IMMUNOPROPHYLAXIS IN LEPROSY

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LEPROSY, which is widely prevalent in several developing countries, is a chronic systemic infectious disease that mainly affects skin, nerves and mucous membranes, particularly of the upper respiratory tract. On the basis of clinico-pathological features, the disease has been classified into 2 polar, namely tuberculoid (TT) and lepromatous (LL), and 3 intermediate forms. This five-stage classification, originally proposed by Ridley and Jopling, is now widely used. Figure 1 depicts the main cellular constituents of the tissue reaction, and immunological features of different forms of leprosy. In the TT variety, which is self-limiting, the patients exhibit 1-2 hypopigmented anaesthetic patch/s. The tissue reaction essentially shows lymphocyte-rich well formed epithelioid cell granulomas containing giant cells. Bacilli, if at all, are rarely seen and the patients exhibit strong cell mediated immunity (CMI) against leprosy antigens but the circulating antibody levels are low. The LL form, on the other hand, is characterized by disseminated lesions. The tissues are laden with M. leprae and show only collection of foamy macrophages containing the bacilli. Lymphocytes are often conspicuous by their absence. A persistent anergy to antigens of M. leprae, not corrected even after years of drug therapy, is a characteristic feature of the LL patients. However, circulating anti-M. leprae antibodies are present in large amounts. Depression of the CMI appears to be highly specific for M. leprae antigens and the response to other antigens is by and large unaffected.

HOST DEFENCE MECHANISMS

Within the leprosy spectrum, the intensity of the CMI is inversely proportional to bacillary load and progressively increases as one proceeds from LL to TT end of the spectrum. The exact mechanism of the defective CMI is still obscure. It could be (a) genetic and/or (b) due to alterations in the host immune system. It is essential to understand the importance of each of these factors, for immunoprophylaxis would be very difficult to achieve, if the defect is genetic.

Genetic factors:

There is hardly any doubt that leprosy is caused by a microbe. However, differing susceptibility and patterns of the disease observed in different geographic areas do suggest genetic predisposition. This is also strengthened by the observations of the familial clustering of leprosy patients and concordance seen with reference to the type of the disease in identical twins. Recently it has been proposed that the mechanisms of killing of intracellular parasites may be analogous to those involved in graft rejection, in which histocompatibility system, which is genetically determined, plays important role.

Association of IR (Immune Regulatory) genes with H-2 complex in mice is well established. HLA, which is the human counter part of the mouse MHC, may likewise contain IR genes that control immune response to M. leprae. With this

Figure 1. Cellular components of tissue reactions and immunological features in different forms of leprosy.
idea HLA patterns have been investigated in leprosy patients by several groups. But, no clear cut picture has yet emerged. It is possible that the existing genetic markers are not adequate to pinpoint the specific defect.

**Immunology of Leprosy:**

(a) *Humoral factors:* High levels of circulating antigens, antibodies and antigen-antibody complexes are known to suppress CMI. This is particularly recognized in tumour immunology. The LL patients frequently exhibit bacteremia and also have circulating immune complexes. Although high levels of circulating *M. leprae* antibodies have been reported, the sera from LL patients do not suppress *in vitro* lymphocyte transformation to *M. leprae*. Also, the fact that the defect is observed even in patients, in whom the bacillary load has been markedly reduced by drugs, speaks against this possibility. Further, existence of persistently lepromin negative individuals, in population not exposed to leprosy, likewise does not favour this contention.

(b) *Macrophages:* Macrophages not only function as phagocytic cells but also play a key role in host immune responses. It has been proposed that, in LL patients, the basic defect resides in macrophages which are unable to kill the bacilli. However, except for a few groups, by and large no such defect has yet been convincingly demonstrated. Macrophages are also involved in antigen processing and in initiation and regulation of humoral and cellular immune responses. It is possible that certain critical enzymes, that are essential for proper processing of *M. leprae* antigens, so as to make them strong immunogens, are lacking in macrophages of LL patients. However, search for a specific enzyme defect has been unsuccessful so far.

