ONE APPROACH TO ASSESS THE LIVER RESPONSES IN MURINE LISTERIOSIS AND VISCERAL LEISHMANIASIS.

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The responses of inbred and congenic mice to Listeria monocytogenes and Leishmania donovani (two pathogens known to enter and replicate inside resident mononuclear phagocytes) were shown to fall into discrete phases which proved tractable to genetic analysis (1,2). Those systems in which alleles at a limited number of loci confer resistance or susceptibility offer powerful experimental conditions to dissect the discrete processes set in motion following infection (3).

Thus, the work to be discussed will focus on two loci whose expression in the liver is critical for resistance/susceptibility to L.monocytogenes and L.donovani, namely, respectively, the Lr locus (1) and the H-11-linkage group (2).

After a short review of the data documenting why the liver is the critical organ to analyze the effects of these two loci, methods allowing to recover and analyze non parenchymal liver cells will be described. Briefly, once the gall-bladders were severed, livers of carefully perfused mice were excised. They were dissociated on a stainless steel sieve with a sterile 20 ml syringue pestle in cold buffered saline solution (BSS). After filtration through a 300 mesh nylon gauze the resulting cell suspension was washed once and centrifuged in Percoll 35% in BSS containing calciparin (100 u/ml). On cytocentrifuge preparations stained with May-Grünwald Giemsa, it was possible to demonstrate that after the Percoll step, the recovered cells were leukocytes (lymphocytes and myelomonocytic cells). It is important to note

that, with this method which deliberately avoid use of collagenase and other proteases, no resident Küpffer cells are recovered from livers of naive mice.

Once appropriately labelled with relevant markers of lymphocyte (Thy1, CD4, CD8, SIg) and of myelomonocytes (Mac1, F4/80, 7/4), the resulting non parenchymal liver cells thus recovered were analyzed by flow cytometry and/or sorted for further functional analysis (e.g. limiting dilution analysis of specific T cells detected through their ability to transfer local delayed-type hypersensitivity) (4).

The results of experiments comparing the liver response of Listeria-resistant (C57Bl/6, Lr^r) and Listeria-susceptible (C3H/He, Lr^s) mice will be briefly summarized : they indicated that successful resistance against Listeria in livers of C57Bl/6 mice required the early and coordinated influx of both Listeria specific CD4+ and CD8+ lymphocytes as well as myelomonocytic cells belonging to the mononuclear phagocyte system ($F4/80^{+ve}$).

Results of experiments comparing H-11 linked genetic susceptibility/resistance of Leishmania donovani would be discussed in more details. Indeed as already noted (5,6), this is the first time that a locus is shown to have a profound effect following infection with L.donovani, L.major or L.mexicana, independently of the dose, route or stage of parasite inoculated. Therefore, comparing the numbers of L.donovani reactive T cells in livers of non healing B10 129 (10M) $H2^bH\text{-}11^b$ and healing B10 ScSn H2 , $H\text{-}11^a$ congenic mice following iv. injection of L.donovani amastigotes might provide relevant data to account for mechanisms of action of the H-11 linkage group. It is important to note that these congenic mice bear the susceptibility (Lsh^s) allele at the Lsh locus, a critical locus determining permissiveness of L.donovani replication in resident Küpffer cells (7). The data suggest that maximal reduction of parasite load in livers of healing B10 ScSn

mice might be mediated by L.donovani-reactive CD8+ lymphocytes. Indeed progressive reduction in the parasite load in livers of these B10 ScSn mice appeared only when L.donovani-reactive CD8- lymphocytes entered and persisted in the livers (between days 30 - 100 in our analysis). At least, it remains to be determined (a) whether the L.donovani reactive CD4+ lymphocytes always found at a low number in livers of healing mice are critical effectors favouring influx and persistance of CD8+ T lymphocytes (b) what are the effector functions by which the CD8+ lymphocytes control parasite load and their precise interactions with other blood derived leukocytes composing the liver granuloma (8,9).

We may expect that answers to these questions (a) will be available from quantitative phenotypic and functional analysis of non parenchymal liver cells recovered from either resistant or susceptible mice (b) will allow to reconsider in situ, how the liver granuloma develop as transient functional units with bactericidal activity (listeriosis) or develop and persist as dynamic functional units inhibiting replication of strictly intracellular parasites (visceral leishmaniasis).

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