

THE IMMUNOLOGICAL ASPECTS OF LEPROSY

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We will discuss an area of great concern to those of us committed to the prevention and cure of leprosy, a disease which is rapidly reaching epidemic proportions in the third World, urgently spurring us on to investigate and act with all the means at our disposal. These means however are currently quite inadequate due to the limited available resources the governments of the underdeveloped countries can realistically allocate to this task.

It is estimated at present that perhaps as more than 10 million cases of leprosy exist throughout the world and that these patients are largely found within the Third World. It is a startling statistic.

The disease is predominantly found in the north regions of Brazil and in various African and Asian nations. These endemic areas share a prevalence rate of more than 10 patients per 1,000 inhabitants. However, even in our more highly-developed southeaster region, wich includes both of our most progressive cities, São Paulo and Rio de Janeiro, a mean rate of 2 patients per 1,000 inhabitants is the norm.

The time trends of leprosy in Brazil indicates that between 1954 and 1968, the number of leprosy cases increased only 4% a year presenting a descendent curve. From 1968 on, however the trends reveal an ascendent curve indicating an annual growth rate of 8% (Motta, C. an personal communication).

It is worth to say that Brazil's current population growth rate is over 3% a year, where as the time trends, indicate a leprosy growth rate of about 8% per annum, which is almost 3 times higher than the estimated population growth rate as a whole.

Leprosy is perhaps that human disease which most amply demonstrates a clinical spectrum directly related to the cell-mediated immune response.

One must take into account that the great majority of persons who have been exposed to *Mycobacterium leprae* probably develop effective immunity which arrests bacteriae growth or mantains it at a sub-clinical stage (Ridley, 1974).

In most persons natural healing occurs and no manifestations of the disease are detected. A very small percentage of people do in fact contract the disease frequently developing lesions designated as the indeterminate form. During the indeterninate phase, the disease may either move toward the highly-resistant tuberculoid form. Tuberculoid leprosy patients present a few well-defined skin lesions that histologically resemble delayed hipersensitivity granuloma (Ridley, 1974). Contrary to tuberculosis in which delayed hipersensitivity exist together with bacillary proliferation, in tuberculoid leprosy a low level or even total absence of bacilli is the rule. In the least resistant forms, namely lepromatous, the lesions are multiple and diffuse, and consist of histiocytes containing large numbers of bacilli. Lack of cellular immunity is the rule in lepromatous forms (Godal, 1978). In the medium intermediate forms, a continuous and histopathological spectrum termed borderline leprosy is observed in which the cellular immune response decreases progressively from borderline tuberculoide to borderline lepromatous (Bloom, 1986). Exception to this rule have been described and some authors have pointed out that borderline lepromatous patients with active lesions are immunologically more responsive than tuberculoid patients in whom silent lesions are observed (Bach et al., 1981).

Although the tuberculoid form is considered to be the most highly-resistant form of leprosy, the truly immune person who never develops lesions beyond the indeterminate stage has been found to display only T lymphocyte infiltration in the skin without granuloma formation.

An interesting clinical finding observed in the case of some borderline lepromatous forms is referred to as "reversal reaction" in which tuberculoid features develop with relatively rapid elimination of bacilli. A rapid increase in cellular-immunity appears to be involved and this is commonly known as "up grading reaction" (Ridley, 1969a, b).

It is also accepted that some borderline patients may move towards the lepromatous

pole after having interrupted their treatment; this phenomena is called "downgrading reaction" (Ridley, 1986).

The availability of monoclonal antibodies specific to human leucocyte antigens has made it possible to identify subsets of these cells in blood and within the leprosy lesions.

There has been general agreement as to the distribution of the T-cell subsets within the lesions. The number of OKT4 (Leu 3a) positive cells has been found to be higher at the tuberculoid pole of the spectrum while drastically lower at the lepromatous pole whereas the number of OKT8 positive cells remains almost constant in all clinical forms (Narayanan et al., 1983).

Therefore from our studies the ability to control bacillary multiplication in the tuberculoid forms seems to be correlated with the absolute number of OKT4, Leu 3a positive cells in the lesion but was not related to the absolute number of OKT8, Leu 2a positive cells (Van Voorhis et al., 1982).

