SPECIAL ARTICLE

Immunopathology of leprosy granulomas—current status: a review

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Introduction

The term granuloma was first introduced by Virchow as a tumor like mass or nodule of granulation tissue. Granulomas have been defined in different ways. One of the common definitions could be 'A granuloma is a localized collection of cells of the mononuclear phagocyte system with or without the admixture of other inflammatory cell types.' The 'Koch phenomenon' was probably the first experimentally induced granuloma. It is an accelerated enhanced dermal reaction evoked by live or dead tubercule bacilli in guinea-pigs presensitized with tubercle bacilli. This is probably due to the release of lymphokines following, the interaction between the tubercle bacilli and specifically sensitized T cells in the blood or tissues. The infiltrates of this reaction contain lymphocytes and epithelioid cells. The relationship between epithelioid cells and macrophages was recognized by Metchnikoff as early as 1888. In this context, leprosy is an interesting disease for investigation for two main reasons: a, it affects a large population in the world; and b, the histopathological features of lesions and cells in the granulomas bear a positive correlation with the delayed hypersensitivity reaction seen in these patients. Thus it could serve as a useful model for studying the immunopathological aspects.

Leprosy is a chronic infectious disease caused by Mycobacterium leprae where granulomatous inflammation affecting the skin and nerves is a prominent manifestation. This disease exhibits a spectrum with tuberculoid leprosy, a high immune, paucibacillary form at one end and lepromatous leprosy, a low immune, multibacillary form at the other end. The lesions in tuberculoid leprosy are characterized by an epithelioid cell granuloma with abundant lymphocytes forming dense collections around the epithelioid cells. On the other hand, lepromatous leprosy is characterized by a granuloma composed of sheets of macrophages loaded with M. leprae along with plasma cells and a few lymphocytes diffusely distributed into the granuloma. Most of the studies to understand the immunological mechanisms in leprosy have been mainly carried out using in vitro tests on peripheral blood derived lymphocytes and monocytes. However, the major site of immunological reaction in leprosy, i.e. the skin has been largely uninvestigated due to methodological limitations. This has been overcome to a considerable extent with the use of monoclonal antibodies directed against cell surface antigens. They have proved to be an important tool to analyse the nature of lymphocytes and other cell types in humans. The present review is limited to the progress made in the last five years in understanding the immunopathology of leprosy granulomas. Most of the
Characteristics of lymphocytes in leprosy granulomas

NONREACTATIONAL STATES

The characterization of lymphocytes in the leprosy granulomas was done using rosetting techniques. The majority of lymphocytes formed rosettes with sheep erythrocytes indicating the presence of T cells. They were mostly seen to be associated with epithelioid cell granulomas and showed maximal density in tuberculoid leprosy. A graded reduction was observed in borderline leprosy with a severe reduction in lepromatous leprosy. This was further evident by lymphocytes not forming EA and EAC rosettes, in tuberculoid lesions. However, the lymphocytes in the BL lesions rosetted with EA and EAC suggesting that they were mainly B cells. The lymphocytes also exhibited nonspecific esterase activity (marker of T cells) in the leprosy granulomas.

The above observations has been further supported by the immunohistological analysis of leprosy granulomas carried out, using monoclonal antibodies defining T cell subsets and Ia like antigens by four groups of workers. Most lymphocytes in leprosy lesions were positive for OKT3 and Ia like antigens indicating thereby the presence of activated T cells. Maximal numbers of these cells were seen in tuberculoid granulomas in close association with the epithelioid cells. A decline in their numbers were observed over the leprosy spectrum. In disseminated multibacillary lepromatous leprosy, only a few positive cells were visualized. The ratio of OKT4+/OKT8+ cells was higher in tuberculoid lesions in comparison to lepromatous lesions. In treated lepromatous patients, a high proportion of T cells was noticed. An important difference, was observed in the microanatomical distribution of the functional subsets of lymphocytes. In the tuberculoid lesions, the lymphocytes expressing phenotype of helper T cells (T4+) were diffusely scattered being present both amongst the epithelioid cells as well as in the lymphocytic cuff surrounding these cells. In contrast, lymphocytes expressing phenotype of suppressor T cells (T8+) were arranged in 'ring like' manner mainly in peripheral lymphocytic cuff. However, no such demarcation of lymphocyte subsets was observed in lepromatous lesions. That the microanatomical pattern was more important than the numerical value of lymphocytes was further indicated by studies on reactional leprosy lesions. During both ENL and type I reactions of BL which occur in patients with lepromatous type of leprosy, T cells of helper phenotype entered the lesions in large numbers, sometimes reaching levels observed in BT lesions, yet the microanatomical pattern showed scattering of both T8+ (suppressor) and T4+ cells and nonformation of organized granuloma. However, such a distribution was not noticed in the studies of Van Voorhis et al. This could be because a, fewer numbers of patients in each group, or b, sampling error. It would appear therefore that not only the presence of helper or suppressor T cells but their distribution may indicate the development or nondevelopment of effective immunity in the lesions. Such a type of concept is brought out by various workers in other conditions where epithelioid cell granulomas are prevalent, e.g. sarcoidosis, Kveim reaction, by our studies on Mitsuda skin tests in leprosy patients and in American cutaneous leishmaniasis. Similar findings of the presence of T cells and their distribution has been observed in the lymph nodes of leprosy patients. The lymphocytes in both the tuberculoid and lepromatous granulomas expressed M. leprae specific antigens (shown using monoclonal antibodies to soluble antigens of M. leprae) and also expressed fibronectin. This leads to the question as to what is responsible for the organization and maintenance of effective immunity in tuberculoid granulomas. Modlin et al. and Longley et al. have made an attempt to answer it using monoclonal antibodies defining interleukin 2 (IL2) and T cell subsets. Their study reveals that disorganized lepromatous granuloma contains significantly fewer densely staining IL2+ cells than highly organized tuberculoid granuloma, thus leading to diminished production of
IL2 in lepromatous leprosy. Further, IL2+ cells also stained with antileu 4, antileu 3a antibodies but not with antileu 2a antibodies. This finding suggests that IL2 bearing cells were helper/inducer T cells. Working independently, Nilsen et al.59 have made similar observations in the immunohistological analysis of nerve granulomas in leprosy.