(c) *Modulation in immuno-regulation:* *T* lymphocytes are not a homogeneous population of cells but, depending on the function, are now sub-classified into *T* helper, *T* suppressor, *Tkiller* and *T* uncommitted. Several workers have demonstrated generation of suppressor cells during the course of infection by *M. lepraemurium* in mice. Recently Mehra *et al.* have reported lepromin induced suppression of Con-A stimulation in LL and BT/BL patients but not in TT or normal individuals. Using antithymocyte globulin, it is shown that the suppressor cells belong to TH2 subclass of *T*-cells. Many questions need to be answered before full implications of these observations, in terms of human leprosy, are accepted. Thus it would be essential to know whether the phenomenon is specific for *M. leprae*. For this purpose, it would be necessary to show that other mycobacteria, such as BCG, do not bring about similar changes. Interestingly, using identical technique, Nath and Singh have failed to demonstrate generation of suppressor cells in LL cases. Similarly, using a slightly different system, Stoner *et al.* have failed to show immune suppression in lepromatous patients. Thus conflicting results have been obtained with reference to this rather attractive hypothesis that the immune defect in LL is due to increased suppressor cell activity.

(d) *Absence of reactive clones:* A reduction in the *T* cells has been reported by a number of workers. Washed *T* cells from LL patients cultivated in the presence of autologous plasma or plasma from normal individuals are unable to respond to *M. leprae* antigens. Also plasma of LL patients is unable to suppress the lymphocyte transformation in TT patients. These observations indicate that the basic defect is cellular and that no serum factors are involved. According to Godal *et al.*, the main defect is the 'central failure' due to lack of specifically reacting *T* cells. This would explain continuation of anergy even when patients are almost completely treated. Also the observation of anergy existing in population never exposed to *M. leprae* antigens will be in favour of this view which would probably be the most acceptable proposition. However, CMI response is polyclonal, involving more cultivated, large quantities are now available from difficult to visualize that all clones are genetically absent.

CONTROL STRATEGIES

Control strategies in any infectious disorder are aimed at (i) attacking the causative agent and
reducing the bacillary load, mostly through drugs, and (ii) increasing the host resistance by stimulation of the immune system with vaccines.

Anti-leprosy Drugs:

A number of drugs, which are effective against \textit{M. leprae}, are now available. Drugs reduce bacillary load in the patient and, since man is possibly the only reservoir for the leprosy germs, it should be theoretically possible to wipe out leprosy by treatment of patients. However, this modality faces several problems. In the first instance, because of a very long incubation period, the patient is diagnosed late and by that time he would have spread the disease to others. Also, drug resistance is occurring at an alarmingly high rate\textsuperscript{26}. In one study from Ethiopia, primary resistance to DDS was observed in 51\% of the cases\textsuperscript{26}. Resistance to other drugs has also been reported. There is also the problem of persistence of the organisms in the patients' tissues in spite of years of drug therapy\textsuperscript{26}. Moreover, at the community level, all susceptible individuals have to take drugs at least so long as they reside in the endemic area. Chemoprophylaxis is therefore an infeasible proposition.

\section*{IMMUNOPROPHYLAXIS—VACCINES}

Development of an anti-leprosy vaccine would involve, in the first place, proper selection of an immunogenic microbe. Further, it would be essential to show that the vaccine offers protection against \textit{M. leprae} in suitable animal models and stimulates protective immunity in susceptible subjects.

Selection of the Microbe:

In general, attenuated live organisms are most effective. Although \textit{M. leprae} have not yet been cultivated, large quantities are now available from armadillo. The bacilli recovered from armadillo (\textit{M. leprae} A) are not only antigenically similar to \textit{M. leprae} but also exhibit a growth pattern characteristic of the latter in the mouse foot-pad\textsuperscript{27,28}. Lepromins prepared from the germs obtained from patients as well as armadillo give almost identical skin reactions\textsuperscript{29}. Further, leprosy has been successfully induced in monkeys using \textit{M. leprae} grown in armadillo\textsuperscript{30}. \textit{M. leprae} A thus meets the requirement of Koch's postulates for a causative agent. In the present state of our knowledge \textit{M. leprae} A is considered to be identical to \textit{M. leprae}. For obvious reasons, only killed bacilli, which should be free from armadillo tissue proteins, could be used in vaccine preparation. \textit{M. leprae} is perhaps antigenically the weakest mycobacteria, pathogenic to man\textsuperscript{31}. Attempts could be made to enhance its antigenicity by suitable chemical treatment. Yet another approach could be to use a live or killed cultivable non-pathogenic mycobacterium that cross reacts with \textit{M. leprae}, with reference to CMI antigens that induce protective immunity.

