Suppression of ADCC by Immune Complexes Formed in Vitro in Mycobacterium leprae-Infected Mice

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Received February 10, 1992; in revised form, October 9, 1992. Accepted November 5, 1992

Abstract: Antibody-dependent cellular cytotoxicity (ADCC) was assessed in mice infected experimentally with Mycobacterium leprae and injected simultaneously with in vitro-formed immune complexes (IC). Significant decrease in the ADCC function was observed in animals given IC at zero day (0dIC) and 3 months (3mIC) post inoculation with M. leprae, when ADCC activity was assessed at 3, 6 and 9 months period. From the data obtained we believe that ADCC is suppressed by IC formed in vitro.

Key words: ADCC, Immune complexes, Leprosy

Mycobacterium leprae, the causative microorganism of leprosy, occurs intracellularly and so cell-mediated immunity (CMI) is of prime importance for its elimination. When an antigen present on cell surface combines with its specific antibody, cell demise may occur by promoting contact with phagocytes. Cytotoxic killing may occur either (a) by reduction in surface charge (b) by opsonic adherence directly through Fc receptors, (c) by immune adherence through bound C3 or (d) by the activation of full complement system (10). Antibody-dependent cellular cytotoxicity (ADCC) is an operation of quite distinct cytotoxic mechanism. Non-specific killing occurs through an extracellular non-phagocytic mechanism involving non-sensitized lymphoreticular cells. The effector cells bind to target cells by specific receptors for Cγ2 and Cγ3 domains of IgG Fc. ADCC mechanism is exhibited by phagocytes, non-phagocytic myeloid cells and by large granular lymphocytes with Fc receptors dubbed the 'K' cells which are identical to natural killer (NK) cells (10). Human NK cells have been observed to possess potent antibacterial activity (3). Similar lytic activity against intracellular microorganism by human blood leucocytes has also been reported (1). The role of NK cells in leprosy is uncertain. Cytotoxic/lytic mechanism may be very important in containing lepra bacilli in leprosy patients. The importance of NK mechanism in the elimination of cells harboring M. leprae is highlighted by the finding of non-T cytotoxic cells influx at purified protein derivative (PPD)-induced delayed-type hypersensitivity (DTH) site in lepromatous leprosy (LL) patients (5). However, immune complexes (IC) formed due to the massive antigenic assault and an equally evoked antibody load could be deleterious to the functioning of the immune system. In an earlier study, we reported that ADCC was largely unaltered in M. leprae-infected animals (2). In the present study we have investigated the effect of IC formed in vitro on the ADCC activity in experimental leprosy. From the data obtained we believe that ADCC is suppressed by IC formed in vitro.

Materials and Methods

Swiss albino mice (Lacca strain) maintained in...
the Central Animal Facility of the Institute were employed for the present investigation. The strain has been found to be susceptible to *M. leprae* infection (13). Thymectomy was carried out at four weeks of age in the thymectomized/irradiated group, and inoculation of *M. leprae* (as described later on) was carried out a week later. Animals in the normal (unthymectomized) group were also age-matched. The experimental study, divided into two main phases, comprised animals not given IC (Phase I) and those to whom IC were administered (Phase II). The result of the Phase I study has already been published as mentioned above (2).

Phase II animals were given *in vitro*-formed IC either on zero day (0dIC batch) at 3 months (3mIC batch) or at 6 months (6mIC batch) after injection of *M. leprae* in the infected groups and at a corresponding period in the uninfected control groups. The following groups were thus formed:

**Group 1**: Normal control (NC) and thymectomized/irradiated control (TRC) uninfected animals given IC and subgrouped as follows:

a) NC/0dIC and TRC/0dIC (30 animals each).
b) NC/3mIC and TRC/3mIC (25 animals each).
c) NC/6mIC and TRC/6mIC (20 animals each).

**Group 2**: Normal infected (NI) and thymectomized/irradiated infected (TRI) animals were given IC and divided into the following batches:

a) NI/0dIC and TRI/0dIC (30 animals each).
b) NI/3mIC and TRI/3mIC (25 animals each).
c) NI/6mIC and TRI/6mIC (20 animals each).

*M. leprae* isolated from the skin biopsies of untreated lepromatous patients were inoculated into the right hind footpads (1 × 10^4 acid-fast bacilli (AFB/footpad) of all the animals in the infected groups by the method described earlier (14). Immune complexes prepared *in vitro* (6) as described below were administered (1 mg protein) in 0.3 ml of saline intravenously every week for a period of one month at different times as mentioned above. Five animals were sacrificed periodically at 3, 6 and 9 month periods from 0dIC batches, at 6 and 9 month periods from the 3mIC batches and in 6mIC batches at 9 months period. The data for the remaining period was obtained from Phase I study itself.

