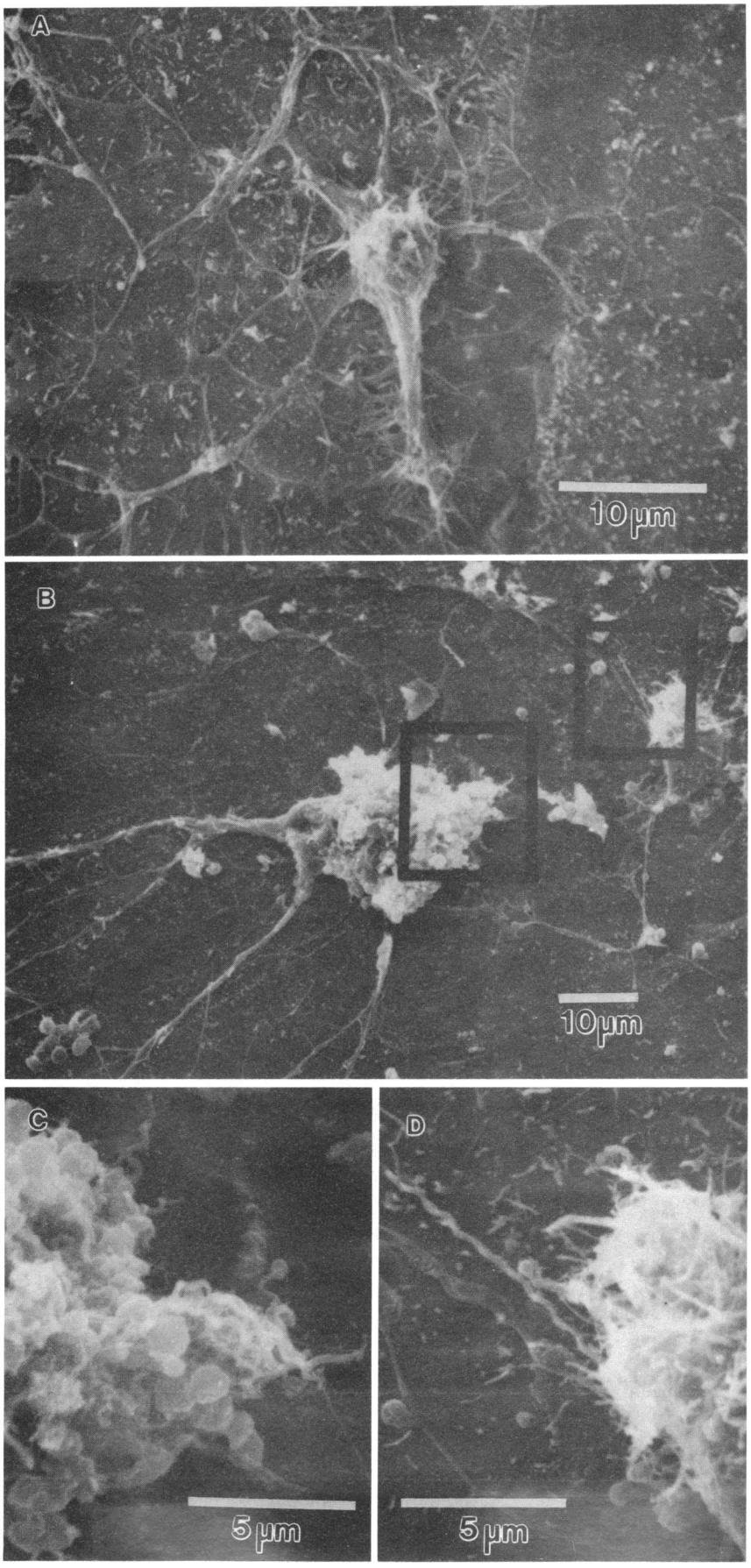


FIG. 2. Association of *B. burgdorferi* with human umbilical vein endothelial cells and subendothelial matrix. (A) A spirochete penetrating an endothelial monolayer at an intercellular space between adjacent cells. (B) Spirochetes adherent to subendothelial extracellular matrix.

(126). Antibodies against collagens type I and III and von Willebrand factor did not alter spirochete adherence. Adherence of the spirochetes to the subendothelial matrix was unaffected in the presence of soluble human plasma fibronectin. It has been found that *B. burgdorferi* does not adhere to plastic coated with fibronectin (128). The ability of *B. burgdorferi* to recognize subendothelial fibronectin and not plasma fibronectin-coated plastic may reflect differences between HUVEC-derived fibronectin and plasma fibronectin, as well as conformational differences between the native molecule present in the extracellular matrix and the protein absorbed to plastic. Pretreatment of HUVEC monolayers with polyclonal antifibronectin antibody did not significantly reduce spirochete adherence to the cell surface, suggesting


A
10μm
B
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that attachment to HUVEC and their extracellular matrix is mediated by different receptor molecules. The ability of the spirochetes to adhere to the extracellular matrix may explain the rather nonspecific tissue tropism of this organism.

Two studies have shown that *B. burgdorferi* penetrate endothelial cell monolayers following adherence to HUVEC. A human blood isolate of *B. burgdorferi* (HBD1) was shown to migrate across monolayers in the region of the intercellular space (126) (Fig. 2). This mechanism of transendothelial migration has been observed for another spirochete, *Treponema pallidum* (130). Transmission electron microscopy revealed the presence of *B. burgdorferi* at the apical surface of the endothelium, passing through the monolayer between adjacent cells and beneath the endothelial monolayer. This process occurs within 10 min following attachment of the spirochetes to the apical surface of the endothelial cells. Treatments that disrupt endothelial monolayer integrity (10 mM EDTA or physical scraping) increased spirochete passage through the cell monolayers (126). This would indicate that spirochete penetration of endothelium during dissemination is slowed by the presence of the cells and that abrogation of endothelial integrity may be necessary for spirochetal penetration of tight endothelial monolayers, such as those present in the central nervous system.

In a separate study, the passage of another isolate of spirochetes from human blood (HB19) through the cytoplasm of the endothelial cell and not the region between adjacent cells was reported (37). The penetration of endothelial cell monolayers by a transcytotic process has also been described for another spirochete, *Leptospira interrogans* (129). *B. burgdorferi* penetration of the endothelial monolayer and an underlying polycarbonate filter occurred at a measurable rate, indicating that the spirochetes do not remain in an intracellular location. As was demonstrated for adherence, heat-killed borreliae did not penetrate HUVEC monolayers (37). This result indicates that the spirochetes participate actively in the penetration process. Spirochete penetration was reduced when experiments were performed at 4°C.

Subsequent to attachment, spirochetes cause damage to certain cells in vitro. Tick cells cocultured in the presence of 10^7 *B. burgdorferi* per ml lost surface microvilli, appeared lysed, and became detached (80). Results of chromium release assays and scanning electron microscopy have indicated that coculture with spirochetes induced cytopathic effects on macroglial cells from neonatal rat brain cultures (46a). The oligodendroglial cells demonstrated a retracted appearance and had numerous membranous blebs coincident with the presence of spirochetes (strain B31) (Fig. 3). The absence of detectable chromium release and the lack of morphologic changes in HUVEC cocultured with *B. burgdorferi* (37; unpublished observation) would indicate that the cytopathic effects observed may be cell specific. Alternatively, longer periods of coculture may be necessary to detect injury to endothelial cells.

While it is apparent that *B. burgdorferi* adhere to cells of different origins, evidence in support of a tissue tropism comes from studies of spirochete adherence to glycolipids. The ability of spirochetes to adhere to membrane lipid and glycolipid preparations revealed a greater adherence to galactocerebroside than to other structurally related molecules, including glucocerebroside, ceramide, and sphingosine (46a). Galactocerebroside is located on the surface of oligodendroglia and Schwann cells, which are myelin-producing cells located in the central nervous system and peripheral nervous system, respectively. The adherence of *B. burgdorferi* to galactocerebroside-bearing cells and the apparent damage to oligodendroglial cells in vitro may explain aspects of the neurologic involvement during Lyme disease (Fig. 3).

