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Research Note

DOES MACROPHAGE DEACTIVATING FACTOR PLAY A ROLE IN THE MAINTENANCE AND FATE OF INFECTIOUS GRANULOMATA?

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Since the discovery of macrophages by Élie Metchnikoff scientists have considered the possibility that these cells could be manipulated to increase their capability of killing microorganisms. In fact, a large number of factors and conditions were demonstrated to increase macrophage microbicidal and tumoricidal capacity (J. M. Papadimitriu et al., 1989, *Ultrastructural Path. 13:* 343-372). In many infectious disease, however, macrophages are systemically activated but for unknown reasons, these cells are unable to eliminate the antigens from the tissues (G. B. Mackaness, 1964, *J. Exp. Med., 120:* 105-113). A good example of this situation is Koch's phenomenon, a typical example of concomitant immunity. A hundred years ago Koch discovered that tuberculous guinea pigs could resist a second infection with the same tuberculous bacilli. As he described, the inoculation of virulent mycobacteria into guinea pigs induces a chronic disease characterized by the development of multiple granulomatous lesions in different organs of the animals. These granulomata grow, become confluent and persist until the animal's death. However, if the same bacteria are reinoculated in the skin of these animals, a prompt non granulomatous inflammatory reaction develops, becomes indurated and cures in about seven to ten days (R. Koch, 1891, *Dutsh. med. Woch., 17,* 101. p. 648 Apud. A. Calmette, 1928, *Infection Bacillaire et Tuberculose,* Masson et Cie, Editeurs, Paris, p. 648). The efficiency of phagocytic cells within the secondary reaction to kill mycobacteria is explained by the presence of peripheral specific T lymphocytes in tuberculous animals. Activated T cells recognize mycobacteria antigens in the tissues and mediate the secondary inflammatory response and macrophage activation by lymphokine secretion. Paradoxically, the lesions which resulted from the first inoculation persist, dispite the presence of different types of activated T lymphocytes in the mantle of small round cells which surrounds these lesions. Although the ultimate mechanism which impair the development of an efficient microbicidal activity of macrophages within the persistent granuloma remains to be elucidated, it has been suggest that the imbalance between helper and suppressor activated T lymphocytes might determine the outcome of the lesion (R. L. Modlin et al., 1988, *Proc. Natl. Acad. Sci.*, USA, *85:* 1213-1217). As an alternative hypothesis, epithelioid cells from within the granuloma might secrete one or more factors (V. C. P. C. Camarero et al., 1990, *J. Cel. Physiol., 145:* 481-487) which would deactivate the already activated macrophages which continuously migrate into the granuloma to maintain the turnover of the lesion (A. M. Dannemberg Jr et al., 1975, In: *Mononuclear phagocytes in immunity infection and pathology.*, p. 959-967. Oxford, Blackwell.). The preliminary results herein described support this hypothesis.

The implantation of round glass coverlips into the subcutaneous tissue of mice provides the isolation of inflammatory macrophages, giant cells and epithelioid cells (G. B. Ryan & W. G. Spector, 1970, *Proc. R. Soc. Lond.* (Biol.), *175:* 269-292; M. Mariano et al., 1976, *J. Pathol. 120:* 151-159). We have shown that inflammatory macrophages adherent to the surface of glass coverlips implanted for seven days into the subcutaneous tissue of mice, spontaneously release detectable amounts of superoxide anions. Curiously, cells on the surface of coverslips implanted for fourteen and twenty-one days, which have developed into pre-epithelioid and epithelioid cells fail to release detectable amounts of superoxide anion but secrete a factor which promptly deactivates

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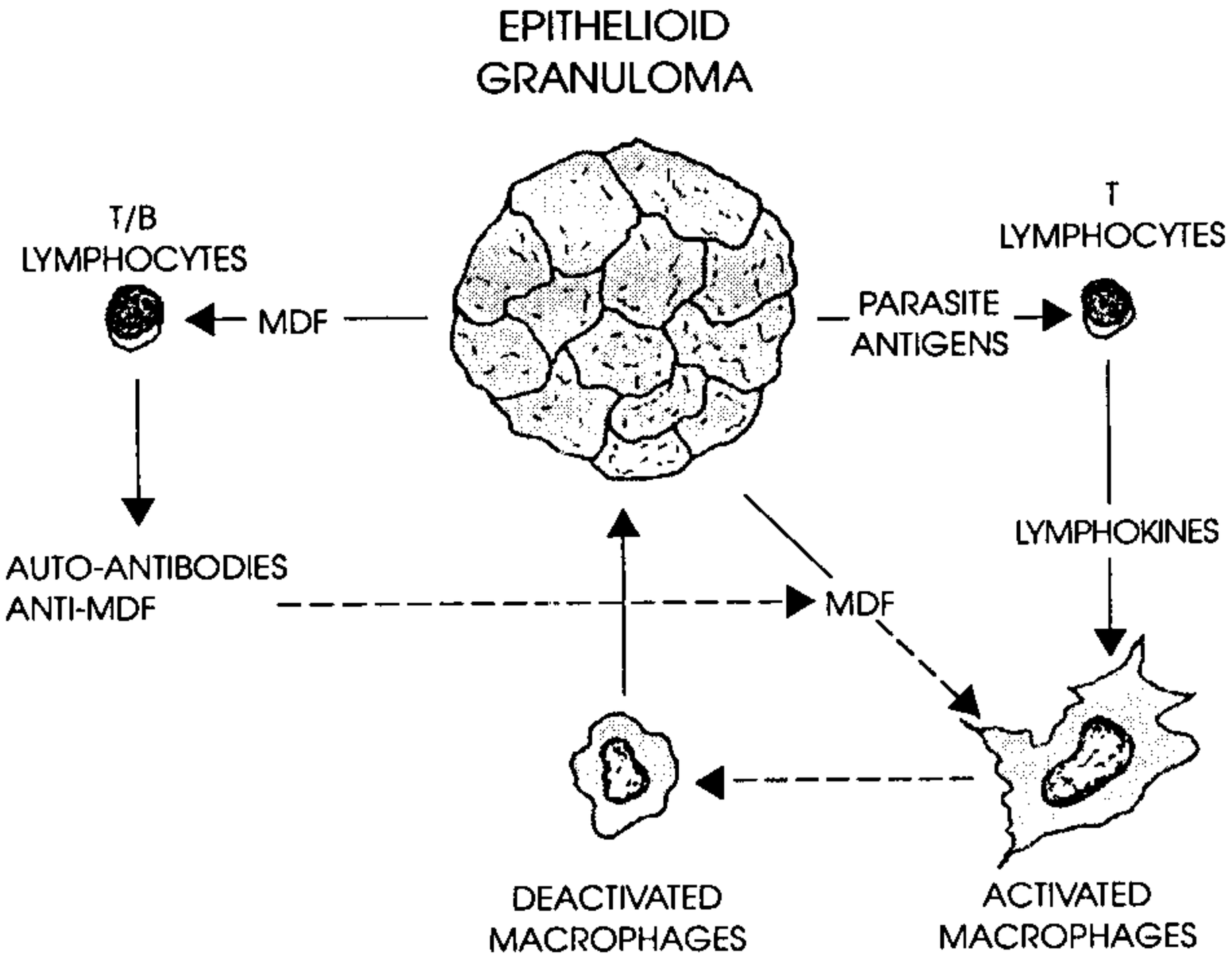