\section*{Animal Models:}

\textit{Mouse foot-pad:} Pioneering work of Shepard\textsuperscript{32} has shown that \textit{M. leprae} grow, to a limited extent, in the mouse foot-pad. An interesting feature of the mouse model is that irrespective of the initial inoculum, the bacilli reach a plateau at $1 \times 10^8$ per foot-pad\textsuperscript{32}. Higher growth curves are obtained in \textit{T}-deprived mice, in which levels of $1 \times 10^9$ per foot-pad are observed and even disseminated lesions are seen\textsuperscript{33}. The growth pattern is characteristic of \textit{M. leprae} and is not exhibited by any other organism. Using this technique, Shepard has shown protection in mice given BCG as well as killed \textit{M. leprae}\textsuperscript{34}. Although mouse foot-pad is an excellent model, particularly to study drug resistance and viability of the germs, its utility for testing of vaccine is restricted. Rook\textsuperscript{35} has recently commented upon some of the limitations of the model.

\textit{Armadillo:} Kirchheimer and Storr\textsuperscript{36} reported that 9 banded armadillo permitted enormous multiplication of \textit{M. leprae} and developed lesions similar to those seen in man. However, only 40\% of the animals develop leprosy and there is no way as yet to identify the susceptible population of armadillo. Vaccination with heat killed \textit{M. leprae} A administered in Freund's
incomplete adjuvant gave protection to the animals. All animals used in the study could be sensitized to *M. leprae* A. What would be the response of those which might be unable to develop delayed hypersensitivity to *M. leprae* A needs to be investigated. Such animals are yet to be found.

**Limitations of laboratory models:** In man, the vaccine is needed most, for those who exhibit anergy specifically to *M. leprae*. Immunologically non-responsive animals capable of permitting almost unrestricted bacillary growth have not yet been found. It is obvious that there is no good model of human leprosy. As such extrapolation of the laboratory data to man would be extremely difficult. Stanford is right in stating that the efficacy of the anti-leprosy vaccine should be tested in the only animal that is susceptible to leprosy: Man.

**DETERMINANTS OF PROTECTIVE IMMUNITY**

Leprosy has a very long incubation period. As such, it would take years before immunoprophylactic effects, if any, of a vaccine become evident. Before initiating field trials it would, therefore, be essential to establish that the vaccine is able to bring about, in susceptible subjects, immune changes that are consistent with protective immunity. The determinants of protective immunity are often difficult to be defined. Pioneering work of Mackness has established the importance of CMI in handling of intracellular parasites. The available evidence, both clinical and epidemiological, clearly shows that CMI is also the main defence mechanism against *M. leprae* and that circulating antibodies have little role.

Amongst the laboratory parameters of CMI, lymphocyte transformation test (LTT), developed by Godal et al. correlates well with hypersensitivity. But it is not a measure of protective immunity. Also the LTT is not suitable for field studies.

Skin reaction, Mitsuda type, to particulate antigens of leprosy germs, on the other hand, has excellent correlation with the capacity of the host to handle *M. leprae*. Thus, as mentioned earlier, within the leprosy spectrum, the pauci-bacillary forms are associated with a positive reaction, while the patients suffering from multibacillary forms are consistently lepromin negative. Epidemiological studies, likewise indicate that the Mitsuda negative individuals, in endemic area, run high risk of contracting the multibacillary forms. Dharmendra and Chatterjee, studied incidence of leprosy in 587 adults, who were subjected to lepromin test, over a period of 15 years. Only 17 (3.24%) of the 524, who were lepromin positive, developed leprosy; all non-lepromatous type. The incidence of the disease in the 63 lepromin negative individuals was not only 8 times but 80% of the affected ones developed the LL variety. Similar conclusions were drawn by Walters in his study of BCG trials in Burma.

A simple, and probably, more convenient 48 hr skin test, based on reaction to soluble protein antigens (SPA) of *M. leprae*, has been developed by Convit et al. Unlike lepromin, the test does not lead to sensitization. However, its relationship to protective immunity is yet to be established.

In the existing state of our knowledge, it appears, that amongst all-the available laboratory parameters, the Mitsuda reaction has the best correlation with protective immunity both in patients and in healthy subjects.