Experiments performed in adult Lewis rats have shown that *B. burgdorferi* penetrated the blood-brain barrier (BBB) in these animals 24 h following intravenous inoculation (49). The detectable presence of spirochetes in the CSF of the rats was preceded by an increase in the permeability of the BBB. Increased BBB permeability was observed 12 h after spirochete inoculation, as measured by the movement of ^{125}I-labeled rat albumin from the blood to the CSF. No detectable alteration in BBB permeability resulted when spirochetes were directly inoculated into the CSF. As few as 10^7 spirochetes per rat altered BBB permeability. Only whole, viable spirochetes were effective; heat-killed spirochetes or sonicates of the bacteria did not alter BBB permeability. A low-passage tick isolate (J31) was more effective than a high-passage isolate (B31) in causing BBB permeability changes. Detection of OspA and OspB in the CSF of patients with early-stage Lyme disease provided evidence for the early invasion of the CNS in humans (49).

Since increased BBB permeability was dependent upon the inoculation of viable *B. burgdorferi* and preceded the detection of spirochetes in the CSF, some interaction with the host during dissemination appears to be necessary to elicit this change. As mentioned previously, IL-1 was produced by cells in response to live *B. burgdorferi*. IL-1 has been shown to increase vascular permeability and cause blood vessel damage (41). It is possible that the elaboration of IL-1 or another agent that alters vascular permeability is a prerequisite to the penetration of the CNS by *B. burgdorferi*. The delay in spirochete penetration of the CNS in the rat (24 h following inoculation) supports such a mechanism.

IMMUNE RESPONSE

The association of *B. burgdorferi* with Lyme disease was first indicated by serology (31). Since that time, Lyme disease has been diagnosed in the laboratory by detection of specific antibodies to the organism. The B-cell response in Lyme disease has been extensively characterized. The temporal development of antibodies to the various antigens of the organism has been elucidated, with significant reproducibility among the groups who have investigated this aspect of the immune response (6, 35, 38). The evolution of the specific antibody response can be concurrent with a polyclonal activation of B cells, as determined by elevated total IgM levels, circulating immune complexes, and cryoglobulins (59, 60, 106, 120).

Studies have shown that the first antibodies produced in infected humans were of the IgM class and developed 3 to 6

FIG. 3. Association between *B. burgdorferi* and rat oligodendrocytes. Spirochetes were cocultured for 12 h with macroglial cells derived from rat brain. (A) Oligodendroglial cell located on top of a layer of astrocytes. (B) Oligodendrocyte following coculture with *B. burgdorferi*. (C and D) High-magnification micrographs of the boxed areas in panel B, revealing the presence of spirochetes on an oligodendroglial cell in an area containing numerous membranous blebs and retracted cellular processes.

weeks following the start of infection (6, 35, 38). These IgM antibodies were directed against a polypeptide of 41 kDa, which is a subunit of the flagellum (7). The IgM response in patients with Lyme disease, as in other infections, may persist or disappear. The IgG response, also to the flagellar subunit, has been detected within 6 weeks of infection (6, 35, 38). Chronically infected patients eventually developed additional antibodies to other spirochetal proteins (6, 35, 38). The immunoblot patterns of patients' sera at various stages of the disease have been analyzed in order to detect trends which could be useful at a clinical diagnostic level. In general, no correlation has been observed between disease state and a specific pattern on Western blots. However, in untreated individuals, the response usually expanded to include many *B. burgdorferi* proteins (6, 35, 38). Although the immune response continued to expand, suggesting prolonged survival of the spirochetes, little or no humoral response has been detected against the major outer surface proteins OspA and OspB. The vast majority of the antispirochete IgG class antibodies were of the IgG1 and IgG3 subclasses (61). An IgE response to *B. burgdorferi* has been observed (24).

The mononuclear cell response in Lyme disease patients has been studied, and the frequencies of the reactive cell types have been determined (39, 85, 88, 101). Compartmentalization of the immune response at sites of active inflammatory response has been described (109, 110). The earliest indication of localized B-cell responsiveness came from the observation of intrathecal antibody production in excess of that found in the serum in certain patients with neuroborreliosis (58, 64, 87, 115, 135). Compartmentalization of the mononuclear response in this disease has also been documented in cells from the synovial fluid and from CSF of patients with Lyme arthritis (110) and neuroborreliosis (90), respectively. In both studies, cells collected from the fluids proliferated more vigorously than peripheral blood mononuclear cells. B-cell hyperactivity has been documented in patients at various stages of Lyme disease by means of both in vivo and in vitro IgM production and antigen stimulation (109). Antigen-specific cellular responses have been measured by using electroeluted OspA, OspB, and the 41-kDa flagellar subunit, with both normal and patient cells as well as with laboratory animals (20). In these studies, the cellular reactivity correlated temporally with the B-cell response, although marked differences in reactivity to antigens were noted between humans and laboratory animals. A dissociation between the T- and B-cell response has been reported and resulted in a phenomenon termed seronegative Lyme disease (40), which, as the name implies, involves patients whose mononuclear cells can proliferate after specific antigen stimulation but who have no detectable antibodies. It has been suggested that in some of these patients, the specific antibodies may be sequestered in complexes (106).

Other functional immune system compartments may play an important role in Lyme disease. Natural killer (NK) cell function was inhibited in patients with both early and chronic Lyme disease but was normal in patients who were in remission (54). In this study, it was noted that the organisms themselves inhibited NK cell function. Such an interference could allow the organism to escape an early, nonspecific protective response.

In a recent study, it was found that T cells from the peripheral blood of patients with reactive arthritis who also had antibodies to *B. burgdorferi* proliferated specifically after stimulation with macrophages previously exposed to the organism. The suggestion was made that the immune response in the joint compartment could be triggered by persistent agents (133)

If the immune response in Lyme disease is not fully effective in eradicating the organisms, does it contribute to the causation of injury? In the next section of this review, we will discuss the evidence for autoreactivity. In general, however, the lack of evidence for immune dysfunction suggests that the organism evades the host immunological response. In this regard, it would have been anticipated that control of the borrelial infection would be lost in a human immunodeficiency virus (HIV)-infected patient. The one reported case of concurrent HIV and Lyme disease did not appear to show significantly more severe borreliosis (51).

AUTOIMMUNITY AND ANTIGEN MIMICRY

One hypothesis for the production of chronic disease following *B. burgdorferi* infection is the development of an immune response in the host to self-determinants which contain structural similarities to spirochetal antigens. An immune response to spirochetal determinants shared with host proteins could lead to chronic inflammatory conditions and tissue damage. Such instances of molecular mimicry have been documented for other viral and bacterial infections (89). MAbs prepared against several different viruses have been shown to react with normal tissues from uninfected mice (112). The role of self-reactive T cells in the development of disease following *Mycobacterium tuberculosis* infection has been described recently (131). It is apparent that autoimmunity develops in certain individuals with Lyme disease. Recent evidence suggests that two spirochetal proteins may induce an autoimmune response, the 41-kDa flagellin subunit and members of a family of proteins called heat shock proteins (1, 33, 84, 111).

The flagella of procaryotes, including the spirochetes, are markedly similar in structure (42, 73, 74). These proteins contain conserved regions in their amino and carboxy termini, with a central variable region. N-terminal sequence similarity between the flagella of *B. burgdorferi* and *T. pallidum* has been documented (36). The flagella of species within the genus *Borrelia* contain cross-reactive epitopes (7). As mentioned earlier, the 41-kDa flagellar subunit is the first antigen recognized in individuals infected by *B. burgdorferi*.

The early response to this antigen may be the result of its similarity to flagella from other bacteria, representing an anamnestic immune response. It has been demonstrated that IgM antibodies from patients with neurologic manifestations in Lyme borreliosis reacted with normal human axons, as determined by indirect immunofluorescence (111). This activity could be absorbed out of the antisera by preincubation with *B. burgdorferi* or spinal cord homogenates but not with lysates of several other bacterial species. An MAb, H9724, directed against *B. burgdorferi* flagellin (7) was also tested and shown to detect a determinant present in human axons (111). MAbs directed against OspA (H5332) or OspB (H6831) did not react with axonal preparations. In a separate study, the MAb H9724 reacted with antigen on myelinated fibers of peripheral nerve, neurons in the central nervous system, hepatocytes, synovial cells, and cardiac muscle (1). In this study, several antibodies directed against the flagellin subunit, OspA, or OspB were tested, but only H9724 reacted with human tissues. By using immunogold electron microscopy, the determinant recognized by H9724 was localized to the Schwann cell cytoplasm and the outermost lamella of myelin sheaths. This MAb also reacted with peripheral

nervous tissue homogenates and myelin in an enzyme-linked immunosorbent assay.

