Immunology and Hypersensitivity in the Development of Hansen's Disease

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Abstract

The primary immune response to M. *leprae* was therefore studied in rhesus monkeys with advanced SIV infection, and in SIV [-] controls. The percentages of CD4+ and CD8+ T cells were determined over 63 days post-inoculation in blood and in cutaneous inoculation sites of live *M. leprae* and compared with flowcytometric determinations of CD4 and CD8 levels in peripheral blood and with assessment of M.leprae-specific lymphocyte proliferation in vitro. Tissue CD4+ percentages rapidly peaked during the first two weeks after *M. leprae* inoculation in SIV[-] monkeys, then declined to a low plateau level by day 27. Tissue CD4+ cell percentages did not reach a peak in the SIV[+] group until day 27. In both groups, however, a similar CD4 maximum of approximately 45% was observed. By day 63 the ratios and percentages in issue had declined to similar levels in both groups. During the first month after *M. leprae* inoculation in SIV[+] animals, CD4+ cells continued to increase at inoculation sites even though the percentage of circulating CD4+ cells declined steadily to very low levels. Compared to SIV[-] animals, SIV[+] animals also showed delayed local expression of IL-2 mRNA, and delayed onset of responsiveness to specific to M. *leprae* antigens in vitro. These results suggest that SIV (and 1-11V) infection disrupts early events in the response to mycobacteria which may be critical in the development of effective cellular immunity to these pathogens, but which may not be readily evident in assessment of old, established lesions.

Introduction

M. leprae causes a wide variety of lesions in man, which are now understood to be the result of a broad, continuous spectrum of human immune response to this organism (1). The seeds of this concept are found in the description of lesions in the Atlas of Danielsen and Boeck in 1847 (2), but the theoretical basis for an immunopathologic spectrum in leprosy was first fully formulated by Skinsnes in 1964(3). A practical classification system based on clinical and pathologic criteria was devised soon thereafter by Ridley & Jopling (4), and is the system widely used today. This theoretical model of leprosy is thus a gift to contemporary investigators present at this Congress. The challenge to investigators today is to explain the mechanisms by which one pathogen, *M. leprae,* can elicit such a broad range of responses.

Although the spectral concept addresses many of the critical issues involved in the pathogenesis of the disease, it may not include some important variables when it is used to assess the possible role of various immunologic mediators. The standard design for such studies is to assess the quantity of the proposed cell type or mediator in biopsies from lesions across this spectrum. Implicit in this approach, but seldom acknowledged, is the assumption that each sub-group (LL, BL, BT, etc) is homogeneous. Moreover, this approach overlooks the fact that the 'new', untreated lesions observed on first presentation to the physician are well-established lesions, usually several years old (5). Because the time of initial infection with *M. leprae* cannot be determined by any current methods, and the resulting skin lesions develop very slowly, are usually indolent in nature, and do not attract medical attention, it is not possible to study the earliest sites of infection nor the earliest host responses to *M. leprae* in man. Assessment of the earliest local and systemic responses to *M. leprae* must therefore be done using animal models.

We have studied the early evolution of the immune response to *M. leprae* in non-human primates using rhesus monkeys *(Macaca mulatta)* inoculated intravenously and intradermally

with M. leprae freshly obtained from experimentally infected armadillos (Dasypus novemcinctus) (6). To further explore the effects of cellular immune dysfunction on this immunodeficiency virus (SIV+) and had developed clinical signs and symptoms of simian AIDS (SAIDS), and a control group of animals which had not received SIV (SIV-). Abundant evidence indicates that SIV and SAIDS are excellent animal models of their human counterparts, HIV and AIDS (7).

Materials and Methods

Twelve rhesus monkeys (Macaca mulatta) were studied: 4 SIV[-1 healthy controls, and 8 SIV[+], infected with SIV eight months previously. Each animal received M. leprae (freshly harvested from armadillo spleen) inoculated intravenously and at 6 sites intradermally.