Also depletion of the OKT8, Leu 2a positive "suppressor" cells was unable to reverse unresponsiveness to *M. leprae* in mononuclear blood cell culture obtained from leprosy patients (Kaplan et al., 1985). This finding agrees with the lack of correlation between numbers of OKT8 positive cells in the lesions and the immune status of leprosy patients. This suggests that the defect may lie in the OKT4 cell function and that this defect may be crucial factor in determining the clinical form of the disease. Since the monoclonal antibodies define cell types according to phenotypic surface markers, the actual function of these cells remains unknown.

Why the helper T-cell is absent in lepromatous lesions considering that normal numbers exist in the blood is not yet known.

Recently, an increase of OKT4, Leu 3a positive cells together with other features of delayed type hypersensitivity have been achieved by Gilla Kaplan and her collaborators by injecting gamma interferon into lepromatous lesion (Kaplan et al., 1987). This has resulted in an apparent decrease in acid-fast bacilli. The capacity of gamma interferon to induce gene expression in some tissue cells suggests a more complex response in the milieu of the delayed type hypersensitivity (Nathan et al., 1986). The role of the local environment in delayed type hypersensitivity may be such that tuberculoid

granuloma are frequently observed in the lymph nodes of lepromatous patients and not in the skin.

These "in vivo" observations have led us to investigate the immune process using "in vitro" tests to evaluate T-cell reactivity of leprosy patients using lymphoproliferative tests and gamma interferon production assays.

Peripheral blood mononuclear cells isolated on Fycoll gradient were cultured in the presence of *M. leprae*. At the end of 5 days tritiated thymidine was added to measure proliferative response or the supernatant was removed to quantitate the gamma interferon production. The *M. leprae* used as antigen in all assays were donated by Dr. Nadia Nogueira and Dr. Gilla Kaplan. Both samples were obtained from IMMLEP.

Gamma interferon was measured using an assay kit (IMRC Corp. Centocor Malvern PA), in collaboration with Dr. Nadia Nogueira.

We consider less than 50U/ml as no response; more than 50 and less than 100 as low response and more than 100U/ml as a high response.

Initially the cellular immune response of 98 leprosy patients to *M. leprae* was studied; 52 of these patients were not under treatment. Patients were classified clinically and histologically according to the Ridley-Jopling scale (1966). Patient data was distributed as follows: 9 tuberculoid, 17 borderline tuberculoid, 5 mid borderline, 21 borderline lepromatous, 38 polar lepromatous and 8 indeterminate patients.

Although the lymphoproliferative assay using tritiated thymidine incorporation is in common use to evaluate cellular immunity, its correlation with the ability to activate macrophages seems to be an indirect one.

Much evidence has indicated that gamma interferon is an effective mediator in lymphocyte-macrophage interactions and in the effector end of the cellular immune response. It appears quite certain that gamma interferon can stimulate both hydrogen peroxide formation and intracellular killing of bacteria (Horwitz et al., 1984).

In general, lepromatous patients classified as low responders to *M. leprae* on antigen-induced proliferation tests also failed to produce gamma interferon. However, individual comparison between the two tests did not always concur. Some patients classified as low re-

sponders according to the lymphoproliferative assay released high levels of gamma interferon.

Similar to what was observed in lymphoproliferative tests, the gamma interferon assay also showed an increased mean in the spectrum ranging from lepromatous to tuberculoid forms. We consider less than 50U/ml as no response, more than 50 and less than 100U/ml as low response and more than 100U/ml as a high response.

In polar lepromatous patients the mean of gamma interferon was 34.55U/ml; in borderline lepromatous the mean was 75.05; in mid borderline, the mean was 65.75; in borderline tuberculoid the mean was 92.68; in polar tuberculoid, the mean was 132.45; and in the indeterminate form the mean was 109.28. With the exception of the polar tuberculoid form the standard deviation of the means was large in comparison to the actual mean values in all forms.

Three polar lepromatous patients demonstrated high levels of gamma interferon production, although reversal reaction could be clinically proven in only one patient. Studies are being carried out in an effort to more fully understand how the immune response functions during clinical episodes of reaction.