Antineuronal antibodies have been detected in patients with Guillain-Barré syndrome and lymphocytic meningoradiculitis (97). Antibodies to myelin and myelin basic protein have been detected in the CSF and sera of some patients with Lyme borreliosis (48, 124). Anticardiolipin antibodies as well as rheumatoid factor have been detected in patients with Lyme disease (79, 82). The prevalence of antibodies to host cell determinants in the nervous tissue and the reactivity of an MAb directed against the flagellin suggest a role for this molecule in autoimmune sequelae in the nervous system.

Cellular immune responses in certain patients also indicate an autoimmune phenomenon. T-cell lines reactive towards peripheral myelin have been derived from patients with Lyme radiculomyelitis (83). In addition, T-cell lines have been shown to react with myelin basic protein, cardiolipin, and galactocerebrosides (83). A small proportion of the T-cell lines reacted with both *B. burgdorferi* antigen and myelin basic protein. However, the fact that these are cell lines and not clones does not preclude the possibility that more than one T-cell specificity accounts for this finding.

Recent interest has been focused on the role of heat shock proteins, or stress proteins, in the development of chronic Lyme disease. Members of this family of proteins have been shown to evoke autoimmune responses following experimental infection with *Mycobacteria* spp. (137). It has been demonstrated that the 65-kDa mycobacterial stress protein has immunologic and N-terminal sequence homology with a protein antigen of 58 to 60 kDa from *B. burgdorferi* (57, 84, 108). In general, stress proteins are highly conserved molecules found everywhere from procaryotic organisms to highly specialized eucaryotic cells (102, 136). Although first described as a class of proteins produced in response to an elevation in temperature, these proteins are also synthesized in response to a variety of other perturbations, including oxygen and nutrient deprivation, exposure to oxygen radicals, and viral infection (137). The function of these highly conserved proteins appears to be to stabilize proteins and protein complexes intracellularly and perhaps at the cell surface (46, 102). In this regard, they have been called molecular chaperones (46). Based on molecular mass, stress proteins have been divided into four families of 90, 70, 60, and 10 to 30 kDa (81). Members within the 60-kDa and 70-kDa families are highly homologous at the amino acid level and are also dominant antigens following infection by many microorganisms (81).

During infection, *B. burgdorferi* is exposed to stressful environments. First, the spirochetes are exposed to an increase in temperature upon leaving the poikilothermic vector (22, 25, 30, 32, 93, 96, 138) and entering the warm-blooded host (78, 134). Another stressful environment would be encountered in inflammatory foci. Recent studies have shown that synthesis of several proteins is increased when *B. burgdorferi* is cultured at elevated temperatures (33, 34). In one study, stress proteins of 75, 42, 39, and 27 kDa were reported when spirochetes were cultured at higher temperatures (34). In a separate report, major heat shock proteins of 72, 66, 60, 43, and 27 kDa were observed (33). The 75-kDa and 72-kDa proteins were the most abundant proteins induced by increased temperature and may represent the same protein. The 72-kDa protein demonstrated immunologic cross-reactivity with well-characterized members of the 70-kDa heat shock protein family from both *Escherichia coli* and *Mycobacterium tuberculosis* (33). While members of this family are antigenic, no reactivity to the 72-kDa protein from *B. burgdorferi* was observed in a limited number of antisera from patients with Lyme disease (33). The 66-kDa and 60-kDa proteins were found to be related to members of the 60-kDa stress protein family, including the 65-kDa protein of *M. tuberculosis*, by immunologic cross-reactivity (33). This protein may be similar if not identical to the 60-kDa common antigen, which was found to be immunologically related to proteins from *Pseudomonas aeruginosa*, *T. pallidum*, and *Legionella micdadei* (57). The 58- to 60-kDa protein was also a target of the humoral immune response in patients with Lyme disease. It was also one of only four antigens which reacted with antibodies from the synovial fluid of a patient with Lyme arthritis (84).

Because of the similarity between procaryotic and eucaryotic stress proteins and the strong immune response to a member of the 60-kDa family in patients with Lyme disease, it is tempting to speculate that this protein induces an autoimmune response in the joints during Lyme arthritis. Recent work with *Mycobacterium* spp. has shown that the 65-kDa stress protein, which is homologous to the 58- to 60-kDa protein of *B. burgdorferi*, is associated with autoimmune phenomena leading to experimental arthritis in rats (136, 137). This autoimmune response can be transferred by a T-cell clone (A2b), which was found to react with an epitope found in both the mycobacterial 65-kDa protein and cartilage proteoglycans (131). In addition, a second clone (A2c) can suppress the development of disease (132). These results indicate that different regions in the stress proteins may invoke immune responses which are both harmful and protective (65). The 65-kDa antigen also stimulated T cells from the synovial fluid of patients with inflammatory arthritis (53, 95). $CD8^+$ T cells, which react to the 65-kDa antigen, can lyse macrophages exposed to stressful stimuli, including gamma interferon treatment and viral infection, indicating that these proteins or portions thereof may be exposed on the surface of cells (76).

In the case of Lyme disease, which would not be too different from the mycobacterial model, highly conserved stress proteins from *B. burgdorferi* could act as immunogens or as autoimmunogens, or perhaps both. As is speculated in a recent article, the stress proteins of *B. burgdorferi* could have variable (species specific) regions which could induce a protective response if B and T cells recognized determinants within this region (33). On the other hand, if the immune response is directed towards conserved regions in the stress proteins, tissue destruction could occur. Clearly, this is a thought-provoking and plausible theory to explain chronic Lyme disease.

ANIMAL STUDIES

Several animal models have been developed to study experimentally induced Lyme disease. These include studies with rabbits, hamsters, rats, and mice. Thus far, these studies have produced valuable information regarding the infectivity of different strains of *B. burgdorferi* and the in vivo sensitivity of the organism to antibiotics. Also, passive immunization studies have provided some insight into vaccine development. Moreover, certain models, especially those using immunocompromised or neonatal animals, have demonstrated disease progression. A closer look at these models is warranted on the basis of the nature of the histopathological changes noted in the affected organs, notably the joints, and the need for immunosuppression or, as

in the case of the neonatal rat, an undeveloped immune system in order to precipitate the signs of disease.

Rabbits were the first animals used to establish laboratory infections with *B. burgdorferi*. These animals developed erythematous skin lesions, which resembled erythema migrans, following injection with the organisms (30). In general, however, these lesions were unlike the ones in humans in their morphology and the composition of the cellular infiltrate (30, 77). In the rabbit, the lesion was found to be indurated, of limited size, and infiltrated by neutrophils and macrophages, thus resembling an acute dermal inflammatory response rather than the expanding erythema migrans of humans with its mononuclear cell infiltrate (27). The rabbit has been used to infect ticks with spirochetes in the laboratory, indicating either a blood-borne phase or persistence within the skin (30). In general, however, there has been no specific disseminated disease in the rabbit following inoculation with *B. burgdorferi*.

The Syrian hamster proved to be the first laboratory animal which provided a reproducible model for the recovery of spirochetes from the blood and other organs following inoculation (71). Subsequent to intraperitoneal injection of a human CSF isolate of *B. burgdorferi*, the hamsters became spirochetemic within 24 h of inoculation, followed by a persistent disseminated infection (71). After hematogenous dissemination, spirochetes could be found in many organs, notably the spleen and kidneys, but also in the gonads, liver, brain, and eyes (70, 71). Spirochetes were also isolated intermittently from the blood, suggesting the spirochetemia, if not a constant phenomenon, can occur repeatedly during the infection period in these animals (70, 71). *B. burgdorferi* inoculated into the skin of hamsters also disseminated to other organs and persisted in the skin for relatively long periods of time. Recovery of the spirochetes from infected hamsters was shown to diminish with subculture of the organisms in vitro (71). This finding provided the first indication that subculture of the spirochetes results in changes which may be related to the virulence of the organism. Although this hamster model has proven to be quite useful in studies on antibiotic effectiveness and passive immunization, signs and symptoms of disease have not been documented. In this regard, *Peromyscus leucopus*, the natural reservoir for the spirochetes in nature, do not show signs of illness (43). In fact, the organ histopathology in these infected hamsters has shown only minimal changes limited to moderate nonspecific inflammation (44).

