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CUTANEOUS AND DISSEMINATED BORRELIOSIS: DIFFERENCES IN PROTEIN PATTERNS BETWEEN *B.BURGDORFERI* ISOLATES

Alje P.van Dam, Herman Kuiper* , Koen Vos, Anneke Widjojokusumo, Anton C.P.Ramselaar, Barthold M.de Jongh, Michael D.Kramer and Jacob Dankert, Depts. of Med. Microbiol., Neurol. and Dermatol., University of Amsterdam, NL, and Inst. Immunology, University of Heidelberg, FRG.

We studied by SDS-PAGE and immunoblotting whether differences exist between protein patterns of *B.burgdorferi* strains from ticks and patients with cutaneous or disseminated manifestations of Lyme borreliosis. Strains were cultured from skin biopsies taken from 39 patients with erythema migrans (EM), 5 with acrodermatitis chronica atrophicans (ACA), 2 with neuroborreliosis (NB) and one with arthritis and cardiac involvement (AC). Additional isolates were obtained from cerebrospinal fluid (CSF) of 2 patients with NB; from one of them also a skin isolate was available. Twenty-three strains were cultured from ticks.

Of the 67 EM, ACA and tick isolates, 63 had MWs of Osp B and Osp A of 35 and 32 kD, respectively. Two isolates lacked an Osp A protein, one had an Osp B with a MW of 36 kD and an Osp A of 32 kD and one, from a patient with an atypical EM and arthralgies had an Osp B of 34 and an Osp A of 31 kD. Two NB isolates and the AC isolate had Osp B migrating as a 34 kD protein; from another patient with NB, skin and CSF isolates both showed an Osp B with an MW of 33 kD. The MW of Osp A of these five isolates was 32.5 kD. None of the strains reacted with the anti-Osp A Mab LA-31, except those from AC and NB patients and the strain from the patient with atypical EM .

In conclusion, the phenotypes of *B.burgdorferi* strains isolated from patients with disseminated Lyme borreliosis differ from those isolated from ticks and patients with only cutaneous Lyme borreliosis.

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**COILING PHAGOCYTOSIS IS THE PREFERENTIAL PHAGOCYTIC MECHANISM FOR *BORRELIA BURGDORFERI***

Michael Rittig*, Andreas Krause, Thomas Häupl, Ulrich E. Schaible, Manuel Modolell, Michael D. Kramer, Markus M. Simon, Gerd R. Burmester, University of Erlangen-Nürnberg, Max-Planck-Institut für Immunbiologie, Freiburg, DKFZ Heidelberg, Germany

The phagocytic mechanism for the spirochete *Borrelia burgdorferi*, the causative agent of Lyme disease, was investigated by electron microscopy for human and murine phagocytes. Spirochetes of both a low- and a high-passage strain were preferentially internalized by coiling phagocytosis rather than by conventional phagocytosis. The spirochetes engulfed by coiling phagocytosis were found to disintegrate without the participation of lysosomes. Preincubation of *B. burgdorferi* with IgG2a monoclonal antibodies specific for the spirochetal outer surface protein A (OspA) did not change the relation between both types of phagocytic mechanisms consistantly. Quantitative and kinetic differences concerning the rate and mechanism of uptake were evident between phagocytes from different lineages, different human individuals, and between the two species. In general, the smaller the number of cells ingesting spirochetes, the more coiling phagocytosis was used as uptake mechanism. These results suggest that coiling phagocytosis of *B. burgdorferi* leads to intracellular events distinct from conventional phagocytosis, and that coiling phagocytosis plays a critical role in the control of spirochetal infection. More detailed studies on the molecular basis of this phagocytic mechanism may lead to new insights into the pathogenesis of Lyme borreliosis, a disease which is frequently characterized by the host's inability to eliminate the pathogenic spirochete.

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THE FATE OF *BORRELIA BURGDORFERI* IN MOUSE MACROPHAGES: DESTRUCTION, SURVIVAL, RECOVERY. Ruth R. Montgomery*, Michael H. Nathanson, and Stephen E. Malawista. Yale University School of Medicine, New Haven, CT 06510.