Schematic representation of the possible rôle played by MDF and natural antibodies directed to MDF on the persistence of granulomatous inflammation.

superoxide release by activated macrophages and neutrophils (V. C. P. C. Camarero et al., 1990, *loc. cit.*). Camarero et al. (in preparation) further characterized this epithelioid cell-derived macrophage deactivating factor (ECD-MDF) as a heat stable, trypsin sensitive 11 kDa protein which interferes with the membrane oxidase activity of leukocytes. Substances similar to ECD-MDF have being detected in the culture supernadants of murine (A. Szuro-Sudol & C. F. Nathan, 1982, *J. Exp. Med. 156:* 945-961) and human tumor cells, fibroblasts (D. S. Nelson et al., 1982, *Aust. J. Exp. Biol. Med. Sci. 60:* 493-502) and alveolar macrophages (R. B. Zeidler et al., 1987, *Inflammation 11:* 371-379). TGF-β1 and TGF-β2 (S. M. Tsunawaki et al., 1988, *Nature* (Lond.) *334:* 260-262) as well as calcitonin gene-related peptide (CGRP) (Y. Nong et al., 1989, *J. Immunol. 143:* 45-49) and IL-4 (M. Lehn et al., 1989, *J. Immunol., 143:* 3020-3024) were also found to suppress macrophage respitatory burst activity.

In order to elucidate whether ECD-MDF is involved in the dynamic of infectious granulomata, we have obtained a polyclonal rat antiserum against the secretory products of epithelioid cells obtained on the surface of coverslips implanted for fourteen days in the subcutaneous tissue of mice. The immunoglobulin fraction of this serum was shown to react, by immunocytochemical methods, with epitopes in the cell membrane of macrophages on the surface of coverslips implanted for fourteen but not for five days. The same antiserum revealed a doublet band of about 12 kDa by immunoblotting when epithelioid cell secreted substances were used as antigens. This doublet was also recognized by normal rat serum, only when tested in low dilution. Furthermore, the immunoglobulin fraction was able to block completly the macrophage deactivating activity of epithelioid cell culture supernatants on BCG activated mouse peritoneal macrophages, evaluated by superoxide anion release. Moreover, the administration of the immunoglobulin fraction to C5 deficient mice, bearing BCG induced granulomas in the footpad for fourteen days, significantly reduced the size of these lesions. Finally, when mice were immunized by intrasplenic route with antigens secreted by mouse epithelioid cells and challenged in the footpad

with viable BCG, the size of immunized animal lesions was significantly reduced. This result, strongly suggest that epithelioid cell secretory products are able to stimulate autoreactive antibodies production to regulate the evolution of the lesion (M. Mariano et al. in preparation).

These results support the hypothesis that once epithelioid cells are formed, their secretory products such as MDF exert, by a feedback mechanisms, a suppressive effect on the major effector cell of the immune system, the activated macrophage. This might expalin why the lesions which result from the first inoculation of the mycobacteria in guinea pigs persist and kill the animal dispite the intense reactivity of T sensitized lymphocytes. On the other hand, since epithelioid cells are not formed in the second inoculation of the bacteria, T cell activated macrophages are able to kill the bacteria and promote tissue healing. The inhibitory activity of these substances on the methabolic activity of activated macrophages might, on the other hand, be self controlled by autoantibodies directed to these substances (Fig.).

The proposed feedback cell circuit might not be restricted to the granulomatous type of inflammatory response but may represent a general property of macrophages. In this direction, Zeidler et al. (1987, *loc. cit.*) isolated from normal pig lungs, a subpopulation of macrophages which secretes an inhibitory factor for superoxide release by macrophages, similar to the ECD-MDF we have described. These findings demonstrate that even in normal conditions macrophages can be modulated at least in two different directions of activity. First, the well known cell activation process, which enhances microbicidal and tumoricidal capacity of the cell. Secondly, they might be modulated to secrete factors which inhibit macrophage activation controling on one hand tissue healing and, on the other, facilitating parasite persistence in the tissues.

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