The role of hematopoietic growth factors in the fate of an infection with Leishmania mexicana amazonensis: an attempt at a unifying hypothesis.

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Several recent evidences point to the fact that hematopoietic growth factors produced by macrophages or by activated T-cells are instrumental in defining the fate of a leishmanial infection in experimental models of cutaneous leishmaniasis (1, 2, 3, 4). The effect observed in vivo can be explained by recruitment of immature cells to the lesion site where they become "safe-targets" for the growth of the organism (5). This hypothesis however does not explains the enhancing effect observed in the intracellular growth of the organism in vitro cultivated macrophages (1, 2).

In order to analyse the effects of hematopoietic-growth factors on the course of leishmanial lesions, we compared the in vivo and in vitro production of some of those hormones in genetically resistant (C57B110-B10) and susceptible (BALB/c) mice as well as in mice of the same genetic background. To this end, resistance and hypersusceptibility were artificially induced in BALB/c mice, by respectively sub-lethal irradiation (550r) and subcutaneous immunization with formaldehyde fixed parasites (ffp). Our results show that the production of GM-CSF by BALB/c macrophages infected in vitro is higher than that of B10 macrophages. Also the productinon kinetics is different: in macrophages from susceptible animals the GM-CSF activity in the supernantants of infected cells raises sharply in the first hours post-infection and is maintened high throughout the infection period; on the contrary, in cells from the resistant strain, the initial rise is followed by a decrease in activity, returning to approximate basal levels at 72h post-infection. Those date were obtained by assessing the proliferative activity induced in bone-marrow cells as well as in DA-1 cells. This activation is inhibited by an anti-mouse GM-CSF antiserum whith does not cross-react with M-CSF, G-CSF. IL-3 and IL-2 (kindly donated by Dr. Jolanda Schreurs, DNAX Research Institute of Molecular and Cellular Biology, INc. Palo alto, Ca.). In our hands this antibody completely supresses the DA-1 proliferative activity induced by the infected macrophage supernatants (and also of a lung conditioned medium) while it has no effect on the proliferation of the same cells induced by a WEHI supernantans (rich in IL-3). We also observed that the appearance of polycarions during the infectivity curve is more intense in BALB/c macrophages than in B10 cells. It is interesting to note that GM-CSF has been shown to be a potent fusogenic factor (6).

Our in vivo studies also shown, confirming and reinforcing previous ones (4, 5), that hematopoietic growth factors are differentially produced in vivo infected mice. The

difference depends on their lesion being self-healing or progressive and not on the genetic background of the experimental mice. In addition to the above described findings we have shown that a lung conditioned medium (LCM), a classical way of obtaining GM-CSF (7), is an extremely potent growth factor for promastigotes of Leishmania mexicana amazonensis. Several molecular and cellular evidences point to the fact that the Leishmania growth factor (LGF) present in LCM is GM-CSF itself, as follows: both GM-CSF and LCF have the same synthesis kinetics and are equally sensitive to trypsin and to heat (resistant to 56°C and sensitive to 100°C). In addition supernatants from T-cell clones and lines and from permanent cell lines that display GM-CSF activity also display LGF activity and vice-versa. More importantly, depletion of GM-CSF from LCM by immunoprecipitation with the above mentioned anti-murine GM-CSF antibody also equally abrogates the LGM activity of the supernatant. Studies of co-elution of the GM-CSF and LGF activities on a Superose 12 column in FPLC as well as the use of recombinant human and murine GM-CSF are underway. Also, very preliminary results indicate that LCM induces a striking modification on the protein profiles of Leishmania when analysed by two-dimensional gel electrophoresis. The modifications may be similar to the one induced by a heat-shock. Finally it is worth stating that infective promastigotes (recently collected from infected animals) are more sensitive to the effects of LGF than a non-infective form (maintained in culture for more than 300 passages).

These results permit us to propose that the observed effect of GM-CSF are, at least in part, due to a direct effect of the hormone on the intracellular growth of the parasite. Of course one severe restraint of our data is that all the results were obtained with promastigotes and not with amastigotes. We will very soon begin to look for GM-CSF binding in amastigotes of LMA. In any case, our hypothesis is that GM-CSF produced by activated T-cells is internalized together with the parasite, and once in the vacuole it begins to exert its growth promoting activity, or, that GM-CSF synthesized by the infected cell gets in contact with the endocyted parasite. It has been elegantly shown, in a completely different system, that the exocytic pathway taken by synthetised proteins intersects the endocytic route (8). Several different examples of the production by and the response to mammalian hormones by microrganisms have been published (reviewed in 8). To the best of our knowledge this is the first time that such a mechanism is proposed to define infectivity of the responding parasite.

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