The macrophage is a known reservoir for a number of infectious agents, and is therefore a likely candidate for a privileged site where *B. burgdorferi*, the Lyme spirochete, may persist. We have shown that *B. burgdorferi* can enter macrophages, resulting in one instance in degradation and in another in apparent intracellular persistence. We studied uptake of *B. burgdorferi* by the mouse macrophage cell line, J774, by simultaneously labeling infected cells with antibodies to *B. burgdorferi* and to sequential components of the endocytic pathway: the late endosome and lysosome marker, lysosomal glycoprotein (lgp) 110, or the lysosomal hydrolase, cathepsin L. We examined optical sections (0.5-1.0 μm in thickness) of these double-labeled macrophages by confocal fluorescence microscopy at multiple time points after infection. We found that only 5 minutes of incubation at 37°C were required for nearly 100% of *B. burgdorferi* to enter an lgp-positive compartment, while 60 minutes were required for 90% of the spirochetes to appear in a cathepsin L-positive compartment under the same conditions. We also labeled infected living cells with acridine orange to distinguish live from killed intracellular organisms and examined them using confocal microscopy. Although the large majority of spirochetes within a given cell were dead, we saw occasional live ones up to 24 hours (the longest interval examined) after all extracellular organisms had been lysed in distilled water. Moreover, we can reculture spirochetes from both J774 and primary mouse macrophages (strain C3H) for at least a week after infection (the longest interval examined). Persistence of spirochetes within mouse macrophages provides a possible pathogenetic mechanism for chronic or recurrent Lyme disease in man.

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**B. BURGDORFERI OUTER SURFACE PROTEINS INDUCE CHRONIC ARTHRITIS IN RATS.**

*Klaus B. Gondolf*, Steven Batsford, John Dunn and Christiane Rasiah, Dept. of Immunology, Inst. f. Med. Microbiology, 7800 Freiburg, Germany.*

Cationic proteins can induce chronic arthritis in rodents. The outer surface proteins (Osp) of B. burgdorferi are highly cationic and we tested the Osp's from a skin isolate (Freiburg area) for arthritogenic potential in rats. Membrane proteins were isolated by extraction with n-butanol, the resulting complex of about 800 kD consisted mainly of Osp A and B and minor amounts of other proteins (22 and 60 kD). After appropriate immunization male Wistar rats received 100 μg of the complex directly into the right knee joint, the left joint serving as control. Further controls were rats injected with the same antigen without prior immunization and rats immunized and challenged with dimeric ferritin (anionic molecule of similar size). The degree of inflammation was assessed from histology and by comparing $^{99m}$Tc-pertechnetate uptake in both knee joints. Chronic arthritis, still persisting at day 60, could be induced with the Osp-complex after immunization; in the group without prior immunization inflammation was mild and transient (≤21 days), no inflammation was seen in the other controls (difference significant at the 5% level). Experiments to examine the pathogenic potential of individual Osp's, using recombinant antigens, are in progress.The presence of bacterial antigens in affected joints has been shown in Lyme arthritis; our results suggest that outer surface proteins are etiologic agents in this context.

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EXPERIMENTAL LYME BORRELIOSIS IN DOGS.

Max J. Appel,* Sandra Allan, Richard H. Jacobson, Tsai Ling Lauderdale, Sang J. Shin and Brian A. Summers, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

The objectives were to establish a canine model for Lyme disease for immunization and pathogenesis studies.

Specific pathogen free (SPF) beagle dogs from the Baker Institute colony were exposed to Borrelia burgdorferi infected ticks (nymphs or adult Ixodes dammini) that were collected in Westchester County, NY. Recurrent lameness, fever, and depression was observed in 9 of 20 infected dogs between 2 and 5 months post exposure. The incidence was higher in dogs exposed to adult ticks (6 of 8) than to nymphs (3 of 12). Single joints (elbow, carpus, or stifle) were involved when lameness occurred. There was a slight swelling of the joints with an increase in synovial fluid that contained up to 20,000 cells/mm$^3$ with up to 85% polymorphs. A suppurative synovitis was observed on post mortem inspection. There were no significant changes in other tissues. B. burgdorferi spirochetes were isolated from muscle and adrenal and occasionally from other tissues.

All symptomatic dogs produced antibodies to B. burgdorferi (tested by ELISA and western blots) before the onset of clinical signs, usually by 6 weeks post exposure.

Dogs that were not euthanized recovered spontaneously without treatment and remained without symptoms for at least one year.