CLINICAL IMMUNOLOGY IN LEPROSY
— ETIOLOGY OF ERYTHEMA NODOSUM LEPROSUM —

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Generally speaking, in the serum of a leprosy patient free from reactional phases, no peaks other than the physiological macroglobulin component with a sedimentation coefficient of 19S were presented in the ultracentrifugal pattern. The sera of patients in reactional phases of leprosy, especially, reactional borderline leprosy or lepromatous leprosy with erythema nodosum leprosum (ENL), showed the peak of macroglobulin components divided into 2 to 4 parts including the normal macroglobulin. The pathological macroglobulin components sedimented between the G (globulin) component with 7S and physiological M (macroglobulin) component with 19S in the ultracentrifugal picture, and had a sedimentation coefficient of approximately 10.2S to 17.8S. Such pathological components were not observed in the sera of lepromatous leprosy cases without ENL. The unusual high molecular weight components could not be observed by ultracentrifugal analysis when the reactive state had subsided.

On the other hand, in our studies of micro-immunoelectrophoresis of serum proteins of patients with leprosy, remarkable elevations of serum glycoprotein fractions (α_1 -antitrypsin, α_1 -acid glycoprotein, haptoglobin, ceruloplasmin, α_2 -macroglobulin and β_2 -glycoprotein), serum lipoprotein fractions (α_1 -, α_2 -, and β -lipoprotein) and immunoglobulin fractions were noted in cases of borderline and of lepromatous leprosy. These changes became noticeable at lepromatous leprosy with ENL. But, in most cases of indeterminate and of tuberculoid leprosy the levels of serum proteins seemed to be within normal limits. In immunoelectrophoretic tracing, the most striking facts were a marked increase of the IgG- and IgM-immunoglobulins, and an exceedingly frequent and prolonged appearance of serum paraproteins in leprosy reactions.

Latex agglutination tests were made on leprosy sera using cardiolipin, sphingomyelin, nucleoprotein, human gamma-globulin and thyroglobulin as antigens respectively. In the cases with ENL, there were marked positive agglutination reactions of high antibody titers. The antibody titers were of lower levels in lepromatous leprosy cases without ENL than in those with ENL. Those antibodies in serum from lepromatous leprosy cases, especially from those with ENL were heat-labile. These antibodies were found to be sensitive to the treatment with mercaptoethanol as indicated by the complete loss of latex agglutinating activity. In addition, it was found that the circulating antibodies is present in a IgM-immunoglobulin.

And then immunofluorescent staining was performed on cryostat sections of skin eruptions of ENL and skin lepromas of lepromatous leprosy without ENL. In the indirect incubation method, the antibody labelled with fluorescein isothio-

cyanate to the pooled serum immunoglobulins of the lepromatous leprosy with ENL was prepared in rabbits. In the sera of ENL, the prominent fluorescence indicated that immunoglobulins were presented within the cytoplasm of the plasma cells, small lymphocytes and of histiocytes at the site of eruptions of ENL. These reactions were not observed, however, in the sera of lepromatous leprosy without ENL and of tuberculoid leprosy.

The results of the previous reports suggest that there are immunological tissue damage at the stage of ENL. It is probable that tissue cell degeneration in case of persistent lepromatous inflammatory changes is responsible for the production of antibodies. In advanced lepromatous leprosy, especially in those of the systematic or diffuse forms in which the episode of ENL are frequently observed, the presence of delayed hypersensitivity due to degenerated leprosy bacilli or altered self cell components may rather be important in causing the production of many humoral self factors (antibodies). One of the interesting facts is that phospholipids may be the intrinsic factors for the occurrence of ENL at the later stage of lepromatous leprosy. *M. leprae* has phospholipid antigen similar to that of human heart, blood vessels, nerve tissues and joints. Needless to say, antigens of the bacillary components presumably become cross antigens to those tissues, and *M. leprae* causes autoantibody formations against heart, vessels, nerve, joints, testis and/or iris. It is conceivable that the autoantibodies found in the sera of ENL may arise through this mechanism.

CULTIVATION AND ANIMAL-TRANSMISSION OF *MYCOBACTERIUM* *LEPRAE* (*M. LEPRAEMURIUM*)

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1. Cultivation of *M. lepraemurium*

In the past three decades, our various trials to cultivate *M. lepraemurium* on bacteriological solid and liquid media favorable for the growth of *M. tuberculosis* have not shown any growth of the bacilli.

However, recently we were able to observe a limited multiplication of *M. lepraemurium* in cultures of mouse foot-pad cells up to the third sub-cultures.

After 96 hr of incubation at 33°C for phagocytosis, infected cells were washed to remove unphagocytized bacilli and transferred to new culture vessels. There was a 4.9-fold increase in the number of bacilli during 70 days of successive cultures up to the third culture in flasks, giving an overall generation time of 30.4 days.

Acid-fast stained cover-slips taken from the culture in Leighton tubes revealed intracellular multiplication of *M. lepraemurium*, frequently in bundles of hundreds of bacilli, and gave an average generation time of 16.4 days in the primary, 15.7 days in the secondary, and 8.1 days in the third culture.