Articular manifestations have been induced in LSH hamsters after injection of *B. burgdorferi* (CSF strain) into the hind paws. These inflammatory changes were noted by histopathology (63, 105). Irradiated hamsters (600 rads of gamma radiation) inoculated in the same manner developed severely inflamed joints in the hind legs, resulting in erosive bone destruction (63, 105). Another model that used neonatal (3 day old) Lew/N rats has shown that an arthritis can develop after intraperitoneal inoculation with spirochete strain N/40 (12, 13). In addition to arthritis, these rats developed multiorgan invasion by spirochetes. The arthritis, which developed by day 14 after inoculation, appeared in multiple joints and was associated with the presence of the organism (12, 13). Killed organisms did not induce articular changes (12, 13), indicating that viable spirochetes are required for disease progression. This model offers additional insights into the role of the rat genotype in terms of disease manifestations. Studies have shown that both inbred and outbred rats can be infected with *B. burgdorferi* and develop arthritis (12, 13) and that the younger the rat at the time of inoculation, the more severe the articular manifestations. In this regard, the susceptibility of neonatal and weanling mice to *B. burgdorferi* infection has been found to be genotype dependent (11).

Recently, the mouse has been the subject of intensive study. Inoculations of *B. burgdorferi* (high passage) into several inbred mouse strains resulted in mild histopathological changes in various organs (99). A more severe arthritis was noted in severe combined immunodeficient (SCID) mice inoculated with low-passage strains of *B. burgdorferi* (100). These animals also developed evidence of carditis, a finding which had not been noted in the previous models. In the SCID mouse model, low-passage live spirochetes produced inflammatory changes in the joints and the heart which were not observed when the high-passage B31 isolate or UV-irradiated low-passage strain was used. Furthermore, normal C.B-17 mice (not immunodeficient) did not develop signs of disease (100).

The arthritis-producing models, i.e., irradiated hamsters, neonatal rats, and SCID mice, have one remarkable feature in common: of all the organ systems, the joints are most readily and obviously affected. This is a very significant finding in that it emphasizes the natural arthritogenic tendency of this organism, in line with one of the major manifestations of Lyme disease. Yet the histopathological features in these animal models are similar in some respects but differ in other ways from the features of the disease in human joints.

It has been demonstrated that in both the irradiated hamster and the neonatal rat, objective swelling of joints developed with exudation of fibrin and infiltration of neutrophils into the periarticular areas and into the joint space. In the rat model, synovial epithelial hypertrophy with erosion followed by villous hypertrophy of the synovium with a mononuclear cell infiltrate has been described (12, 13, 86). The mastocytosis, lymphoid follicles, extensive fibrin deposition, and marked angiopathic changes seen in human Lyme arthritis were not present in any of the animal models (45, 72, 117). The SCID mouse model shows a prominent cellular infiltrate composed primarily of macrophages (100). This model is also unique in that significant pathology is noted in other organs, indicating that this immunodeficiency permits the invasion of most organ systems (100).

Normal Syrian hamsters passively immunized with anti-*B. burgdorferi* antiserum raised in rabbits or in hamsters failed to be infected following challenge; that is, spirochetes could not be recovered from the organs of the animals (68). In contrast, if the antiserum was administered after inoculation of the spirochetes, the hamsters developed disseminated infection (68). Protection by passive immunization was shown to be strain specific, since antiserum directed against one strain of *B. burgdorferi* did not prevent spirochete infection caused by a challenge strain from a different geographic location (70). Passive immunization was also achieved in irradiated LSH hamsters (104). In this study, irradiated hamsters were given immune serum intravenously and then challenged with live *B. burgdorferi*. Passively immunized hamsters did not develop gross histopathological arthritis, and their organs did not yield spirochetes (104). Likewise, active immunization with killed or lyophilized organisms prior to challenge with viable *B. burgdorferi* prevented colonization by the spirochetes (69).

A recent report demonstrated that a murine MAb raised against OspA, given at the time of spirochetal challenge, can protect SCID mice from developing arthritis and carditis (98). This MAb but not others directed to the flagellin of *B.*

burgdorferi was able to mitigate the severity of the disease and inhibit the colonization of tissues in these animals. As in the normal hamster, passive immunization of SCID mice after challenge with spirochetes resulted in loss of the protective effect.

It has been shown that mice and other laboratory rodents developed antibodies to OspA and OspB very soon after a single inoculation with intact, viable *B. burgdorferi* (20). This rapid and well-defined B-cell response is in contrast to the response of human patients, who generally develop antibodies to OspA and OspB late in disease if at all (6, 35, 38). Thus, it is not surprising that either immune serum or an MAb to OspA could provide passive protection in laboratory animals. The fact that humans and laboratory animals react to these antigens in fundamentally different manners (i.e., OspA is an immunodominant antigen in rodents but not in humans) indicates that OspA may not be an appropriate vaccine candidate for humans. If antigen processing and presentation of OspA and OspB in humans were rapid, perhaps this infection would be shorter lived.

Do these models truly represent Lyme disease? The objective clinical observations are reminiscent but do not entirely recreate the features of human disease. Collectively, these studies demonstrate the tropism of *B. burgdorferi* for joint tissue. In this regard, these studies are major breakthroughs not only for Lyme disease, but also for the field of infection-associated articular diseases. On the other hand, the need to immunosuppress these animals in order to produce disease remains a feature which is contrary to the natural history of this disease in humans. These disease models, including the use of SCID, neonatal, and irradiated animals, indicate that an effective immune response needs to be suppressed in order to permit colonization and development of clinical manifestations. Given that Lyme disease affects thousands of people, it is unlikely that preexisting immunological disorders are a significant risk factor. The differences among the various animal models and human infection may reflect nothing more complex than different levels of innate resistance. In this regard, it is noteworthy that arthritis develops in humans possessing the HLA-DR4 phenotype (118).

SUMMARY

The chronic inflammatory condition that develops after infection by *B. burgdorferi* is a complex process resulting from host responses to a limited number of organisms. Amplification mechanisms driven by potent proinflammatory molecules, i.e., IL-1, may explain the vigorous response to a paucity of organisms. Spirochete dissemination to distant locations involves adherence to and penetration across endothelium and may be facilitated by host responses that increase vessel permeability. The apparent lack of tissue tropism in Lyme disease is reflected in the organism's ability to adhere to different eucaryotic cell types in vitro and the wide distribution of *B. burgdorferi* in various organs of infected humans and experimentally infected animals. While phagocytosis and complement activation have been observed in vitro, the specific immune response that develops in humans is inefficient in eradicating the organisms, which may possess some mechanism(s) to evade this response. There is significant evidence for host autoreactivity based on antigenic cross-reactivity between the 41-kDa flagellar subunit and stress proteins of the spirochetes and endogenous host cell components. Although the outer surface proteins appear to be suitable candidates as targets for vaccination in animal studies, fundamental differences in the immune response to spirochetal components may preclude their use in humans.