**Reactional States**

Histologically it has been shown that a large number of lymphocytes enter the lesion during type I reaction of BL and ENL reactions. The nature of lymphocytes has been examined with monoclonal antibodies. The lymphocytes entering the reactional leprosy lesions were predominantly activated T lymphocytes with a preponderance of T4+ cells. The ratio of T4+/T8+ cells was increased in reactional BL and ENL lesions in comparison to nonreactional states. The distribution pattern of T8+ cells was similar to T4+ cells both being diffusely scattered among bacilli laden macrophages in these lesions. Reactional BT lesions showed a mild increase in pan T cells and the microanatomical distribution of T4+ and T8+ cells was identical to that seen in nonreactional tuberculoid lesions. Though ENL and reversal reactions of BL were thought to have different mechanisms of initiation, yet they showed similar T cell types and pattern in the lesions.12,19,20 There was an increase of IL2 positive cells in ENL lesions in comparison to nonreactional lepromatous lesions.45 This clearly suggests that T cells (particularly helper/inducer T cells) are involved in the pathogenesis of these reactions, in leprosy.

These results in turn strongly support one of the earliest findings of the pathogenesis of Type I leprosy reactions in mice. It was shown that T cells were responsible for the elicitation of these reactions. The inflammatory infiltrates contained predominantly small lymphocytes and activated macrophages.52 So, the presence of increased numbers of T4+ cells in these lesions may cause the activation of macrophages due to delayed hypersensitivity reaction and thus the fragmentation of the bacilli. This is particularly seen in ENL lesions. These macrophages may also release increased amount of pyrogenic factors. Further, the T4+ cells could help in the production of antibodies which ultimately results in the formation of immune complexes. All these phenomenon are evident in patients of leprosy undergoing reaction.53,54,56

**In vivo skin reaction to killed Mycobacterium leprae**

Lepromin reaction is one of the parameters which may be used; a, in the assessment of the immune status of a leprosy patient or a contact; b, to test the efficacy of an immunoprophylactic agent; and c, to study the immunological mechanisms involved in the formation of hypersensitivity mediated epithelioid cell granulomas. The type of lepromin reaction depends upon the method of preparation of the antigen. For example, whenever the organisms are sonicated and soluble components are injected, it gives only an early reaction. However, if the killed organisms are inoculated intact, it gives a late reaction.21 Our group has carried out studies to understand the mechanism of elicitation of lepromin reaction by characterizing the cells in the infiltrates with monoclonal antibodies. Standard Dharmendra lepromin has been used which elicits both the early and late reaction. Both these reactions were positive in tuberculoid patients and negative in lepromatous patients. The infiltrates of early reaction comprised of lymphocytes and polymorphonuclear leucocytes. A high proportion of cells in these infiltrates were activated T cells expressing OKT11, Leu 3a, OKT8 and Ia-like antigens. Ia-like antigens were not discernible on polymorphonuclear leucocytes.15 Similar types of observations were made in other skin reactions, e.g. delayed hypersensitivity reaction to PPD in humans,22,23 described earlier and our studies using armadillo derived leprosin,15 purified mycobacterial antigen (MY1) from M. leprae.24 The granulomas of Mitsuda reaction was characterized by the presence of lymphocytes and epithelioid cells. The immunological characteristics of cells in the Mitsuda reaction was similar to that seen in tuberculoid leprosy lesions.15,43 This
has been further confirmed using armadillo derived leprosin coupled to liposomes as antigen in leprosy patients. Further the immunohistology of skin responses have been recently used: a, to distinguish direct reactions from cross-reactions in delayed hypersensitivity reactions in humans elicited by various antigens; b, to understand the entry of T lymphocytes in the lepromatous granulomas; and c, to study the kinetics of the elicitation of lepromin reaction in leprosy patients. Thus these experiments are of potential importance to the vaccination studies undertaken in leprosy.