In order to evaluate possible cross reactions between the response *M. leprae* and other mycobacterium among these leprosy patients we quantitated the gamma interferon in the supernatant removed from blood mononuclear cell cultures stimulated with BCG (from the Pasteur Institute).

The level of gamma interferon in each group was similar to that observed when we used *M. leprae* as an antigen. The tuberculoid patients presented higher levels than the lepromatous ones. Five of the 37 patients considered to be low responders to *M. leprae* released high levels of interferon to BCG. These results suggest important cross reactions between the antigens present in both bacteria. Nevertheless, the individual variability to BCG antigens was greater than that observed to *M. leprae*. This can be demonstrated by the very large standard deviation seen in all clinical forms. It is difficult to ascertain whether or not vaccination previous infection or contact with tuberculosis is responsible for such a variable response.

Hypothetically we might say that some common epitopes present in both mycobacteria might be able to stimulate suppressor mechanism, however this does not explain the higher levels

of gamma interferon that were produced as a result of BCG stimulus than to *M. leprae*.

Considering that the high risk individuals are household contacts of patients with the disease, we decided to analyse the gamma interferon release in 155 contacts. 44 contacts (28.3%) were classified as non responders with a mean of 20.82U/ml; 26 contacts (16.7%) were classified as low responders with mean of 75.78U/ml; 85 contacts (54.8%) were classified as high responders with a mean of 198.15U/ml.

It is unlikely that these non responder contacts had never been previously exposed to *M. leprae* antigen. Thus a current subclinical infection capable of triggering suppressor mechanisms can not be ruled out. Similar to the leprosy patients the contacts have also shown a correlation coefficient higher than 0.5 when the response to *M. leprae* and BCG were plotted on the same scale. Perhaps the most important observation made in this connection was that the majority of nonresponders contacts was also unresponsive to BCG and this same correlation existed for high responders. This correlations suggest that an important cross reaction exists in the mechanisms controlling defense against mycobacteria even before the onset of clinically detectable disease. Whether this reflects active suppression induced by low doses of leprosy bacilli or is a consequence of innate, defective resistance mechanisms remains to be clarified.

The correlation between the gamma interferon release by the blood mononuclear cells and skin tests was also analysed in leprosy patients and contacts. A correlation of 82.8% was seen when lepromin tests and gamma interferon releases to *M. leprae* were compared in leprosy patients. Among the high responder contacts this correlation was also observed, while among the low responder contacts this correlation however, was not observed. Forty-one contacts with low levels of interferon had positive lepromin tests. These results indicate that the delayed hypersensitivity expressed by the skin tests may not express an effective immune response in this contact group or the skin test is more sensitive to diagnose subclinical infection than the Y-IFN release. This might explain the contradictory results obtained when lepromin positive tests were used to indicate the level of resistance to *M. leprae* infection.

In light of these results we might speculate that suppressor response seem in some patients and even in the contacts is not a stable response.

Immune intervention may help to direct it toward better resistance. Some authors have achieved, "in vitro", a reversal of low responsiveness through the following procedure: 1) adding IL-2 to a peripheral blood mononuclear cell culture (Haregewoin et al., 1983; Nogueira et al., 1983); 2) maintaining these cells in medium without antigen for 48 hours in culture before being triggered by *M. leprae* (Mohaghepour et al., 1987) and 3) in a few instances even depleting population of cell subsets (Kangh, 1982).

Common antigens found in other mycobacteria may be very useful in triggering a stimulatory response. We are at present interested in pursuing investigations which might lead to a reversal of low responsiveness to *M. leprae* "in vitro" and also to see if any correlation can be obtained with the response "in vivo". In this connection we have been engaged in using BCG as a primary stimulus in peripheral blood mononuclear cell cultures from low responder patients and contacts.

In addition are indications that immune intervention "in vivo" could provide higher resistance and protection from infection in contacts. In studies carried out in Africa using vaccination with BCG (Stanley et al., 1981) conferred protection against leprosy disease that varied from 20% in Bruma up 80% seen in Uganda. In Venezuela studies the vaccination with the soluble antigen from *M. leprae* and BCG seems that also confer protection. Many others vaccination trials are being carried out in this field and experimental basis is necessary in order to be accepted as a recommendation of controlling the spread of leprosy in the endemic areas.

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