(i) Preparation of immune complexes. In brief, freeze-dried *M. leprae* (obtained as a gift from IMMLEP) were sonicated to prepare antigen and healthy rabbits were immunized with the sonicate to raise anti-*M. leprae* serum (AMLS). The antiserum was adsorbed with homogenized human skin tissue and also with *M. bovis* (BCG), *M. tuberculosis* (H37Rv) and *M. avium*. The production of antibodies against *M. leprae* antigens was confirmed by immunodiffusion. The zone of equivalence (ZE) was determined by incubating in a 37°C shaking water bath increasing quantities of *M. leprae* antigen (supernatant of sonicated *M. leprae*) with a fixed volume of AMLS. After overnight incubation at 4°C and ultracentrifugation, immune complexes were formed. Maximum precipitate (IC) was obtained with 240 µg of *M. leprae* antigen which was taken as ZE. Twice the ZE gave complexes in 2× antigen excess. The precipitate was washed thoroughly and a uniform suspension was made. IC in 2× antigen excess were administered to all the animals.

(ii) ADCC function. Lymphocytes for use as effector cells were obtained from the spleen of the sacrificed animals and viability of the cells was checked by Trypan Blue exclusion. ADCC was carried out according to the method described by Perlman and Perlman (8), employing antibody-coated and chromium-labeled fowl red blood cells (FRBC) as the target cells. Among the two controls that were set up, in one, the anti-FRBC serum was replaced by heat-inactivated normal rabbit serum for spontaneous release of isotope. In the other, the maximum release of isotope was determined by the addition of distilled water to the target cells. All tests were done in duplicate. The damage to the target cells by the effector cells was expressed as the percentage of the total radioactivity released from the target cells into the medium. Detailed methodology has been described in the earlier study (2).

**Results**

(a) ADCC Function (Table 1)

**0dIC batch.** The basal value for ADCC on day zero was 67.7 ± 0.9 for NC and 56.6 ± 1.3 for TRC batches respectively. The values obtained for NC/0dIC batch were 68.9 ± 1.7, 69.6 ± 2.6, and 66.1 ± 3.7 at the 3 study periods, respectively. Compared to the NC/0dIC batch, significant decrease in the cytotoxicity in NI/0dIC batch was
obtained at 3 (P<0.05) and 6 month (P<0.01) study only and no change was observed at 9 months. In TRC/OdIC batch the mean counts obtained during the three different periods of 3, 6, and 9 months were 58.7±1.9, 54.4±3.5, and 58.5±5.2, respectively. The decrease was significant (P<0.05) at 3 and 9 months study in the TRI/OdIC batch compared to TRC/OdIC and only at 9 months period (P<0.01) compared to NI/OdIC.

3mIC batch. Significant decrease in values (P<0.01) was evident in NI/3mIC batch at 9 month study period compared to NC/3mIC. No significant difference was seen between any other groups at any period of study.

6mIC batch. No significant change was seen between any of the group when IC were administered at 6 month period post inoculation with M. leprae.

(b) Acid-Fast Bacilli (AFB) Counts (Table 2)
The results of AFB counts have already been published earlier (15). In the NI/0dIC batch, establishment of infection was evident by as early as 3 months in contrast to NI group not administered immune complexes. A steep increase in the bacillary counts was seen at 6 months which remained static at 9 months. IC administration at 3 months did not bring about any appreciable increase in the bacterial counts compared to NI/0dIC batch. However, there was a significant increase in the AFB harvest at 9 months period in the 6mIC batch. Enhancement of infection was more evident in the immunosuppressed group (TRI) of animals administered IC.

Discussion
In the ADCC mechanism, non-sensitized effector cells bearing Fc receptors bind to antibody-coated target cells and induce their lysis. The ADCC mechanism was seen to be largely unaltered in the Phase I animals not administered IC with an increase in the cytotoxic activity observed in the lymphocytes of NI mice at the end stage of sacrifice (2).

In the 0dIC batch the lysis of target cells by cytotoxic cells was seen to be significantly decreased in both the groups of infected (NI and TRI) animals compared to uninfected (NC and...
The impaired CMI observed in human leprosy could be due to massive infection by *M. leprae*, although the possibility is not excluded that some constitutional difference might favor the initial development of the lepromatous rather than the tuberculoid form of the disease (9). No explanation for this difference of host behavior has been found as yet. Rook (11) suggests two possibilities. Firstly, there may be a delay before the onset of CMI, so that when it appears the bacilli are already established in vulnerable tissues which are subsequently damaged by inflammation. Secondly the CMI could be directed against antigenic components which are not released by live organisms, so that the immunological attack occurs in the wrong places, around dead or leaking bacilli.

The presence of circulating IC formed during the course of the disease with specific mycobacterial antigens and their evoked antibodies could similarly bring about a suppression of the ADCC function particularly in the LL patients. Thus the disease may be impossible to be contained by the immunological defense system of the body under these circumstances. There is a definite relation between the decreased ADCC and increased bacterial counts. The deficiency in NK activity seen in patients with ENL cannot be attributed to any single mechanism. There is a possibility that decreased ADCC and NK cell activity may be inhibited but it is to be noted that ADCC depression is present in all types of leprosy, whereas NK cell activity depression occurs not in stable but in IC-mediated ENL. However, decrease in ADCC is part of the general immune suppression seen after injection of IC, which in turn was responsible for the enhanced bacterial counts.

The Scientific Pool Grant given to one of the authors (C. Vaishnavi) by the CSIR, New Delhi is gratefully acknowledged. The authors also acknowledge the technical help given by Mrs. Harinderjit Kaur.

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