**IMMUNOGLOBULINS AND COMPLEMENT COMPONENTS**

Ridley et al. have evaluated the immunoglobulins, complement components, plasminogen, lysozyme, C-reactive protein and α-1-antitrypsin, in leprosy granulomas using immunoperoxidase staining. These factors were produced in higher amounts in TT and LL with a dip in the BT-BB region (except C-reactive protein and α-1-antitrypsin). The immunoglobulins were present mainly in plasma cells and lymphocytes.

**Accessory cells in leprosy granulomas**

**MACROPHAGES**

Macrophages are involved in antigen presentation and elicitation of immune response. They carry receptors for C3 component of complement, FC component of IgG, exhibit esterase activity and express Ia-like antigens. Two groups of workers have assessed the macrophage membrane characteristics using EA and EAC rosetting in leprosy lesions. Ridley et al. have observed maximal adherence of EAC to cells of the mononuclear phagocyte series (MPS) at the tuberculoïd end of the spectrum with less adherence towards LL. No EA adherence to MPS cells was seen in tuberculoïd patients but increased adherence in the lepromatous granulomas. This suggests that epithelioid cells possess only C3 receptors and no receptor for FC component of IgG. However, Gupta et al. have reported that both epithelioid cells to tuberculoïd leprosy and foam macrophages containing AFB showed adherence to EA and EAC. Further, the presence of nonspecific esterase was uniformly observed in the lesions across the leprosy spectrum. The difference observed in the two studies may be due to type of red cells and immunoglobulin used for preparing EA.

A generalized marker (using monoclonal antibodies to Ia like antigens) have been used to characterize the macrophages in the granulomas. Most of the macrophages from the granulomas of both tuberculoïd and lepromatous leprosy expressed Ia-like antigens. This was a feature even in reactional states. However, in certain large granulomas of tuberculoïd leprosy, the central epithelioid cells lacked the expression of Ia-like antigens. This expression of Ia-like antigens in leprosy lesions was further quantitated. A significant difference in the expression was noticed. The lack of Ia antigen expression was also observed in the granulomas of Mitsuda reaction and in epithelioid cells of experimental mycobacterial granulomas in guinea-pigs. In contrast, Ridley & Ridley claim that Ia antigens are expressed only by cells in the granulomas of tuberculoïd leprosy but not in lepromatous leprosy. This discrepancy in the results was due to the latter having used formalin fixed tissues (which could destroy some of the antigens) and a different type of Ia antibody which was not monoclonal. Further, macrophages from both the granulomas expressed fibronectin and *M. leprae* specific soluble antigens. The presence of fibronectin in these granulomas, may suggest that, this molecule when released within lesion may play some role in the regulation of granuloma formation and in the resolution of the lesion. It has been demonstrated that the majority of mononuclear cells in the granulomas of BL leprosy express the phenotype of macrophages. Two other populations expressing phenotypic characteristics of interdigitating cells and Langerhans cells were also present. More important is that the monoclonal antibodies have
been used recently to differentiate between epithelioid cells and macrophages in the granulomas of leprosy and sarcoidosis.\textsuperscript{51}

\textbf{LANGERHAN’S CELLS (LC)}

Accessory cells other than macrophages have been shown to play an important role in the presentation of antigen to T cells.\textsuperscript{37} Langerhan’s cells present in the skin have been shown to participate in experimental allergic contact dermatitis and delayed hypersensitivity reactions.\textsuperscript{37,38} These cells bear receptors for Fc component of IgG, and C3 component of complement, express Ia like antigens and contain high concentrations of ATP-ase enzyme.\textsuperscript{29} LC can be defined by a specific OKT6 monoclonal antibody.\textsuperscript{30}

