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Regulation of cell-mediated immunity in lepromatous leprosy

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Patients with lepromatous leprosy demonstrate a selective T cell unresponsiveness to *M. leprae* and fail to mobilize appreciable numbers of T cells-- particularly of the T4 (helper) phenotype, into their dermal lesions. In the absence of lymphokine production, cutaneous macrophages serve as permissive hosts for the bacilli and extensive intravacuolar replication takes place. In our studies we wished to examine the factors leading to T cell and monocyte emigration into the skin and the role of interferon-γ. For this purpose we generated delayed hypersensitivity reactions in the dermis of control and lepromatous patients and examined the nature and host-parasite interactions of the cells accumulating at these sites. In addition, we have administered recombinant, human interferon-γ into the skin of patients with lepromatous leprosy. The nature of these reactions at the local and systemic level will be presented.

Immunopathology of leprosy lesions

The dermal lesions of lepromatous patients are characterized by the presence of large numbers of heavily infected foamy macrophages and a sparse infiltrate of lymphoid cells. Using monoclonal antibodies specific for human leukocyte antigens we have shown that the vast majority of the T cells found in these lesions were of the Leu2a/OKT8 (suppressor/cytotoxic) subset of T lymphocytes (1). Only few Leu3a/OKT4 (helper) T cells were present, and the T4/T8 ratios were considerably less than unity. As one progressed towards the tuberculoid pole, the number of T cells increased and many of them were of the Leu3a/OKT4 phenotype. This was associated with a reduction in the extent of parasitization of the cutaneous macrophages and a differentiation of these into epithelioid and multinucleated giant cells organized within granulomas (2).

The exuberant growth of *M. leprae* within macrophages of lepromatous lesions stands in striking contrast to the paucibacillary macrophages of tuberculoid leprosy. The permissive nature of the macrophages of the lepromatous lesions together with the selective absence of helper T cells suggested a lack of local lymphokine production and inadequate macrophage activation.

The cellular kinetics of delayed type hypersensitivity reactions in the lesions of lepromatous leprosy

The lack of accumulation of helper T cells in the lesions of lepromatous leprosy focused our attention on the ability of the dermal milieu of these patients to provide the neccesary conditions for normal cellular immune responses. We wanted to establish whether T4 cells were inhibited from emigrating into the sites of *M. leprae* infection and whether they could be retained and activated to release lymphokines in these lesions. For this purpose we generated

delayed type hypersensitivity reactions in the dermis of lepromatous patients by the use of the purified protein derivative of tuberculin (PPD). The nature and interactions of the cells accumulating in the delayed reaction was studied by immunocytochemistry at the light and electron microscopic level.

Studies have been carried out from 18 hours to 14 days after the administration of antigen and biopsies of the delayed reactions and adjacent uninjected skin taken in 60 patients. This has allowed us a temporal, longitudinal analysis. The majority of lepromatous patients appeared to respond normally to intradermal injection of PPD (3). During the first 18 hours significant numbers of helper T cells and monocytes entered the lesions and their numbers increased until stabilizing at 96-120 hours (4). OKT8 T cells which were initially predominant in the lepromatous lesions became a minor component of the lesions. Foamy, bacilli laden macrophages were reduced in numbers, extracellular *M. leprae* liberated by the disruption of macrophages were evident and reactive T cells were prominent in these areas. Of particular interest was the appearance of potent accessory cells of the T6 phenotype in the delayed reactions at 72 hours (5). These cells, at the electron microscopic level, contained Birbeck granules and were T6 positive indicating that they were Langerhans cells. The accumulation of these cells in the dermal infiltrates was associated with a marked decrease in Langerhans cells in the overlaying epidermis. As the lesions matured (41-60 hours) the overlying epidermal keratinocytes began to proliferate and by 68-72 hours the epidermis was thickened (2-3 fold) (6). This was attributed to increases in both size and numbers of keratinocytes. In addition, the phenotype of the keratinocytes overlaying the delayed reactions was changed from Ia negative to Ia positive. We suspect the production of potent epidermal growth factors by the cells of the dermal reaction. By 7 days granulomas with epithelioid and giant cells were seen. At 14 days the dermal reactions appeared quiescent with a normalization of T6 cells in the epidermis. The epidermis had also undergone normal differentiation and sloughed the cornified layers. It is of interest that *M. leprae* were difficult to locate in these lesions (see below).

