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Why Is Chronic Lyme Borreliosis Chronic?

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Chronic Lyme borreliosis (CLB) can present not only in different organs but also in different patterns. Although many theories exist about the mechanisms leading to CLB, it is known that viable *Borrelia burgdorferi* can persist for decades and cause late skin manifestations of acrodermatitis chronica atrophicans (ACA). Thus, the immunopathogenetic findings in ACA can serve as a model for studying the chronic course of Lyme borreliosis. Recent findings indicate that the most important cell for antigen presentation, the epidermal Langerhans cell (LC), is invaded by *B. burgdorferi* in early Lyme borreliosis. Therefore, LCs were stained immunohistochemically with different markers to investigate their functional activity. Numbers of CD1a$^+$ LCs were reduced in erythema migrans but normal or slightly elevated in ACA. In both diseases there was also a marked downregulation of major histocompatibility complex class II molecules on LCs, as measured by staining of human leukocyte antigen DR. This phenomenon might be a mechanism that protects against the presentation of autoantigens and may be the cause of the impaired capacity of LCs to eliminate *B. burgdorferi* antigens, thus explaining why CLB is chronic.

Lyme borreliosis is a multisystem inflammatory disease caused by *Borrelia burgdorferi*. Patients treated adequately with antibiotics in the early phase of infection show a good clinical response. However, the time of infection and mode of development of various organ manifestations often cannot be followed. Moreover, it is evident that the type of *Borrelia* strain and the immunosusceptibility of the host influence the expansion of clinical symptoms, which may explain differences between the manifestations of chronic Lyme borreliosis (CLB) seen in patients in the United States and Europe.

Several recent articles have described the symptoms of CLB [1–7]. Studies of CLB in the rhesus monkey model have revealed characteristic clinical, histopathologic, and laboratory findings [3]. Although not yet reported to occur in animal models, acrodermatitis chronica atrophicans (ACA) of the skin, caused by *Borrelia afzelii*, a subtype of *B. burgdorferi* sensu lato, develops gradually. Therefore, ACA may be a good model for studying the immunopathogenic events that lead to chronic disease.

Histopathologic and Immunohistologic Findings in the Skin

The first stage of Lyme borreliosis, erythema migrans, is a self-limited dermatosis. Nevertheless, the erythema can either persist and gradually progress into ACA over a period of months to years or can disappear. However, *Borrelia* organisms can survive in the skin, leading to chronic inflammation and thus causing ACA, the final stage of CLB.

At first, the cellular immune response seen locally in erythema migrans is mainly composed of CD3$^+$ T cells, with a predominance of CD4$^+$ helper/inducer cells, 30%–40% CD8$^+$ cytotoxic/suppressor T cells, and numerous macrophages [8]. The composition of this mononuclear infiltrate persists into the ACA stage. The percentage of CD4$^+$ T lymphocytes, however, increases up to 90% [8–10]. Although the presence of many plasma cells is histologically pathognomonic, the predominance of CD22$^+$/CD20$^+$ B cells varies [9, 10].

But what happens as a consequence of this active inflammation? Studies of persistent erythema migrans lesions have shown that the mononuclear cellular elements cluster around vessel walls. These findings, which can be compared with histologic changes in CLB as described with regard to various organs, are suggestive of an angiopathy accompanied by lymphocytic infiltrate [3, 11]. Moreover, an interstitial myositis of the smooth arrector muscles of hair develops, and these gradually become atrophic in ACA [12]. Immunohistochemical studies have shown that in ACA, both the hair muscles and the muscle of vessel walls can no longer be stained with antibodies against smooth muscle actin [12].

Deposition of immunocomplexes in dermal vessel walls as well as granular IgM or C3 deposits can sometimes be seen at the dermoepidermal junction [10]. The inflammatory infiltrate is also arranged interstitially between connective tissue fibers. Ultrastructurally, numerous macrophages are seen to engulf degenerated elastic and collagenous tissue, leading in turn to the degeneration of connective tissue [13, 14]. The basal kera-

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tinocytes show vacuolization, as also seen in lichen planus or graft-versus-host disease of the skin [10, 13, 14].

