VIII ANNUAL SCIENTIFIC CONFERENCE ON LYME BORRELIOSIS

Call For Abstracts

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- ☐ Clinical manifest.
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- ☐ Veterinary Issues
- ☐ Epidemiology
- ☐ Other spirochetal, tick-borne diseases
- ☐ Other (list) _____

ACTIVATION OF NEUTROPHILS BY LYME DISEASE *BORRELIA*

Hiroshi ISOGAI, Emiko ISOGAI[1], Koichi KIMURA[2], Shunji HAYASHI[2], Koichi TAKAHASHI[3] and Nobuhiro FUJII[2]

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The function of polymorphonuclear leukocytes (PMNs) is essential for host defense against infectious agents. Chemiluminescence responses is considered to be indicative of the generation of reactive oxygen, such as super oxide, hydrogen peroxide, hydroxy-radicals and singlet-oxygen. This generation is an important mechanism in the killing of bacteria. Although it is evident that overall, PMNs exert a protective effect, at times they may produce localized tissue damage. In this study, chemiluminescence responses of neutrophiles induced by stimulation of Lyme disease *Borrelia* were examined.

Bacterial strains used were *Borrelia burgdorferi sensu stricto* B31, *B. garinii* strains TN and SIKA2, *B. afzelii* strains ACA-1 and BFOX and *B. japonica* strain O612. Viable and inactivated bacteria and soluble extracted antigens were used as stimulants for neutrophils. Luminol-dependent chemiluminescense responses were assessed by human whole blood and isolated neutrophiles.

All *Borrelia* strains induced chemiluiminescense responses of neutrophils. The responses were dose-dependent in each stimulant. Similar levels of response were observed between the experiments when viable and inactivated bacteria were used for stimulants. It was significantly higher when cells were stimulated by whole bacteria than soluble antigens. The intensities of the responses were different among the bacterial strains used. Chemiluminescence responses responses of neutrophils were enhanced by serum antibodies which obtained from patients with Lyme disease. The data indicated that the Lyme disease *Borrelia* was enable to induce oxygen radicals from neutrophils and suggested that these oxygen radicals affected to development of local tissue damage.

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ROLE OF PLATELET ACTIVATING FACTOR IN THE PATHOGENESIS OF LYME BORRELIOSIS

Emiko ISOGAI, Hiroshi ISOGAI, Koichi KIMURA, Takeshi NISHIKAWA, Norihisa ISHII, Daniele POSTIC, Guy BARANTON and Nobuhiro Fujii
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Both C3H/HeN and BALB/c mice were inoculated subcutaneously with various species of Lyme disease Borrelia low passage isolates; B. burgdorferi sensu stricto B31 (American strain), B. garinii TN (European strain), B. garinii SIKA2 (Japanese strain), B. afzelii ACA-1 (European strain), B. afzelii BFOX (Japanese strain) and B. japonica 0612 (Japanese strain). Appearance of initial hemorrhagic subcutaneous lesion was examined in mice at 7 days after inoculation. All infected mice showed the lesion at the inoculation-site. The lesion size and dissemination pattern were different among mouse groups inoculated with distinct species. Disease-resistant BALB/c mice showed the subcutaneous lesion similar to disease-susceptible C3H/HeN mice inoculated with the same strains, but the lesion size was significant smaller than that in C3H/HeN mice. Appearance of skin lesions including subcutaneous inflammation and secondary erythema was examined at 45 days after infection. The rate was different among C3H/HeN mouse groups inoculated with distinct species. The levels of infectivity and of various organ dissemination varied according to the inoculated bacterial species. These facts could be reflect the different rates of appearance of erythema migrans (EM) among various places in the world.

To determine whether endogenous PAF contributes to the pathogenesis of erythema in Lyme borreliosis, CV-3988, a specific PAF antagonist was injected to the mouse model before and after infection. CV-3988 treatment inhibited B. burgdorferi-induced initial hemorrhagic erythema.

Precise at the beginning they are in vitro experiments. A mixture of human polymorphonuclear leukocytes (PMN) and platelets was stimulated with B. burgdorferi. Both viable and inactivated B. burgdorferi elicited platelets aggregation. Both platelets and PMNs were required to induce such aggregation. The aggregation was dependent upon the numbers of both PMN and the organisms. It was suppressed by pretreatment of PMN-platelet mixture with CV-3988. Platelets aggregation could be initiated via endogenous PAF from PMN. These findings suggest that PAF might play a central role in the pathogenesis of Lyme borreliosis symptoms including EM.

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ENDOGENOUS INTERLEUKIN-1, INTERLEUKIN-6 AND TUMOR NECROSIS FACTOR LEVELS IN DESEASE-SUSCEPTIBLE AND DESEASE-RESISTANT MICE AFTER INFECTION WITH DISTINCT SPECIES OF LYME DISEASE BORRELIA

Takeshi NISHIKAWA, Emiko ISOGAI, Hiroshi ISOGAI, Koichi KIMURA, Norihisa ISHII and Nobuhiro FUJII
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The pathogenesis of Borrelia burgdorferi infection depends on a number of factors; the heterogeneity of the spirochetes, host genetic backgrounds and various immune responses. In SCID and some strains of immunocompetent mice, inflammatory lesions have been reported. Certain inbred strains of mice failed to develope of organ pathology during infection. We have used a mouse model to reveal the principles of pathogenesis induced by various species of Lyme disease Borrelia low passage isolates. In the model, inflammatory cytokine levels were examined.

Both C3H/HeN and BALB/c Mice were infected subcutaneously with distinct species of Lyme disease Borrelia: B. burgdorferi sensu stricto, B. garinii, B afzelii and B. japonica, and then levels of IL-1, IL-6 and TNF in the serum and various organs were examined after infection.

C3H/HeN (disease-susceptible) mice showed 100% infectivity to an isolate of B. garinii and IL-1 and/or TNF were detected in the serum, while BALB/c (disease-resistant) mice showed 20% infectivity and inflammatory cytokines were not detected in the serum. IL-1, IL-6 and TNF were also detected in the parenchemous organs such as spleen of C3H/HeN mice. They were detectable in several organs at low levels in BALB/c mice. Cytokine levels were compared among mouse groups inoculated with distinct species of Lyme disease Borrelia. The levels were different among mouse groups. The genetic control of cytokin production was studied in B10 congenic mouse strains. Cytokine production wa observed in such congenic mice and the levels were different among mouse strains. These results suggest that endogenous cytokines are produced with different levels by different organs in various types of Lyme disease and tha the bacterial species and host genetic background could be related to its production.

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