Four groups of workers have reported on the status of LC in leprosy. Mathur \textit{et al.}\textsuperscript{31} using ATP-ase staining showed that the number of LC were reduced in LL in comparison to TT. Jihe \textit{et al.}\textsuperscript{32} have reported reduced numbers of ATP-ase positive LC in skin lesions of tuberculoid and borderline patients in comparison to normally appearing skin from the same patients. Narayanan \textit{et al.}\textsuperscript{26} have used OKT6 antibody for defining LC. Normal numbers of LC was observed across the leprosy spectrum. However, the dermal granulomas of tuberculoid leprosy showed a high proportion of nondendritic T6 + cells scattered in the mononuclear infiltrates surrounding the epithelioid cells. These cells were not detectable in the lepromatous lesions. Morphologically the T6 + cells in the dermis lacked dendritic appearance. A similar observation was made by Modlin \textit{et al.},\textsuperscript{18} and Kaplan & Cohn.\textsuperscript{48} The discrepancy in results could be where ATP-ase has been used as a marker of LC, it is possible that the differences in the content of this enzyme, may explain the apparent reduction in LL. No difference in the numbers of OKT6 + epidermal LC have been noticed in reactional states of leprosy\textsuperscript{19} and among various types of skin reactions.\textsuperscript{15,24,50} However, a small proportion of T6 + cells was noticed in the infiltrates of these reactions. So, these results particularly using monoclonal antibody (T6) may suggest that the pathogenesis of the tuberculoid lesion may be different from that of lepromatous lesion.

\textit{In vitro} studies on leprosy granulomas

Three groups have been working along these lines. Lai Fat \textit{et al.}\textsuperscript{33} using \textit{in vitro} techniques have demonstrated the synthesis of immunoglobulins and complement in skin lesions across the spectrum. IgG synthesis occurred in small amounts in tuberculoid leprosy, a distinct amount in borderline leprosy and a large amount occurred in lepromatous leprosy. Synthesis of C3 was found only in some cultures of these granulomas. The same group\textsuperscript{34} have shown the synthesis of anti \textit{M. leprae} antibodies \textit{in vitro} from the skin lesions of leprosy patients.

Our group has recently reported that, it is possible to prepare a single cell suspension from the leprosy granulomas by collagenase treatment and study some of the properties of cells \textit{in vitro}. The first part\textsuperscript{15} involves the characterization of cells using rosetting and histochemical techniques. The granulomas were found to contain lymphocytes and macrophages. The number of lymphocytes were significantly higher in the suspensions of tuberculoid granuloma in comparison to the suspensions from the lepromatous granuloma. A high percentage of lymphocytes from the tuberculoid granuloma formed rosettes with sheep erythrocytes and also showed the presence of esterase as dots in the cytoplasm. However, the lymphocytes did not form rosettes with EAC. This indicates that these lymphocytes appear to be T cells. Most of the macrophages from both the granulomas were esterase positive, exhibited peroxidase activity and did not carry receptors for C3 component of complement. The macrophages from tuberculoid granuloma were nonadherent to plastic surface, while those from the lepromatous granuloma were adherent. In the second part\textsuperscript{56} these cells have been further characterized using monoclonal antibodies. The results showed a good correlation with \textit{in situ} characteristics as described earlier. Modlin \textit{et al.}\textsuperscript{45} have also made single cell...
suspension from the leprosy granulomas and separated T4 and T8 positive cells. These cells were exposed to IL2 and the T4, T8 cell lines were established. The T8 cell lines from lepromatous granulomas showed significant suppressor activity in comparison to the T8 cell lines derived from tuberculoid granulomas. Further, a comparison has been made of the characteristics of dermal granulomas of tuberculoid and lepromatous leprosy by culturing them in vitro. It was observed that lepromatous granulomas release soluble factors which significantly reduced the viability and division of lymphocytes derived from peripheral blood of normal individuals in comparison with tuberculoid granulomas.

Conclusions

The nature and characteristics of infiltrating cells in leprosy granulomas has been elucidated. It has been possible to understand some of the features of antigen presenting cells like macrophages and LC in these lesions. In particular, the lack of expression of Ia-like antigens by epithelioid cells in situ and in experimental mycobacterial granulomas could suggest that these cells may not be involved in antigen presentation. More interestingly, macrophages from lepromatous granuloma express abundant Ia-like antigens and therefore may possess the ability to present antigen. Incidentally, a large proportion of these macrophages were adherent to plastic surface. It is known that adherent cells are involved in antigen presentation. Nonreactional lepromatous lesions contain only occasional positive T lymphocytes while during rectional phase, there was an influx of large numbers of T lymphocytes. Recent attempts to isolate the cells from the dermal granulomas have proved to be successful and has given way for studying functional characteristics of lesional cells. With these available facts, it is hoped that an investigation along the following lines will be possible in the future: a, Mechanism leading to lymphocyte deficit in lepromatous granulomas; b, To clone the T4+ and T8+ cells from lesions and to assess their characteristics; c, To study the characteristics of lesional macrophages and their products; and d, Role of Langerhans cells and T6+cells in the development of leprosy lesions.

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