The end stage of ACA is atrophy of epidermis, collagen, and elastic fibers. In half of the cases, ACA is associated with neuropathy. Perineural lymphocytic infiltrates severe enough to obliterate blood vessels, destroy myelinated fibers, and thus lead to axonal nerve degeneration are typical of this late manifestation [15].

Immune Response in ACA

Patients with ACA mount an immune response but cannot eliminate the pathogen *B. burgdorferi*. In the sera of such patients, high titers of IgG antibody to *B. burgdorferi* can still be detected years after treatment [16, 17]. Although most of these antibodies are of the IgG1 isotype, there is a distinct IgG2 and IgG3 component [18]. In a minority of patients, IgM antibodies can also be detected by ELISA [16], although these findings are mostly associated with a positive rheumatoid factor. On the other hand, polyclonal hyperimmunoglobulinemia, mostly of the IgM isotype, is often seen in ACA [19].

The cellular immune response of peripheral blood lymphocytes seen in CLB [20] is not regularly found in ACA. For example, in lymphocyte proliferation tests performed by Breier et al. in 19 ACA patients, only 3 patients exhibited a marked stimulation index against viable *B. burgdorferi* subspecies *garinii* organisms, while 4 patients showed no stimulation at all [21]. In contrast, Büchner et al. reported positive lymphocyte transformation tests in most ACA patients when sonicated *B. burgdorferi* sensu stricto was used as antigen [22]. A similar test using *B. afzelii* as antigen has, to the best of our knowledge, not yet been performed. Since this strain has been isolated from patients with ACA, the highest proliferation rates could be expected if the latter strain were used as antigen.

The Role of Epidermal Langerhans Cells

Antigen-presenting cells play a central role in immune defense mechanisms. One particular type of antigen-presenting cell, epidermal dendritic Langerhans cells (LCs), have been studied in several chronic infectious diseases, including syphilis, leprosy, leishmaniasis, and HIV infection [23–26]. Ultrastructural studies of skin biopsy specimens from patients with erythema migrans have shown that *B. burgdorferi* selectively invades and destroys LCs [27].

Moreover, recently performed in vitro studies proved that skin- and blood-derived dendritic cells are also able to efficiently engulf *B. burgdorferi* (Figueira L., Nestle F., Rittig M., Groscurth P. Phagocytosis and antigen-processing of *B. burgdorferi* by human dendritic cells. Presented at the 4th International Workshop on Langerhans Cells [Venice]. October 1996, p. 132). Although intracellular survival of *B. burgdorferi* in LCs has not yet been described, the parasitism of LCs in the early stage of borreliosis suggests that their function might be altered in Lyme borreliosis.

In a recent study, skin biopsy specimens from seven patients with erythema migrans and 19 with ACA were investigated immunohistochemically for their expression of CD1a—the most reliable antibody to identify LCs [28]—human major histocompatibility complex (MHC) human leukocyte antigen (HLA) subregions HLA-DR, -DQ, and -DP, and several other different leukocyte differentiation antigens. These studies were performed in order to determine whether the number or function of epidermal LCs was altered (M. Silberer, F. Koszik, E. Aberer, manuscript in preparation).

Semiautomatic image analysis of CD1a staining revealed a decreased density of epidermal LCs in erythema migrans sections (598 $\pm$ 240 per mm^2 of epidermis), vs. a normal or even higher density in ACA sections (835 $\pm$ 317/mm^2) (figure 1), as compared with their density in normal skin (460–1,000/mm^2 [29]).

More important, MHC class II molecule expression, as evaluated by HLA-DR expression, was considerably downregulated in erythema migrans and ACA (by 28% and 18%, respectively). In contrast, CD1a and HLA-DR were equally expressed in normal skin [29]. In addition, HLA-DP and -DQ molecules were downregulated on epidermal dendritic cells.