Effect of recombinant interferon-γ on monocyte-derived macrophages from patients with lepromatous leprosy

Interferon-γ (IFN-γ) appears to be the principal factor secreted by antigen-stimulated lymphocytes that activates macrophages, in the sense that the phagocytes more efficiently inhibit or kill pathogenic microorganisms (reviewed in 7). Deficient macrophage activation may be a characteristic of borderline and polar lepromatous leprosy. We have shown that monocytes from the blood of lepromatous leprosy patients respond normally to recombinant IFN-γ with an enhanced secretion of H_20_2 (8). H_20_2 release can be triggered in these cells both by phorbol myristate acetate and by intact irradiated *M. leprae*. The uptake of *M. leprae* by cultured monocytes from lepromatous leprosy patients is dose dependent in a similar way to that observed with cells from normal control donors. Prior ingestion of *M. leprae* does not interfere with the ability of macrophages to respond to rIFN-γ by the production of oxygen intermediates. These observations have led us to conclude that the immune defect in lepromatous leprosy probably results from a lack of response to *M. leprae* by the patients' T cells. This would result in a reduced or absent release of lymphokines including IFN-γ (9, 10) leading to a local lack of macrophage activation and the absence of killing of intracellular *M. leprae*.

Local and systemic effects of intradermal recombinant interferon-γ in patients with lepromatous leprosy

Evidence that macrophage activation may be defective in lepromatous leprosy, led us to test the effects of intradermal injection of low doses of recombinant IFN-γin patients with this

disease (11). IFN-γ was administered into cutaneous lesions and the site biopsied 24-168 hours after the first injection. Immunocytological analysis of the biopsy sites revealed that IFN-γ elicited local effects similar to certain features of delayed hypersensitivity reactions. Twenty-four hours after the administration of intradermal IFN-γ into lepromatous leprosy lesions newly emigrated T cells and monocytes were observed locally. T4 cells predominated whereas T8 cells had previously been the major T lymphocyte (12). Keratinocyte proliferation, diminution of epidermal Langerhans cells, and dermal and epidermal Ia antigen expression were observed. In addition the blood monocytes of these patients were shown to be activated systemically to release enhanced levels of H_20_2 when triggered by phorbol myristate acetate or intact *M. leprae*.

Local production of IFN-γ in delayed type hypersensitivity reactions in the lesions of leprosy patients

Many of the above mentioned changes induced by PPD and recombinant IFN-γ occur naturally in the skin lesions of tuberculoid leprosy patients. The keratinocytes overlaying these lesions are Ia positive (6) and T6 cells are prominent in tuberculoid granulomas (5, 13). In a recent study carried out in this laboratory INF-γ was shown to rapidly induce the expression of a new group of genes in macrophages, fibroblasts, and endothelial cells (14). One of these genes has been isolated and its DNA sequenced. The polypeptide deduced from the nucleic acid sequence (γIP-10) has been synthesized and an antiserum raised against this peptide. We have used this antiserum to demonstrate the expression of the γIP-10 peptide in the recombinant IFN-γinjected lesions. By 18 hours after the injection of rIFN-γ into the dermis of lepromatous patients, cells in the basal layer of the epidermis showed clear immunohistological staining for the induced peptide (15). Identification of IFN-γ induced peptides in inflammatory lesions provides us with a direct way of demonstrating the local production and release of IFN-γ.

When PPD responses were examined we observed that the IFN-γ induced peptide was expressed by 24 hours in some of the basal cells of the epidermis (4). At 48 hours all the basal cells of the epidermis and many of the mononuclear leukocytes and endothelial cells in the dermal accumulation were positive for γIP-10. We have also shown that the epidermal and inflammatory cells of tuberculoid (but not lepromatous) lesions express the IFN-γ induced peptide. This observation is compatible with our hypothesis that IFN is released locally in tuberculoid leprosy lesions but not in lepromatous leprosy lesions.

Host-parasite interactions in delayed reactions in leprosy lesions

The reduction in numbers of foam macrophages observed during delayed hypersensitivity reactions to intralesional PPD was further studied by electron microscopy (4). At 72-96 hours after PPD injection dead and damaged foam cells were prominent and cell organelles, *M. leprae* and remnants of Mycobacteria were found in the extracellular space. By 1 and 2 weeks few foam cells had survived and epithelioid cells predominated. Very few bacteria were found in these lesions and those that were observed were mostly within Schwann cells and endothelial cells. In only one case was *M. leprae* found within an epithelioid cell. These observations suggested that the delayed reaction to PPD caused a destruction of foam cells, releasing the intracellular bacteria which were then taken up by new monocytes entering the lesions. The bacteria were killed and digested by the new inflammatory monocytes and therefore could not be found in the lesions at the later time points. The killing of parasitized foam cells could be mediated by cytotoxic T cells or monocytes that recognize a *M. leprae* specific antigen on the surface of the parasitized host cells. Alternatively, the foam cells

could be especially fragile (because of their size and volume of vacuoles) and killing could be a bystander effect secondary to monocyte-T cell interactions and granuloma formation. We are currently investigating these observations in more detail.

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