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REFERENCES

1. **Aberer, E., C. Brunner, G. Suchanek, H. Klade, A. Barbour, G. Stanek, and H. Lassmann.** 1989. Molecular mimicry and Lyme borreliosis: shared antigenic determinant between *Borrelia burgdorferi* and human tissue. Ann. Neurol. **26**:732–737.
2. **Asbrink, E., B. Hederstedt, and A. Hovmark.** 1984. The spirochetal etiology of acrodermititis chronica atrophicans Herxheimer. Acta Dermato-Venereol. **64**:506–512.
3. **Asbrink, E., and A. Hovmark.** 1985. Successful cultivation of spirochetes from skin lesions of patients with erythema chronicum migrans Afzelius and acrodermitits chronica atrophicans. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **93**:161–163.
4. **Banfi, E., M. Cinco, S. Perticarari, and G. Presani.** 1989. Rapid flow cytometric studies of *Borrelia burgdorferi* phagocytosis by human polymorphonuclear leukocytes. J. Appl. Bacteriol. **67**:37–45.
5. **Barbour, A. G.** 1988. Laboratory aspects of Lyme borreliosis. Clin. Microbiol. Rev. **1**:399–414.
6. **Barbour, A. G., W. Burgdorfer, E. Grunwaldt, and A. C. Steere.** 1983. Antibodies of patients with Lyme disease to components of the *Ixodes dammini* spirochete. J. Clin. Invest. **72**:504–515.
7. **Barbour, A. G., S. F. Hayes, R. A. Heiland, M. E. Schrumpf, and S. L. Tessier.** 1986. *Borrelia* genus-specific monoclonal antibody binds to a flagellar epitope. Infect. Immun. **52**:549–554.
8. **Barbour, A. G., S. L. Tessier, and S. F. Hayes.** 1984. Variation in a major surface protein of Lyme disease spirochetes. Infect. Immun. **54**:94–100.
9. **Barbour, A. G., S. L. Tessier, and H. G. Stoenner.** 1982. Variable major proteins of *Borrelia hermsii*. J. Exp. Med. **156**:1312–1324.
10. **Barbour, A. G., S. L. Tessier, and W. J. Todd.** 1983. Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. Infect. Immun. **41**:795–804.
11. **Barthold, S. W., D. S. Beck, G. M. Hansen, G. A. Terwilliger, and K. D. Moody.** 1990. Lyme borreliosis in selected strains and ages of laboratory mice. J. Infect. Dis. **162**:133–138.
12. **Barthold, S. W., K. D. Moody, G. A. Terwilliger, P. H. Duray, R. O. Jacoby, and A. C. Steere.** 1988. Experimental Lyme arthritis in rats infected with *Borrelia burgdorferi*. J. Infect. Dis. **157**:842–846.
13. **Barthold, S. W., K. D. Moody, G. A. Terwilliger, R. O. Jacoby, and A. C. Steere.** 1988. An animal model for Lyme arthritis. Ann. N.Y. Acad. Sci. **539**:264–273.
14. **Beck, G., J. L. Benach, and G. S. Habicht.** 1989. Isolation of interleukin-1 from joint fluids of patients with Lyme disease. J. Rheumatol. **16**:800–806.
15. **Beck, G., and G. S. Habicht.** 1988. Isolation and biological activity of *Borrelia burgdorferi* peptidoglycan. Ann. N.Y. Acad. Sci. **539**:365–366.
16. **Beck, G., G. S. Habicht, J. L. Benach, and J. L. Coleman.** 1985. Chemical and biologic characterization of a lipopolysaccharide from the Lyme disease spirochete (*Borrelia burgdorferi*). J. Infect. Dis. **152**:108–117.
17. **Beck, G., G. S. Habicht, J. L. Benach, and F. Miller.** 1984. Interleukin 1: a common endogenous mediator of inflammation and the local Shwartzman reaction. J. Immunol. **136**:3025–3031.
18. **Benach, J. L., and E. M. Bosler.** 1988. Lyme disease and

related disorders. Ann. N.Y. Acad. Sci. **539**:1–513.
19. **Benach, J. L., E. M. Bosler, J. P. Hanrahan, J. L. Coleman, G. S. Habicht, T. F. Bast, D. J. Cameron, J. L. Ziegler, A. G. Barbour, W. Burgdorfer, R. Edelman, and R. A. Kaslow.** 1983. Spirochetes isolated from the blood of two patients with Lyme disease. N. Engl. J. Med. **308**:740–742.
20. **Benach, J. L., J. L. Coleman, J. C. Garcia-Monco, and P. C. DePonte.** 1988. Biological activity of *Borrelia burgdorferi* antigens. Ann. N.Y. Acad. Sci. **539**:115–125.
21. **Benach, J. L., J. L. Coleman, and M. G. Golightly.** 1988. A murine monoclonal antibody binds an antigenic determinant in Osp-A, an immunodominant basic protein of the Lyme disease spirochete. J. Immunol. **140**:265–272.
22. **Benach, J. L., J. L. Coleman, R. A. Skinner, and E. M. Bosler.** 1987. Adult *Ixodes dammini* on rabbits: a hypothesis for the development and transmission of *Borrelia burgdorferi*. J. Infect. Dis. **155**:1300–1306.
23. **Benach, J. L., H. B. Fleit, G. S. Habicht, J. L. Coleman, E. Bosler, and B. P. Lane.** 1984. Interactions of phagocytes with the Lyme disease spirochete: role of the Fc receptor. J. Infect. Dis. **150**:497–507.
24. **Benach, J. L., B. L. Gruber, J. L. Coleman, G. S. Habicht, and M. G. Golightly.** 1986. An IgE response to spirochete antigen in patients with Lyme disease. Zentralb. Bakteriol. Mikrobiol. Hyg. **263**:160–168.
25. **Benach, J. L., G. S. Habicht, and J. L. Coleman.** 1984. Experimental transmission of the Lyme disease spirochete to rabbits. J. Infect. Dis. **150**:786–790.
26. **Benach, J. L., G. S. Habicht, B. L. Gocinski, and J. L. Coleman.** 1984. Phagocytic cell responses to *in vivo* and *in vitro* exposure to the Lyme disease spirochete. Yale J. Biol. Med. **57**:599–605.
27. **Berger, B. W., M. H. Kaplan, I. R. Rothenberg, and A. G. Barbour.** 1985. Isolation and characterization of the Lyme disease spirochete from the skin of patients with erythema chronicum migrans. J. Am. Acad. Dermatol. **13**:444–449.
28. **Bergstrom, S., V. G. Bundoc, and A. G. Barbour.** 1989. Molecular analysis of linear plasmid-encoded major surface proteins, OspA and OspB, of the Lyme disease spirochaete *Borrelia burgdorferi*. Mol. Microbiol. **3**:479–486.
29. **Brandt, M. E., B. S. Riley, J. D. Radolf, and M. V. Norgard.** 1990. Immunogenic integral membrane proteins of *Borrelia burgdorferi* are lipoproteins. Infect. Immun. **58**:983–991.
30. **Burgdorfer, W.** 1984. The New Zealand white rabbit: an experimental host for infecting ticks with Lyme disease spirochetes. Yale J. Biol. Med. **57**:609–612.
31. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davies.** 1982. Lyme disease—a tick borne spirochetosis? Science **216**:1317–1319.
32. **Burgdorfer, W., S. F. Hayes, and J. L. Benach.** 1988. Development of *Borrelia burgdorferi* in *Ixodid* tick vectors. Ann. N.Y. Acad. Sci. **539**:172–180.
33. **Carriero, M. M., D. C. Laux, and D. R. Nelson.** 1990. Characterization of the heat shock response and identification of heat shock protein antigens of *Borrelia burgdorferi*. Infect. Immun. **58**:2186–2191.
34. **Cluss, R. G., and J. T. Boothby.** 1990. Thermoregulation of protein synthesis in *Borrelia burgdorferi*. Infect. Immun. **58**: 1038–1042.
35. **Coleman, J. L., and J. L. Benach.** 1987. Isolation of antigenic components from the Lyme disease spirochete: their role in early diagnosis. J. Infect. Dis. **155**:756–765.
36. **Coleman, J. L., and J. L. Benach.** 1989. Identification and characterization of an endoflagellar antigen of *Borrelia burgdorferi*. J. Clin. Invest. **84**:322–331.
37. **Comstock, L. E., and D. D. Thomas.** 1989. Penetration of endothelial cell monolayers by *Borrelia burgdorferi*. Infect. Immun. **57**:1626–1628.
38. **Craft, J. E., D. K. Fischer, G. T. Shimamoto, and A. C. Steere.** 1986. Antigens of *Borrelia burgdorferi* recognized during Lyme disease. Appearance of a new immunoglobulin in response and expansion of the immunoglobulin G response late in the illness. J. Clin. Invest. **78**:934–939.
39. **Dattwyler, R. J., D. J. Volkman, J. J. Halperin, B. J. Luft, J. Thomas, and M. G. Golightly.** 1988. Specific immune responses in Lyme borreliosis. Ann. N.Y. Acad. Sci. **539**:93–102.
40. **Dattwyler, R. J., D. J. Volkman, B. J. Luft, J. J. Halperin, J. Thomas, and M. G. Golightly.** 1988. Seronegative Lyme disease. Dissociation of specific T- and B-lymphocyte responses to *Borrelia burgdorferi*. N. Engl. J. Med. **319**:1441–1446.
41. **Dinarello, C. A.** 1989. Interleukin-1 and its biologically related cytokines. Adv. Immunol. **44**:153–205.
42. **Doetsch, R. N., and R. D. Sjoblad.** 1980. Flagellar structure and function in eubacteria. Annu. Rev. Microbiol. **34**:69–108.
43. **Donahue, J. G., J. Piesman, and A. Spielman.** 1987. Reservoir competence of white footed mice for Lyme disease spirochetes. Am. J. Trop. Med. Hyg. **36**:92–96.
44. **Duray, P. H., and R. C. Johnson.** 1986. The histopathology of experimentally infected hamsters with the Lyme disease spirochete, *Borrelia burgdorferi*. Proc. Soc. Exp. Biol. Med. **181**:263–269.
45. **Duray, P. H., and A. C. Steere.** 1988. Clinical pathologic correlations of Lyme disease by stage. Ann. N.Y. Acad. Sci. **539**:65–79.
46. **Ellis, R. J.** 1987. Proteins as molecular chaperones. Nature (London) **328**:378–379.
46a. **Garcia-Monco, J. C.** 1990. Experimental approaches to the neuropathogenesis of Lyme disease. Abstract. Book A, p109. IV International Conference on Lyme Borreliosis, Stockholm, Sweden.
47. **Garcia-Monco, J. C., and J. L. Benach.** 1989. The pathogenesis of Lyme disease. Rheum. Clin. N. Am. **15**:711–726.
48. **Garcia-Monco, J. C., J. L. Coleman, and J. L. Benach.** 1988. Antibodies to myelin basic protein in Lyme disease patients. J. Infect. Dis. **158**:667–668.
49. **Garcia-Monco, J. C., B. Fernandez-Villar, J. C. Allen, and J. L. Benach.** 1990. *Borrelia burgdorferi* in the central nervous system: experimental and clinical evidence for early invasion. J. Infect. Dis. **161**:1187–1193.
50. **Garcia-Monco, J. C., B. Fernandez-Villar, and J. L. Benach.** 1989. Adherence of the Lyme disease spirochetes to glial cells and cells of glial origin. J. Infect. Dis. **160**:497–506.
51. **Garcia-Monco, J. C., H. M. Frey, B. Fernandez-Villar, M. G. Golightly, and J. L. Benach.** 1989. Lyme disease concurrent with HIV infection. Am. J. Med. **87**:325–328.
52. **Gassmann, G. S., M. Kramer, U. B. Globel, and R. Wallich.** 1989. Nucleotide sequence of a gene encoding the *Borrelia burgdorferi* flagellin. Nucleic Acids Res. **17**:3590.
53. **Gaston, J. S. H., P. F. Life, L. C. Bailey, and P. A. Bacon.** 1989. *In vitro* responses to a 65-kilodalton mycobacterial protein by synovial T cells from inflammatory arthritis patients. J. Immunol. **143**:2494–2500.
54. **Golightly, M. G., J. Thomas, D. Volkman, and R. J. Dattwyler.** 1988. Modulation of natural killer cell activity by *Borrelia burgdorferi*. Ann. N.Y. Acad. Sci. **539**:103–111.
55. **Habicht, G. S., G. Beck, and J. L. Benach.** 1988. The role of interleukin-1 in the pathogenesis of Lyme disease. Ann. N.Y. Acad. Sci. **539**:80–86.
56. **Habicht, G. S., G. Beck, J. L. Benach, J. L. Coleman, and K. D. Leichtling.** 1985. Lyme disease spirochetes induce human and murine IL-1 production. J. Immunol. **134**:3147–3154.
57. **Hansen, K., J. M. Bangsborg, H. Fjordvang, N. Strandberg-Pedersen, and P. Hindersson.** 1988. Immunological characterization of and isolation of the gene for a *Borrelia burgdorferi* immunodominant 60-kilodalton antigen common to a wide range of bacteria. Infect. Immun. **56**:2047–2053.
58. **Hansen, K., M. Cruz, and H. Link.** 1990. Oligoclonal *Borrelia burgdorferi*-specific IgG antibodies in cerebrospinal fluid in Lyme borreliosis. J. Infect. Dis. **161**:1194–1202.
59. **Hardin, J. A., A. C. Steere, and S. E. Malawista.** 1979. Immune complexes and the evolution of Lyme arthritis. N. Engl. J. Med. **301**:1358–1363.
60. **Hardin, J. A., L. C. Walker, A. C. Steere, T. C. Trumble, K. S. K. Tung, R. C. Williams, S. Ruddy, and S. E. Malawista.** 1979. Circulating immune complexes in Lyme arthritis. Detec-