Bacterial Elimination and Survival

Recent experiments have shed light on the elimination and survival of borreliae. On the one hand, in vitro experiments have shown that borreliae are internalized by macrophages, catabolized in endosomes, loaded with MHC class II molecules, and then presented to CD4$^+$ cells [30]. On the other hand, uptake of borreliae by coiling phagocytosis has also been observed; the borreliae enter the cytosol and join the MHC class I pathway involving CD8$^+$ suppressor/cytotoxic cells for lysis.

Since the majority of infiltrating cells in the skin biopsies we investigated were of the CD4 helper/inducer type and since a high number of CD68$^+$ macrophages in those samples also expressed HLA-DR, we think that the MHC class II pathway rather than the class I pathway is involved in immune defense mechanisms in ACA (Silberer M., Koszik F., Aberer E., manuscript in preparation).

Immunohistochemical staining of ACA skin biopsy specimens with a monoclonal antibody to flagellin has shown that ACA-affected skin harbors several forms of borreliae. Heavily stained, clumped, intertwined forms and granular *Borrelia* structures among collagen fibers (figure 2) are also seen to form after incubation with antibodies to *B. burgdorferi* in vitro, and delicate dispersed forms are found lying in degenerating collagen fibers [31]. The existence of these forms has been confirmed ultrastructurally [27].

Previous studies have shown that ACA can be successfully treated with oral antibiotics given for 30 days [19]; the skin

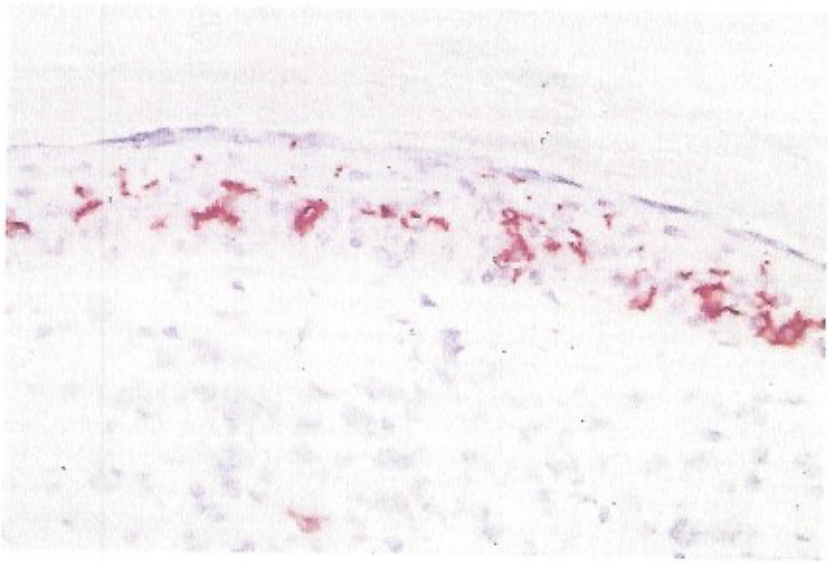

Figure 1. CD1a$^+$ epidermal Langerhans cells in a patient with acrodermatitis chronica atrophicans (ACA). The cells were visualized by immunohistochemical staining with anti-OKT-6 antibody and 2-amino-ethylcarbazole (original magnification, × 40).

lesions regress slowly over the next 6–12 months. Repeated biopsies of ACA-affected skin from two patients treated with ceftriaxone for 15 days showed that one patient still had perivascular lymphoplasmocytic infiltrates 1 year later, although no *Borrelia* DNA could be detected in the urine with a nested PCR method and flagellin primers [19].

In patients treated orally for only 20 days or intravenously for 15 days, either skin involvement or associated symptoms persisted. In 10 patients with ACA treated with a 30-day course of oral antibiotics, no *Borrelia* DNA fragments could be detected in the urine after 6 months, and all patients were free of further clinical symptoms at 12 months. In still other studies, PCR analysis of biopsy specimens of previous skin lesions at a median of 1.5 years after treatment showed no sign of *Borrelia* DNA [32].