**Anti-leprosy Vaccines:**

So far, the following vaccines have been used:

1. BCG
2. *M. leprae* A (killed) + BCG
3. ICRC (killed).

The first one has been used on mass scale while the last two, which are now ready for field trials, have given promising results in patients and susceptible subjects.

**BCG:** There is considerable antigenic cross reactivity between BCG and *M. leprae* with reference to sero-antigens. Even in LTT some cross reactivity has been observed. Epidemiological studies have shown that BCG is able to bring about lepromin conversion in a number of individuals. As mentioned earlier, BCG offers pro-
tection against *M. leprae* in mouse foot-pad\(^{34}\). For these reasons, BCG has been used in immunoprophylaxis of leprosy in man. The results are contradictory\(^{44}\). For example some protection was observed in Uganda but not in Burma, for which several explanations have been offered including the possibility of infection with cross reacting environmental mycobacteria\(^{50}\).

**Vaccine containing a mixture of *M. leprae* A and BCG:** Convit et al.\(^{51}\) made a very interesting observation. They showed that, in patients of indeterminate leprosy, intracutaneous administration of killed *M. leprae* resulted in formation of poor granuloma consisting of only macrophages and the bacillary clearance was extremely slow. However, when a mixture of *M. leprae* and BCG was injected, a totally different picture was seen—the granuloma was well formed and the mycobacteria were quickly eliminated. This observation marked the beginning of a series of well planned experiments that culminated in the formulation of the concept of a vaccine containing a mixture of killed *M. leprae* A + BCG\(^{52}\).

Several years ago Hanks et al.\(^{53}\) had demonstrated, that, in mice, a mixture of BCG plus *M. lepraemurium* induced better immunity than when either of the organisms was given alone. This would probably explain why a mixture of BCG and killed *M. leprae* is effective when neither of them given alone is immunogenic to the patients\(^{52}\).

The vaccine has been tried in a number of patients suffering from different forms of leprosy and also their lepromin negative contacts who would represent a high risk susceptible group\(^{45}\). Skin reaction to SPA has been mainly used as an index of immunity. In addition, in a few cases TTT to lepromin was carried out. Besides clinical observations, morphologic changes in the lesions were also studied. The data are summarised and compared with those obtained with ICRC anti-leprosy vaccine (described later) in table 1.

An interesting feature of the study was that progressively increasing number of patients exhibited a positive SPA reaction\(^{44}\). Thus no conversion were observed in active BL/LL 6 months post-vaccination. However, 20% and 38% patients exhibited a positive SPA reaction between 6-18 months and beyond 18 months respectively. Similar trend was observed in inactive BL/LL. Very high conversion rates (97%) were observed in the indeterminate group. In the majority of the patients, the vaccine had to be repeated 3–4 times, at 3 months interval, before a conversion could be observed. However, most of the contacts were converted within 6 months with only one dose.

In vaccinated patients, there was a reduction in the bacillary load and the tissue reaction presented a picture of 'up-graded' lesions with accumulation of lymphocytes, and macrophages acquired epithelial characters. The vaccinated patients thus showed marked clinical, pathological and immunological improvement. In fact in about 27% of active BL/LL cases, reversal reaction was also recorded.

**ICRC—'Anti-leprosy' Vaccine:** ICRC is a cultivable slow growing mycobacterium that has been repeatedly grown from human leproma since 1958. ICRC lacks antigen 2, which is amongst the most stable and reproducible antigen of *M. leprae*. Likewise, it lacks some determinants of 4 that are specific for *M. leprae*. (Closs, O., personal communication). It is thus evident that, in the present state of our knowledge, ICRC is not *M. leprae*. On the basis of sero-antigens, it has been shown to belong to *M. Avium Intracellulare* group\(^{46}\). However, the sero-antigens have little role to play, in protective immunity in leprosy. Studies carried out, both in man (patients as well as healthy subjects\(^{54},^{55}\)) and in laboratory animals\(^{56}\), have shown that ICRC cross reacts with *M. leprae* with reference to CMI antigens.

Recently, a vaccine has been prepared from ICRC killed by gamma irradiation. After limited studies in mice, in which it offered protection against *M. leprae*, the vaccine has been given to a number of leprosy patients and contacts of lepromatous (LL) cases\(^{46},^{47}\). The results are summarized and compared with those obtained by Convit et al.\(^{45}\) (table 1).