tion by the ^{125}I-C1q binding, C1q solid phase and Raji cell assays. J. Clin. Invest. **63**:468–477.
61. **Hechemy, K. E., H. L. Harris, M. J. Duerr, J. L. Benach, and C. B. Reimer.** 1988. Immunoglobulin G subclasses specific to *Borrelia burgdorferi* in patients with Lyme disease. Ann. N.Y. Acad. Sci. **539**:162–170.
62. **Hechemy, K. E., W. A. Samsonoff, M. McKee, and J. M. Guttman.** 1989. *Borrelia burgdorferi* attachment to mammalian cells. J. Infect. Dis. **159**:805–806.
63. **Hejka, A., J. L. Schmitz, D. E. England, S. M. Callister, and R. F. Schell.** 1989. Histopathology of Lyme arthritis in LSH hamsters. Am. J. Pathol. **134**:1113–1123.
64. **Henriksson, A., H. Link, M. Cruz, and G. Stiernstedt.** 1986. Immunoglobulin abnormalities in cerebrospinal fluid and blood over the course of lymphocytic meningopolyradiculitis (Bannwarth's syndrome). Ann. Neurol. **20**:337–345.
65. **Holoshitz, J., Y. Naperstek, A. Ben-Nun, and I. R. Cohen.** 1983. Lines of T lymphocytes induce or vaccinate against autoimmune arthritis. Science **219**:56–58.
66. **Johnson, R. C.** 1989. Lyme disease. Rheumatic Dis. Clin. N. Am. **15**:627–825.
67. **Johnson, R. C., F. W. Hyde, G. P. Schmid, and D. J. Brenner.** 1984. *Borrelia burgdorferi* sp. nov.: etiologic agent of Lyme disease. Int. J. Syst. Bacteriol. **34**:496–497.
68. **Johnson, R. C., C. Kodner, and M. Russell.** 1986. Passive immunization of hamsters against experimental infection with *Borrelia burgdorferi*. Infect. Immun. **53**:713–714.
69. **Johnson, R. C., C. Kodner, and M. Russell.** 1986. Active immunization of hamsters against experimental infection with *Borrelia burgdorferi*. Infect. Immun. **54**:897–898.
70. **Johnson, R. C., C. Kodner, M. Russell, and P. H. Duray.** 1988. Experimental infection of the hamster with *Borrelia burgdorferi*. Ann. N.Y. Acad. Sci. **539**:258–263.
71. **Johnson, R. C., N. Marek, and C. Kodner.** 1984. Infection of Syrian hamsters with Lyme disease spirochetes. J. Clin. Microbiol. **20**:1099–1101.
72. **Johnston, Y. E., A. C. Steere, M. Kashgarian, J. Boza, S. E. Malawista, and P. E. Askenase.** 1985. Lyme arthritis: spirochetes found in synovial microangiopathic lesions. Am. J. Pathol. **188**:26–34.
73. **Joys, T.** 1985. The covalent structure of the phase 1 flagellar filament protein of *Salmonella typhimurium* and its comparison with other flagellins. J. Biol. Chem. **260**:15758–15761.
74. **Joys, T. M.** 1988. The flagellar filament protein. Can. J. Microbiol. **34**:452–458.
75. **Kochi, S. K., and R. C. Johnson.** 1988. Role of immunoglobulin G in killing of *Borrelia burgdorferi* by the classical complement pathway. Infect. Immun. **56**:314–321.
76. **Koga, T., A. Wand-Wurttenberger, J. De Bruyn, M. E. Munk, B. Scoel, and S. H. E. Kaufmann.** 1989. T cells against a bacterial heat shock protein recognize stressed macrophages. Science **245**:1112–1115.
77. **Kornblatt, A. N., A. C. Steere, and D. G. Brownstein.** 1984. Infection of rabbits with the Lyme disease spirochete. Yale J. Biol. Med. **57**:613–614.
78. **Krinsky, W. L., S. J. Brown, and P. W. Askenase.** 1982. *Ixodes dammini*: induced skin lesions in guinea pigs and rabbits compared to erythema chronicum migrans in patients with Lyme arthritis. Exp. Parasitol. **53**:381–395.
79. **Kujala, G. A., A. C. Steere, and J. S. Davis.** 1987. IgM rheumatoid factor in Lyme disease: correlation with disease activity, total serum IgM, and IgM antibody to *Borrelia burgdorferi*. J. Rheumatol. **14**:772–776.
80. **Kurtti, T. J., U. G. Munderloh, G. G. Ahlstrand, and R. C. Johnson.** 1988. *Borrelia burgdorferi* in tick cell culture: growth and cellular adherence. J. Med. Entomol. **25**:256–261.
81. **Lydyard, P. M., and W. van Eden.** 1990. Heat shock proteins: immunity and immunopathology. Immunol. Today **11**:228–229.
82. **Mackworth-Young, C. G., E. N. Harris, A. C. Steere, F. Rizvi, S. E. Malawista, G. R. V. Hughes, and A. E. Gharavi.** 1988. Anticardiolipin antibodies in Lyme disease. Arthritis Rheum. **31**:1052–1056.
83. **Martin, R., J. Ortlauf, V. Sticht-Groh, and U. Bogdahn.** 1988. *Borrelia burgdorferi* specific and autoreactive T cell lines from cerebrospinal fluid in Lyme radiculomyelitis. Ann. Neurol. **24**:509–516.
84. **Mensi, N., D. R. Webb, C. W. Turck, and G. A. Peltz.** 1990. Characterization of *Borrelia burgdorferi* proteins reactive with antibodies in synovial fluid of a patient with Lyme arthritis. Infect. Immun. **58**:2404–2407.
85. **Moffat, C. M., L. H. Sigal, A. C. Steere, D. A. Freeman, and J. M. Dwyer.** 1984. Cellular immune findings in Lyme disease: correlation with serum IgM and disease activity. Am. J. Med. **77**:625–632.
86. **Moody, K. D., S. W. Barthold, G. A. Terwilliger, D. S. Beck, G. M. Hansen, and R. O. Jacoby.** 1990. Experimental chronic Lyme borreliosis in Lewis rats. Am. J. Trop. Med. Hyg. **42**:165–174.
87. **Murray, N., W. Kristoferitsch, G. Stanek, and A. V. Steck.** 1986. Specificity of cerebrospinal fluid antibodies against components of *Borrelia burgdorferi* in patients with meningopolyneuritis Garin-Bujadoux-Bannwarth. J. Neurol. **233**:224–227.
88. **Neumann, A., M. Schlesier, H. Schneider, and H. H. Peter.** 1989. Frequencies of *Borrelia burgdorferi* reactive T lymphocytes in Lyme arthritis. Rheumatol. Int. **9**:237–241.
89. **Oldstone, M. B. A.** 1989. Molecular mimicry as a mechanism for the cause and as a probe uncovering etiologic agent(s) of autoimmune disease. Curr. Top. Microbiol. Immunol. **145**: 127–135.
90. **Pachner, A. R., A. C. Steere, L. H. Sigal, and C. J. Johnson.** 1985. Antigen-specific proliferation of cerebrospinal fluid lymphocytes in Lyme disease. Neurology **35**:1642–1644.
91. **Peterson, P. K., C. C. Clawson, D. A. Lee, D. J. Garlich, P. G. Quie, and R. C. Johnson.** 1984. Human phagocyte interactions with the Lyme disease spirochete. Infect. Immun. **46**:608–611.
92. **Pfister, H. W., U. Neubert, B. Wilske, V. Preac-Mursic, K. M. Einhaupl, and G. D. Borasio.** 1986. Reinfection with *Borrelia burgdorferi*. Lancet **ii**:984–985.
93. **Piesman, J., T. N. Mather, R. J. Sinsky, and A. Spielman.** 1987. Duration of tick attachment and *Borrelia burgdorferi* transmission. J. Clin. Microbiol. **25**:557–558.
94. **Preac-Mursic, V., B. Wilske, G. Schierz, H. W. Pfister, and K. Einhaupl.** 1984. Repeated isolation of spirochetes from the cerebrospinal fluid of a patient with meningoradiculitis Bannwarth. Eur. J. Microbiol. **3**:564–565.
95. **Res, P. C. M., C. G. Schaar, F. C. Breedveld, W. van Eden, J. D. A. van Embden, I. R. Cohen, and R. R. P. DeVries.** 1988. Synovial fluid T cell reactivity against 65 kD heat shock protein of mycobacteria in early chronic arthritis. Lancet **ii**:478–480.
96. **Ribeiro, J. M., T. N. Mather, J. Piesman, and A. Spielman.** 1987. Dissemination and salivary delivery of Lyme disease spirochetes in vector ticks (Acari: Ixodes). J. Med. Entomol. **24**:201–205.
97. **Ryberg, B., B. Hindfelt, B. Nilsson, and J. E. Olsson.** 1984. Antineural antibodies in Guillain-Barre syndrome and lymphocytic meningoradiculitis (Bannwarth's syndrome). Arch. Neurol. **41**:1277–1281.
98. **Schaible, U. E., M. D. Kramer, K. Eichmann, M. Modolell, C. Museteanu, and M. M. Simon.** 1990. Monoclonal antibodies specific for the outer surface protein A (OspA) of *Borrelia burgdorferi* prevent Lyme borreliosis in severe combined immunodeficiency (scid) mice. Proc. Natl. Acad. Sci. USA **87**:3768–3772.
99. **Schaible, U. E., M. D. Kramer, C. W. E. Justus, C. Museteanu, and M. M. Simon.** 1988. Demonstration of antigen-specific T cells and histopathological alterations in mice experimentally inoculated with *Borrelia burgdorferi*. Infect. Immun. **57**:41–47.
100. **Schaible, U. E., M. D. Kramer, C. Museteanu, G. Zimmer, H. Mossmann, and M. M. Simon.** 1989. The severe combined immunodeficiency (scid) mouse: a laboratory model for the analysis of Lyme arthritis and carditis. J. Exp. Med. **170**:1427–1432.
101. **Scheider, H., A. Vogt, and K. Pelz.** 1988. Lymphocyte proliferation and identification of the responding subpopulation after *in vitro* stimulation with *Borrelia burgdorferi*. Zentralb. Bakteriol. Mikrobiol. Hyg. A **269**:542.