Yet, the question still remains: where do *Borrelia* organisms survive in patients with CLB? An inadequate immune response and an evasion of bacteria into immunologically privileged

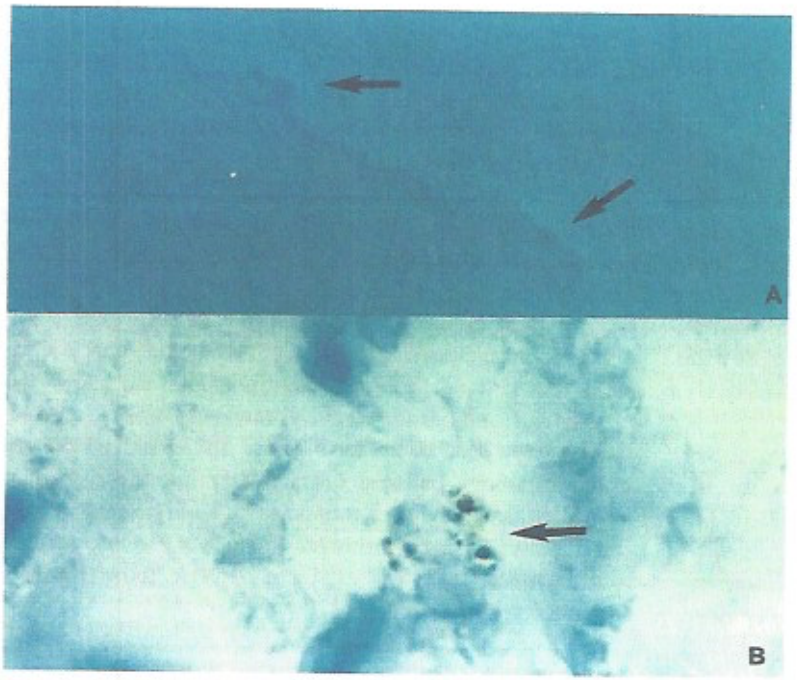

Figure 2. *A*, Culture of *B. burgdorferi* sensu stricto incubated with serum from a patient with ACA for 24 hours. Numerous granules (*arrows*) developed along the axis of borreliae (darkfield microscopy; original magnification, × 2,000). *B*, Granules of different size (*arrow*) among the inflammatory infiltrate in ACA were visualized by immunoperoxidase staining with H9724 flagellar monoclonal antibody and 2-amino-ethylcarbazole (original magnification, × 2,000).

sites may be the cause for microbial persistence. Recently, resistance to complement-mediated killing was reported following the study of an in vitro model in which borreliae were cultured in rat joint tissue [33]. Moreover, survival of borreliae via targeting with fibronectin has been reported [33].

B. burgdorferi has been isolated from skin affected by ACA for >10 years [34], from joint fluids in a patient treated for facial palsy [35], from the myocardium of another patient [36], and from joint tissue of a rhesus monkey 6 months after infection [3]. Numerous researchers have detected *Borrelia* DNA in synovial fluid, CSF, urine, skin, and joint tissue from patients with chronic disease [36–39]. Ultrastructurally, *B. burgdorferi* has also been detected in ligamentous tissue [5].

The intracellular survival of borreliae in mouse macrophages has been suggested by Montgomery et al. [40]. In rhesus monkeys, staining deposits by two monoclonal antibodies to *B. burgdorferi* (a 7.5-kD lipoprotein and OspA LA-31) were detected in macrophages in infected but not control animals, by means of an immunoperoxidase method [3]. In our own immunohistochemical studies using the flagellar H9724 monoclonal antibody, some macrophages stained positively for borreliae, not only in skin biopsy specimens from patients with ACA but also in control biopsy specimens from healthy individuals [31]. These findings were interpreted as being due to cross-reactive epitopes of the monoclonal flagellar antibody and several human tissue components [41].

Discussion

It is not yet established whether viable *B. burgdorferi* organisms are necessary to maintain the inflammatory response seen in CLB. Intracellular survival of *B. burgdorferi* in antigen-presenting cells, although not yet described as occurring in Lyme borreliosis, can be possible only when antigens are not presented appropriately. Besides macrophages and B-lymphocytes, dendritic cells, especially LCs, are capable of functioning as antigen-presenting cells and thus are responsible for the induction of primary antigen-specific immune reactions [42–44]. Later on, other professional and nonprofessional antigen-presenting cells are able to stimulate T lymphocytes.