The intradermal inoculation sites were biopsied on days (2, 5, 9, 14, 21, 27, 63). One half each biopsy was fixed in formalin and the other half was snap frozen in liquid N2 and stored at —70°C. Frozen sections were stained by an inderect avidin-biotin technique, using commercial monoclonal anti-human CD4 or anti-human CD8 antibodies, counter stained with hematoxylin, and percentages of antibody-stained cells were determined manually. Percentages of circulating CD4+ and CD8+ cells were determined by FAGS analysis, using fluorescent conjugates of the same antibodies. Lymphocyte transformation tests were performed on peripheral blood mononuclear cells (PBMC) with or without 100 g/ml M. leprae sonicate antigen under standard conditions, measuring uptake of 3H-thymidine after 6 days. Total RNA was isolated from 10 frozen sections of each biopsy using a commercial kit (Promega). Reverse transcription and PCR amplification of mRNA were performed using commercial primers for human -actin (Clontech), and recently published sequences for rhesus IL-2(8). After 35 cycles of amplification, agarose gel electrophoresis was performed on the PCR products. The identity of IL-2 bands was confirmed on Southern blots or on slot blots of the PCR products, using a biotinylated probe synthesized from published sequences (8).

Results

Routine histopathologic assessment of the biopsies showed the expected development of chronic inflammation and early granuloma formation over the course of the study. Comparison of the histologic features at each time point did not show substantial differences between the SIV[-] and [+] groups.

SIV[-] animals, however, showed a pattern of rapidly increasing CD4+ cell influx during the first week, reaching a median peak of 44% between days 5-14. Then declining steadily until day 27 and remained stable (Figure 1A). In contrast, in SIV[+] animals the median tissue percentage of CD4+ cells increased slowly until it reached a peak of 46% at 27 days, after which it declined to a level similar to that seen in SIV[-] animals (Figure A). median percentages of CD4+ cells in SIV[-] and SIV[+] groups were significantly different at 5 days ($p<0.025$) and at 27 days ($p<0.05$), but day 63 had become stablished at comparable levels which were not statistically different.

Specific lymphocyte proliferation in vitro in response to M. leprae antigens was observed in all SIV[-] animals during the first 4 weeks after M. leprae inoculation (Figure 1 B). The development of antigen responsiveness among SIV[+] animals lagged behind that of SIV[-] animals, and some of the surviving SIV[+] animals were unable to demonstrate lymphocyte responsiveness in vitro even after 15 weeks.
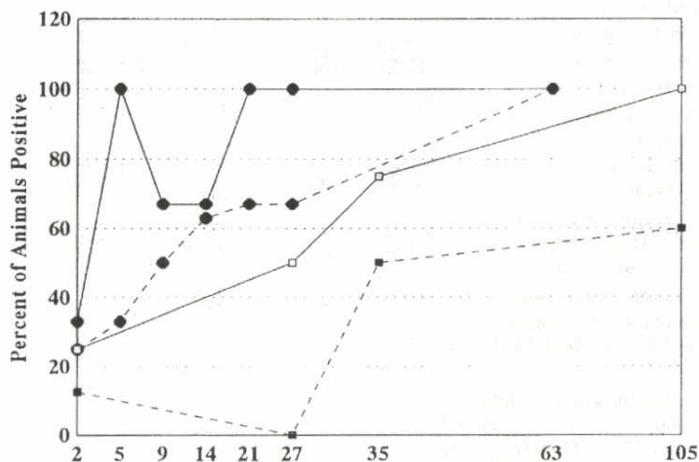
In SIV[-] animals, mRNA for IL-2 was detected at the inoculation site by day 5 in all until day 63 (Figure 1B). In SIV[+] animals, mRNA for IL-2 was detectable in only a few biopsies by day 5, but the percentage of biopsies positive for IL-2 mRNA increased steadily throughout the first month. By day 63 IL-2 message was detectable in biopsies from all surviving animals in this group, also.

Figure 1. Primary Response to M. *leprae* in SIV[-] and SIV+ Rhesus Monkeys.

A. CD4+ cells at inoculation sites. In SIV[-] animals (solid line) within one week, and the percentage of sites positive remained high through 2 months. In SIV[+] animals, (dotted line), the percentage of inoculation sites with detectable IL-2 message rose steadily to reach 100% by 2 months.