102. **Schlesinger, M. J.** 1990. Heat shock proteins. J. Biol. Chem. **265**:12111–12114.
103. **Schmidli, J., T. Hunziker, P. Moesli, and U. B. Schaad.** 1988. Cultivation of *Borrelia burgdorferi* from joint fluid three months after treatment of facial palsy due to Lyme borreliosis. J. Infect. Dis. **158**:905–906.
104. **Schmitz, J. L., R. F. Schell, A. G. Hejka, and D. M. England.** 1990. Passive immunization prevents induction of Lyme arthritis in LSH hamsters. Infect. Immun. **58**:144–148.
105. **Schmitz, J. L., R. F. Schell, A. Hejka, D. M. England, and L. Konick.** 1988. Induction of Lyme arthritis in LSH hamsters. Infect. Immun. **56**:2336–2342.
106. **Schutzer, S. E., P. K. Coyle, A. L. Belman, M. G. Golightly, and J. Drulle.** 1990. Sequestration of antibody to *Borrelia burgdorferi* in immune complexes in seronegative Lyme disease. Lancet **335**:312–315.
107. **Schwan, T., W. Burgdorfer, and C. F. Garon.** 1988. Changes in infectivity and plasmid profile of the Lyme disease spirochete, *Borrelia burgdorferi*, as a result of in vitro cultivation. Infect. Immun. **56**:1831–1836.
108. **Shinnick, T. M., M. H. Vodkin, and J. L. Williams.** 1988. The *Mycobacterium tuberculosis* 65-kilodalton protein is a heat shock protein which corresponds to common antigen and to the *Escherichia coli* GroEL protein. Infect. Immun. **56**:446–451.
109. **Sigal, L. H., A. C. Steere, and J. M. Dwyer.** 1988. *In vivo* and *in vitro* evidence of B cell hyperactivity during Lyme disease. J. Rheumatol. **15**:648–654.
110. **Sigal, L. H., A. C. Steere, D. H. Freeman, and J. M. Dwyer.** 1986. Proliferation responses of mononuclear cells in Lyme disease. Reactivity to *Borrelia burgdorferi* antigens is greater in joint fluid than in blood. Arthritis Rheum. **29**:761–769.
111. **Sigal, L. H., and A. H. Tatum.** 1988. Lyme disease patients' serum contains IgM antibodies to *Borrelia burgdorferi* that cross react with neuronal antigens. Neurology **38**:1439–1442.
112. **Srinivasappa, J., J. Saegusa, B. S. Prabhakar, M. K. Gentry, M. J. Buchmeier, T. J. Wiktor, H. Koprowski, M. B. A. Oldstone, and A. L. Notkins.** 1986. Molecular mimicry: frequency of reactivity of monoclonal antiviral antibodies with normal tissues. J. Virol. **57**:397–401.
113. **Stanek, G., H. Flamm, A. G. Barbour, and W. Burgdorfer.** 1987. Lyme borreliosis. Gustar Fischer Verlag, Stuttgart, Federal Republic of Germany.
114. **Steere, A. C.** 1989. Lyme disease. N. Engl. J. Med. **321**:586–596.
115. **Steere, A. C., V. P. Berardi, K. E. Weeks, E. L. Logigian, and R. Ackermann.** 1990. Evaluation of the intrathecal antibody response to *Borrelia burgdorferi* as a diagnostic test for Lyme neuroborreliosis. J. Infect. Dis. **161**:1203–1209.
116. **Steere, A. C., C. E. Brinkerhoff, D. J. Miller, H. Drinker, E. D. Harris, and S. E. Malawista.** 1980. Elevated levels of collagenase and prostaglandin E_2 from synovium associated with erosion of cartilage and bone in a patient with chronic Lyme arthritis. Arthritis Rheum. **23**:591–599.
117. **Steere, A. C., P. H. Duray, and E. C. Butcher.** 1988. Spirochetal antigens and lymphoid cell surface markers in Lyme synovitis. Comparison with rheumatoid synovium and tonsillar lymphoid tissue. Arthritis Rheum. **31**:487–495.
118. **Steere, A. C., E. Dwyer, and R. Wincester.** 1990. Association of chronic Lyme arthritis with HLA-DR4 and HLA-DR2 alleles. N. Engl. J. Med. **323**:219–223.
119. **Steere, A. C., R. L. Grodzicki, A. N. Kornblatt, J. E. Craft, A. G. Barbour, W. Burgdorfer, G. P. Schmid, E. Johnson, and S. E. Malawista.** 1983. The spirochetal etiology of Lyme disease. N. Engl. J. Med. **308**:733–740.
120. **Steere, A. C., J. A. Hardin, S. Ruddy, J. G. Mummaw, and S. E. Malawista.** 1979. Lyme arthritis. Correlation of serum and cryoglobulin IgM with activity and serum IgG with remission. Arthritis Rheum. **22**:471–483.
121. **Steere, A. C., S. E. Malawista, J. E. Craft, D. K. Fischer, and M. Garcia-Blanco.** 1984. Lyme disease: first international symposium. Y. J. Biol. Med.
122. **Steere, A. C., S. E. Malawista, J. A. Hardin, S. Ruddy, P. W. Askenase, and W. A. Andiman.** 1977. Erythema chronicum migrans and Lyme arthritis. The enlarging clinical spectrum. Ann. Intern. Med. **86**:685–698.
123. **Steere, A. C., S. E. Malawista, D. R. Snydman, R. E. Shope, W. A. Andiman, M. R. Rosi, and F. M. Steele.** 1977. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum. **20**:7–17.
124. **Suchanek, G., W. Kristoferitsch, G. Stanek, and H. Bernheimer.** 1986. Antimyelin antibodies in cerebrospinal fluid and serum of patients with meningopolyneuritis Garin-Bujadoux-Bannwarth and other neurological disorders. Zentralbl. Bakteriol. Mikrobiol. Hyg. **263**:160–168.