Parasitism of LCs has been described in association with several infectious diseases, such as leprosy, leishmaniasis, and AIDS [24, 42, 45]. For example, in leprosy, dermal macrophages and LCs are infected with *Mycobacterium leprae* [24]. These parasitized cells then express weaker MHC class II molecules than do uninfected cells. In addition, the vast majority of epidermal LCs, although not infected and evenly distributed, do not stain with HLA-DR molecules [24]. In lepromatous leprosy, in which macrophages are not able to kill *M. leprae,* there is a lack of T cell–derived lymphokines such as IFN-γ and IL-2 [46]. In fact, injected IL-2 can modify the immune response in leprosy through a cell-mediated pathway that results in a reduced bacterial load and a T-helper type 1 (Th1) cell response. After IL-2 administration, which goes hand in hand with granuloma formation, LCs become prominent in the dermis.

In Lyme borreliosis a similar polarization of the immune response occurs, although in an opposite way. IFN-γ appears to promote disease. This cytokine is produced by Th1 lymphocytes, which proliferate in patients with chronic Lyme arthritis [47]. Moreover, IFN-γ–secreting cells have been identified in the blood and CSF from patients with neuroborreliosis [48].

In the animal model, C3H mice, which are susceptible to Lyme borreliosis, produce higher levels of IL-2 and IFN-γ and lower levels of IL-4 than do Lyme borreliosis–resistant BALB/c mice [49]. Whereas IFN-γ appears to promote disease, recombinant IL-4 administered to susceptible C3H mice enables early control of *B. burgdorferi* infection [49] and decreases the bacterial load. In both mice, $CD4^+$ helper IL-4-secreting T cells play an important role in controlling spirochete growth, since abrogation of this cell type leads to aggravation of arthritis and increase of bacterial load in mice.

$CD8^+$ cells also are involved in the course of the disease. Depletion of IFN-γ–producing $CD8^+$ cells leads to a reduction of both arthritis and spirochete number. Treatment with recombinant IL-4 augments resistance to *B. burgdorferi* in normal susceptible mice as well as mice with severe combined immunodeficiency [49]. This resistance is also accompanied by a significant reduction in IFN-γ response of spleen cells and decreased serum levels of IgG2a and IgG3a antibodies. Since IL-4 regulates B cell function and upregulation of MHC class II molecules on B cells, it is possible that IL-4 promotes antispirochete immunity by augmenting specific antibody production [49, 50].

In other experiments, immunization of C3H/HeNcr mice with *B. burgdorferi* has been shown to result in Th1 cell proliferation, a strong delayed-type hypersensitivity reaction, and an IgG2 response [51]. However, the small amounts of IgG1 antibodies produced in these immunized animals favored also the participation of Th2 cells, which allow the survival of bacteria.

Ultraviolet B radiation prior to immunization suppressed this delayed-type hypersensitivity response by a mechanism involving IL-10 [51]. Injection of mouse antibody to IL-10 abrogated the effect of ultraviolet B radiation. This ultraviolet irradiation of LCs in vitro altered their antigen-presenting capability in that such cells failed to stimulate Th1 clones but preserved the ability to activate Th2 clones. From former studies it is known that ultraviolet B irradiation of BALB/c mice reduces significantly the number of epidermal LCs, whereas the average density of MHC class II molecules on the remaining cells is not reduced [52]. Therefore, it was assumed that Th1 cells might require a higher density of MHC class II–associated antigen for their activation than would Th2 cells.

In further experiments it was suggested that this ultraviolet irradiation–induced immunomodulation was mediated by costimulatory signals, such as IL-1, that can convert LCs from

immunogenic to tolerogenic antigen-presenting cells [53]. Therefore, the possibility exists that IL-1, which is induced by *B. burgdorferi* [54], influences antigen presentation and thus the capability for elimination of this spirochete from the host.

