IN VIVO LEISHMANICIDAL MECHANISMS IN TEGUMENTARY LEISHMANIASIS. ULTRA-STRUCTURAL OBSERVATIONS.

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Activation of macrophages by T lymphocytes or its products are thought to be the most important host defense mechanism in leishmaniasis. Such observations have been predominantly made using in vitro models, with very few in vivo observations of the modifications induced by the agents or procedures used. Although tissular reactions are heterogeneous and dynamic some histopathological changes have been correlated to prognosis in human or experimental leishmaniasis, comparing susceptible and resistant mouse strains. It is difficult, however, to discriminate factors involved in resistance from those merely reflecting diverse genetic backgrounds but not implicated in specific protection. Such difficulty may be overcome by using the same mouse strain altering its susceptibility to Leishmania. Extremely susceptible BALB/c mice may be rendered partially resistant to L. mexicana amazonensis (Lma) by intravenous immunization. We took advantage of this procedure to study the morphological aspects and the kinetics of the tissular response of immunized animals following Lma infection, compared to those of their susceptible counterparts.

Immunization consisted of 3 i.v. doses of 5x10⁷ solubilized Lma promastigotes at weekly intervals. Mice were infected in the foot-pad with 5x10⁸ homologous promastigotes one week after immunization. Lesion fragments at different phases of infection were submitted to optical and electron microscopy. In order to perform a quantitative and qualitative
evaluation of cell types present in the exudate, its interaction with parasites and other cells, a differential counting of a 100 to 200 cells per sample was made. The parasites were also counted taking into consideration its location and rate of integrity.

During the first 24 hours after infection no differences were observed between immunized or unimmunized animals. There was an acute reaction with dense infiltration of neutrophils, eosinophils and several macrophages. The majority of parasites was damaged. These findings suggest that at the beginning of infection the response depends on the natural resistance of the animal, since mice responded similarly, despite previous immunization.

Between one and 4 weeks post-infection macrophages containing parasites predominated in the lesions. Amongst these cells there were several lymphocytes, eosinophils and neutrophils. Frequently a close contact between the plasma membranes of parasitized macrophages and lymphocytes or eosinophils were noted. In these situations most of the parasites exhibited structural alterations. Degeneration, or even lysis, of macrophages with liberation of parasites to the extracellular space was also observed. The presence of parasite in the matrix was associated to accumulation of granulocytes. Lesions from immunized mice showed a few microscopic foci of fibrinoid necrosis. Differences in the number and integrity of parasites between the two groups increased with evolution of the disease. At the 4th week of infection 54% of the macrophages in the immunized group showed signs of activation against only 17% of macrophages in the unimmunized group.
Clear-cut differences between immunized and unimmunized animals were observed after the 7th week post-infection up to the end of the period of study (13 weeks post-infection). At this time the lesions of animals infected without previous immunization were represented by an extensive and almost monomorphic infiltration. Vacuolated macrophages comprised 87% of the cells in the exudate and 66% of them were heavily parasitized. More than 50% of the parasites inside cellular vacuoles were well preserved. Lymphocytes represented only 2% of all the inflammatory cells. This monotonous picture was altered by the presence of granulocytes forming microabscesses around a few necrotic cells. In the immunized animals the lesions were represented by a mixed-cell inflammatory infiltration. Most of the macrophages exhibited signs of activation. Only 13% of the cells were parasitized and the majority of parasites was degenerated. Lymphocytes represented 14% of the cells, and frequently exhibited close contacts with macrophages. Foci of granulomatous reaction formed by accumulation of epithelioid and multinucleated giant cells were found in small clusters surrounding parasitized macrophages. This aspect was correlated to lysis of macrophage and parasites. Focal fibrinoid necrosis was an outstanding feature. Destruction of parasites was seen more often in the extracellular space than inside phagocytic cells. In the group of immunized animals there was an active fibrosis with fibroblast proliferation and collagen deposition.

The results presented here show that immunization induces qualitative different tissue response in animals of the same genetic background. The presence of lymphocytes, granulomatous reactions,
fiorinoid necrosis and fibrosis have been correlated to cellular immune responses, and protection against leishmania in human and experimental leishmaniasis. Our findings seem to confirm that histological picture may reflect host immune status in this disease, and that diverse tissular mechanisms operate in susceptible and resistant mice.

Our data suggest that the most important host defense mechanism against leishmania is the lysis of parasitized macrophages, and amastigote destruction by granulocytes. A close contact of lymphocytes, eosinophils or epithelioid cells was related to macrophage lysis. The role of eosinophils in the destruction of parasites and macrophage activation is unknown. Likely, the action of such cells does not depend much on the immune system, as it was similar in immunized and unimmunized animals. Direct contact of lymphocytes have been considered important for macrophage activation, and it has been recently shown in vitro that even helper T cells may induce a cytotoxic effect in macrophages. Close contact of lymphocytes and parasitized macrophages, with destruction of the latter cell, was also seen by us in human leishmanial infection. In our model there was a positive correlation between number of lymphocytes in the lesion and protection, and parasitized macrophage rupture was seen more frequently than intracellular parasite destruction inside activated macrophages.