Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Suppl. I Vol. 83, November, 1988/Page 407

THE ROLE OF GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR IN THE PATHOGENESIS OF EXPERIMENTAL MURINE LEISHMANIASIS

W. Solbach, B. Bodendorfer, J. Greil & M. Röllinghoff

Institut f. Klin. Mikrobiologie, Universität Erlangen, Wasserturmstr. 3, D-8520 Erlangen, FRG

Experimental local infection of mice with Leishmania major (L. major) promastigotes results in a wide spectrum of diseases depending on the gentic background of the animals. Mice of the BALB/c strain are uniformely susceptible to the infection and develop a systemic disease which usually is fatal. On the other hand, resistant strains such as C57BL/6 develop a localized, self-healing lesion (1).

Experimental evidence convincingly suggests that $CD4^+$ T lymphocytes, activated after infection, play a crucial role in both the progression and the resolution of the disease (for review see (2)). The precise mode of action of the $CD4^+$ cells is not clear yet. Analyses in vitro and adoptive transfer experiments have revealed, however, that distinct lymphokine-profiles of T lymphocytes activated by Leishmania antigens are closely associated with resistance and susceptibility. For BALB/c mice it has been shown that with progression of the infection the amount of synthesized Interferon-y (IFN-y) and Interleukin-2 (IL-2) decreases (3, 4). In contrast, Interleukin-4 (IL-4) and Interleukin-3 (IL-3) production is continuously elevated (4, 5).

IL-3 and Granulocyte-Macrophage Colony-Stimulating-Factor (GM-CSF) are moleules secreted by activated $CD4^+$ T lymphocytes, which stimulate the proliferation, differentiation and end-cell activation of granulocytes and macrophages (MØ) both in vitro and in vivo (6). In addition, it has been shown that these factors contribute to the recruitment of blood-derived MØ to the site of inflammation (7). On the basis of experimentation in vitro it has been sugessted that GM-CSF can activate human and murine MØ populations for antimicrobial activity against Trypanosoma cruzi (8),

Salmonella typhimurium (9), Leishmania donovani (10), and Leishmania tropica (11).We were interested to study whether this effect was reflected in disease progression, when L. major infected mice were treated with GM-CSF in vivo.

BALB/c mice locally (footpad) infected with L. major promastigotes (2 x 10^7) and concomitantly injected daily intraperitoneally with recombinant (r)GM-CSF (1,5 x 10^6/mouse x day) differed from animals not treated with rGM-CSF with respect to the following:

1. rGM-CSF treated mice showed an accelerated lesion development. We never observed a beneficial effect of the agent.

2. At day 21 p. i.,one mg of infected footpad tissue contained approx. 1,5 times more living L. major promastigotes.

3. The number of parasites in the spleens and lymph nodes draining the infection was 2 - 7 fold higher.

4. In addition, the number of mononuclear cells in these organs was significantly enhanced.

These data are consistent with the following findings in vitro. When paraitized splenic MØ obtained from chronically diseased animals (8wk p.i.) were incubated with rGM-CSF (100 U/ml) for 8 days, the number of MØ per culture increased approx. twofold above that in the control cultures. More important, when the percentage of MØ infected with L. major was scored, it clearly became apparent that cells grown in rGM-CSF did **not** differ significantly from those grown without rGM-CSF.

IFN-y has been shown to be of critical importance for the induction of antimicrobial MØ activity (12). It was therefore of interest to compare the effects of rGM-CSF and rIFN-y on the growth and antimicrobial effects of parasitized MØ obtained from chronically diseased mice. It was found, first, that the rGM-CSF induced enhancement of MØ growth (see above) was inhibited by approx. 65% through the action of rIFN-y; second, rIFN-y clearly reduced both the number of L. major infected MØ and the average

number of Leishmania per infected MØ to less than 50% of the values observed in cultures treated with rGM-CSF (without rIFN-y).