The interaction of various cytokines seems to be of central significance in polarizing the immune response in CLB. Although the cytokine profile has not yet been investigated in patients with ACA, studies on immunoglobulin isotypes have shown a distinct IgG2 and IgG3 response but mostly an IgG1 response in the sera of such patients [18]. This points to a simultaneous Th1 and Th2 response in ACA.

The question remains whether downregulation or even loss of MHC class II molecules on LCs might influence a patient's disease susceptibility. It is MHC class II molecules that bind antigenic peptide fragments, present them to $CD4^+$ Th cells, and induce cytokine secretion and IgG secretion by B cells [55]. In vitro investigations have shown that MHC class II molecules are downregulated on antigen-presenting cells after coculture with Th cell clones in the presence of antigenic peptides of tetanus toxoid or staphylococcal superantigen, which elicit a strong HLA-DR-restricted T cell response.

Several hypotheses were suggested as the cause of this downregulation. (1) Downregulation occurs when antigenic peptides catabolized in macrophages are recognized by $CD4^+$ helper T cells, in order to control the size of a T cell clone and provide a homeostatic mechanism [55]. (2) Downregulation occurs for completion of T-B cell collaboration after antigen presentation, limiting excessive T cell help to the triggered B cells, or (3) it occurs for focusing the T cell response to one or a few immunodominant peptides.

(4) LCs of patients with AIDS express decreased amounts of MHC class II molecules. Polyclonal B-cell activation, as seen in these patients and in patients with ACA, could cause the appearance of autoantibodies or immunocomplexes that interact with LCs and block their surface-staining characteristics [45]. (5) IL-10, originally identified as a product of Th2 cells, has a significant inhibitory influence on the antigen-presenting functions of macrophages and LCs by downregulation of MHC class II molecules. In fact, LCs pretreated with IL-10 were converted from specifically sensitizing to specifically tolerogenic antigen-presenting cells in vitro and in vivo [56]. In other studies treatment of LC cultures with IL-10 inhibited the upregulation of HLA-DR [57].

(6) Downregulation is initiated for establishment of self-tolerance. This downregulation can protect the antigen-presenting cell by inhibiting the presentation of self-antigens [58]. On the other hand, the downregulation of MHC class II antigens on LCs could result in inadequate presentation of antigens in lymph nodes, which in turn may reduce activation and proliferation of both B and T cells and the secretion of relevant cytokines. This may be what happens in CLB.

In spite of the marked cellular and humoral immune response in ACA, borreliae can still survive for long periods in tissue. Borreliae may induce the release of factors or cytokines such as IL-10 that can inhibit the expression of HLA-DR molecules on LCs, molecules critical for immune defense. Whereas downregulation of MHC class II molecules on LCs might induce tolerance to *B. burgdorferi,* other MHC class II^+ antigen-presenting cells in the dermis at the same time may maintain an immune response, which can be seen in tissue as heavy lichenoid infiltration.

Recent studies indicated that T cell and humoral immune responses can even be induced in MHC class II–deficient individuals after repeated vaccination with tetanus toxoid [59]. These persons have hypoimmunoglobulinemia and undetectable expression of MHC class II molecules on peripheral blood mononuclear cells. Even after IFN-γ stimulation, HLA-DR expression cannot be induced. Since monoclonal antibodies against HLA-DR, -DP, and -DQ completely block this immune response, traces of MHC class II molecules seem to be sufficient for T cell activation. Thus, even minimal HLA-DR molecules, as expressed on LCs in ACA, are sufficient for establishment of a cellular and humoral immune response.

In leprosy, cross-reactivity between *M. leprae* antigens and human tissues is suspected to be the cause of chronic disease [60]. Similarly, in CLB, molecular mimicry is suspected to lead to several manifestations of disease [40, 61]. In both diseases, downregulation of MHC class II molecules on LCs has been observed. Graft-versus-host–like changes seen in ACA-affected skin do not rule out local autoimmune reactions.

Thus, to prevent manifest autoimmune disease, immunotolerance that includes tolerance of *B. burgdorferi* might be sustained in ACA. Factors that abolish the downregulation of MHC class II molecules might be of importance in new therapeutic strategies against CLB to eliminate the microorganism.

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