124a. **Szczepanski, A.** 1989. Ph.D. dissertation, State University of New York, Stony Brook.

125. **Szczepanski, A., and H. B. Fleit.** 1988. Interaction between *Borrelia burgdorferi* and polymorphonuclear leukocytes. Ann. N.Y. Acad. Sci. **539**:425–428.
126. **Szczepanski, A., M. B. Furie, J. L. Benach, B. P. Lane, and H. B. Fleit.** 1990. Interaction between *Borrelia burgdorferi* and endothelium *in vitro*. J. Clin. Invest. **85**:1637–1647.
127. **Takayama, K., R. J. Rothenberg, and A. G. Barbour.** 1987. Absence of lipopolysaccharide in the Lyme disease spirochete, *Borrelia burgdorferi*. Infect. Immun. **55**:2311–2313.
128. **Thomas, D. D., and L. E. Comstock.** 1989. Interaction of Lyme disease spirochetes with cultured eucaryotic cells. Infect. Immun. **57**:1324–1326.
129. **Thomas, D. D., and L. M. Higbie.** 1990. In vitro association of leptospires with host cells. Infect. Immun. **58**:581–585.
130. **Thomas, D. D., M. Navab, D. A. Haake, A. M. Fogelman, J. N. Miller, and M. A. Lovett.** 1988. *Treponema pallidum* invades intercellular junctions of endothelial cell monolayers. Proc. Natl. Acad. Sci. USA **85**:3608–3612.
131. **Van Eden, W., J. Holoshitz, Z. Nevo, A. Frenkel, A. Klajman, and I. R. Cohen.** 1985. Arthritis induced by a T-lymphocyte clone that responds to *Mycobacterium tuberculosis* and to cartilage proteoglycans. Proc. Natl. Acad. Sci. USA **82**:5117–5120.
132. **Van Eden, W., J. E. R. Thole, R. van der Zee, A. Noordzij, J. D. A. van Embden, E. J. Hensen, and I. R. Cohen.** 1988. Cloning of the mycobacterial epitope recognised by T lymphocytes in adjuvant arthritis. Nature (London) **331**:171–173.
133. **Weyand, C. M., and J. J. Goronzy.** 1989. Immune responses to *Borrelia burgdorferi* in patients with reactive arthritis. Arthritis Rheum. **32**:1057–1064.
134. **Wheeler, C. M., J. L. Coleman, G. S. Habicht, and J. L. Benach.** 1989. Adult *Ixodes dammini* on rabbits: development of acute inflammation in the skin and immune response to salivary gland, midgut, and spirochetal components. J. Infect. Dis. **159**:265–273.
135. **Wilske, B., G. Schierz, V. Preac-Mursic, K. von Busche, R. Kuhbeck, H. W. Pfister, and K. Einhaupl.** 1986. Intrathecal production of specific antibodies against *Borrelia burgdorferi* in patients with lymphocytic meningoradiculitis (Bannwarth's syndrome). J. Infect. Dis. **153**:304–314.
136. **Young, R. A.** 1990. Stress proteins and immunology. Annu. Rev. Immunol. **8**:401–420.
137. **Young, R. A., and T. J. Elliot.** 1989. Stress proteins, infection and immune surveillance. Cell **59**:5–8.
138. **Zung, J. L., S. Lewengrub, M. A. Rudzinska, A. Spielman, S. R. Telford, and J. Piesman.** 1989. Fine structural evidence for the penetration of the Lyme disease spirochete *Borrelia burgdorferi* through the gut and salivary tissues of *Ixodes dammini*. Can. J. Zool. **67**:1737–1748.