Taken together, these findings clearly indicate that rGM-CSF induces MØ proliferation and supports the survival of intracellular parasites but that it does not stimulate MØ to antileishmanial activity. Very similar data from in vivo and in vitro experiments were reported recently by Fengh et al. who investigated the effects of another member of the family of CSF's, IL-3 (13).The discrepancy between the data reported here and those ascribing an antimicrobial activity to rGM-CSF (8 - 11)) cannot be fully explained at present, but are most likely due to variations in experimental design.

In summary, the proposed action of GM-CSF in the pathogenesis of Leihmania infection of BALB/c mice is as follows: T lymphocytes secrete GM-CSF upon stimulation with the parasite. The cytokine supports then the proliferation of local tissue MØ and recruits blood-derived phagocytes, which serve as host cells for multiplying parasites. Due to inappropriate quantities of IFN-y the MØ-accumulating effect mediated by GM-CSF is not counterbalanced. In addition, the MØ are not triggered to develop from host cells to antiparasitic effector cells and thus can serve as "safe targets" (14) for L. major. The consequence would be an aggravated course of the disease.

In a broader concept, the data reported here imply that it may be of disadvantage to induce MØ in high numbers if it is not ensured that they are activated for antimicrobial effector functions. Despite dramatic responses of cellular components to GM-CSF administered in leukopenic patients, convincing evidence that GM-CSF leads to of a reduction of mortality and morbidity due to infections with intracellularly growing microbes is still lacking.

References:

1. **Mitchell, G. F. 1984.**
   Immunol. Today 5: 224 - 226.

2. **Louis, J. and G. Milon (eds). 1987.**
In: Ann. Inst. Pasteur/Immunol. 138: 219 - 277.

3. **Solbach, W., M. Lohoff, H. Streck, P. Rohwer, and M. Röllinghoff. 1987.**
Immunology 62: 485 - 491.

4. **Locksley, R. M., F. P. Heinzel, M. D. Sadick, B. J. Holaday, and K. D. Gardner, Jr. 1987.**
Ann. Inst. Pasteur/Immunol. 138: 226 - 231.

5. **Lelchuk, R., R. Graveley, and F. Y. Liew. 1988.**
Cell. Immunol. 111: 66 - 76.

6. **Metcalf, D., A. W. Burgess, G. R. Johnson, N. A. Nicola, E. C. Nice, J. DeLamarter, D. R. Thatcher, and J.-J. Mermod. 1986.**
J. Cell. Physiol. 128: 421 - 427.

7. **Metcalf, D., C. G. Begley, D. J. Williamson, E. C. Nice, J. DeLamarter, J.-J. Mermod, D. Thatcher, and A. Schmitt. 1987.**
Exp. Hematol. 15: 1 - 9.

8. **Reed, S. G., C. F. Nathan, D. L. Pihl, P. Rodricks, K. Shanebeck, P. J. Conlon, and K. H. Grabstein. 1987.**
J. Exp. Med. 166: 1734 - 1742.

9. **Grabstein, K., S. Reed, K. Shanebeck, and P. Morrisey. 1987**
Lymphokine Res. 6: 1707 (Abstr.).

10. **W. Y. Weiser, A. Van Niel, S. C. Clark, J. R. David, and H. G. Remold. 1987.P**
J. Exp. Med. 166: 1436 - 1445.

11. **E. Handman and A. W. Burgess. 1979.**
J. Immunol. 122: 1134 1137.

12. **Sadick, M., R. M. Locksley, C. Tubbs, and H. V. Raff. 1986.**
J. Immunol. 136: 655 - 661.

13. **Fengh, Z. Y., J. Louis, V. Kindler, T. Pedrazzini, J. F. Eliason, R. Behin, and P. Vasalli. 1988.**
Eur. J. Immunol. 18: 1245 - 1251.

14. **Mirkovich, A. M., A. Galelli, A. C. Allison, and F. Z. Modabber. 1986.**
Clin. exp. Immunol. 64: 1 - 7.