Systemic immune response to M. *leprae* antigens, measured as lymphocyte proliferation in vitro (squares) increased rapidly in SIV[-] (solid line) animals so that all were responsive by 105 days. The percentage of SIV[+] animals able to respond in vitro (dotted line) rose slowly and not all had become responsive by 105 days.
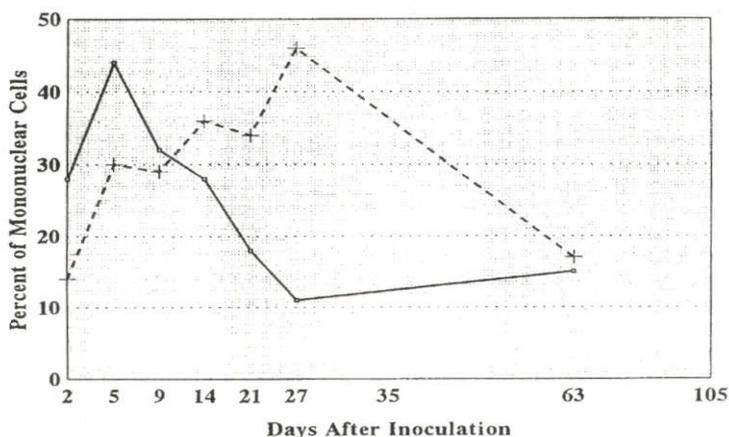
B. IL-2 mRNA at inoculation sites and lymphocyte transformation in vitro. IL-2 mRNA (solid circles) was observed at all inoculation sites in SIV[-] animals (solid line) within one week, and the percentage of sites positive remained high through 2 months. In SIV[+] animals, (dotted line), the percentage of inoculation sites with detectable IL-2 message rose steadily to reach 100% by 2 months.

Systemic immune response to M. leprae antigens, measured as lymphocyte proliferation in vitro increased rapidly in SIV[+] animals so that all were responsive by 105 days. The percentage os SIV[+] animals able to respond in vitro (dotted line) rose slowly and not all had become responsive by 105 days

Discussion

This is the first characterization of events occurring locally during the first month of the primary response to *M. leprae* in primates. The major preliminary findings of this study are that in healthy rhesus monkeys, *the 1^9 immune response to M. leprae* in the tissue is characterized by an early influx of CD4+ T cells and concomitant local production of IL-2 mRNA. These occur within 2 weeks and therefore precede the development of specific immunity to *M. leprae* antigens. These local responses, however, are followed by the development of specific, systemic CMI by 4 weeks, as measured by lymphocyte proliferation in response to *M. leprae* antigens in vitro.

In immunodeficient, SIV[+] animals, the influx of CD4+ cells into the inoculation site is delayed until approximately 4 weeks, as is the local production of detectable IL-2 mRNA. Notably, however, even while the number and percentage of CD4+ cells in the blood is declining, recruitment into the inoculation sites ultimately reached the same maximal percentage of CD4+ cells (approx. 45%), after which the percentage dropped in SIV[+] animals as it did in SIV[-] animals. Systemic CMI, as indicated by lymphocyte proliferation in

response to *M. leprae* antigens in vitro, was also delayed in SIV[+] animals. At the late' time point in this study (2 months), the percentages of CD4+ cells were the same in SIV[-] and SIV[+] groups, as has been described in human leprosy patients with or without AIDS (9).

The rhesus model offers a unique opportunity to examine the earliest, determining events in the primate response to viable (infectious) *M. leprae,* since these events cannot be studied in man. Co-infection models in rhesus monkeys may therefore be especially valuable in studying the immunologic aberrations induced by retroviruses which render the host so susceptible to mycobacteria (10). *M. leprae,* a pathogen of relatively low virulence, may offer a particularly useful tool in such investigations, since it does not overwhelm the host but does appear to elicit different patterns of response in the development of cellular immunity in SIV[-] and SIV[+] macaques.

Acknowledgments

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