All vaccinated subjects, both patients and contacts, developed ulcer at the vaccination site with enlargement of regional lymphnodes. The
Ulcér healed with local treatment. ENL was observed in 30% of LL patients (mostly in high index cases with Bl of 3+ or more) 2–3 weeks after vaccination. The repeat lepromin was performed as and when the patients reported to the hospital anywhere between 2–10 months after vaccination. Lepromin conversion was observed in 57.7% and 90% LL and BB/BL patients respectively. Lepromin test was repeated in lepromin negative contacts 6–8 weeks post-vaccination; 92% exhibited lepromin conversion.

Significant changes were observed in the morphology of skin lesions in vaccinated patients. There was loosening of the granuloma and a reduction in the number of macrophages which were vacuolated and foamy. The granuloma also showed infiltration with lymphocytes and a reduction in the tissue bacillary load. 'Reversal' reaction with up-grading of lesions has been observed, so far, in 5 patients.

The fact that better conversions are observed in the BB/BL patients should not surprising, since

**Table 1**

Comparison of anti-leprosy vaccines

**Effects of chlorophenicol on growth, acidity and invertase activity from A. niger**

<table>
<thead>
<tr>
<th>Convit et al. (Venezuela)</th>
<th>Deo et al. (India)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition and bacillary concentration</strong></td>
<td><strong>ICRC (killed by γ-irradiation) 25–50 μg (0.5–1 × 10⁶ approx.)</strong></td>
</tr>
<tr>
<td>Mixture of <em>M. leprae</em> A (heat killed) 6 × 10⁸ BCG (live) 1 × 10⁶ (0.2 mg)</td>
<td><strong>ICRC</strong></td>
</tr>
<tr>
<td><strong>Source</strong></td>
<td><strong>BCG</strong></td>
</tr>
<tr>
<td><em>M. leprae</em> A — From Armadillo</td>
<td><strong>Cultivable</strong></td>
</tr>
<tr>
<td><strong>Volume of each dose</strong></td>
<td><strong>0.1 ml.</strong></td>
</tr>
<tr>
<td>0.5 ml.</td>
<td><strong>Single in the deltoid region.</strong></td>
</tr>
<tr>
<td><strong>Site/s</strong></td>
<td><strong>(Intradermal)</strong></td>
</tr>
<tr>
<td>Multiple</td>
<td><strong>(Intradermal)</strong></td>
</tr>
<tr>
<td>(Intradermal)</td>
<td><strong>Mitsuda (3–4 weeks)</strong></td>
</tr>
<tr>
<td><strong>Immune Conversion</strong></td>
<td><strong>58% in LL (one shot — 71)</strong>*</td>
</tr>
<tr>
<td><strong>BL/LL (Active)</strong></td>
<td><strong>90% in BB/BL (one shot — 11)</strong></td>
</tr>
<tr>
<td>38% (Multiple Injections — 259)*</td>
<td><strong>92%</strong></td>
</tr>
<tr>
<td>63% (Multiple Injections — 113)</td>
<td><strong>(one shot — 12)</strong></td>
</tr>
<tr>
<td>97% (Multiple Injections — 32)</td>
<td></td>
</tr>
<tr>
<td>100% (Single shot mostly — 25)</td>
<td></td>
</tr>
<tr>
<td><strong>Reactions</strong></td>
<td><strong>30% of LL High index (Bl &gt; 3+)</strong></td>
</tr>
<tr>
<td><strong>i) ENL</strong></td>
<td><strong>In 12% of the lepromin converted</strong></td>
</tr>
<tr>
<td>In a number of cases</td>
<td></td>
</tr>
<tr>
<td><strong>ii) Reversal reaction</strong></td>
<td><strong>Extremely rare, transient, easily controllable</strong></td>
</tr>
<tr>
<td>In 27% of active BL/LL</td>
<td></td>
</tr>
<tr>
<td><strong>Fresh neurologial lesions</strong></td>
<td><strong>Rare, easily controllable</strong></td>
</tr>
<tr>
<td><strong>Likely problems</strong></td>
<td><strong>No such problem</strong></td>
</tr>
<tr>
<td>Possible contamination with armadillo proteins</td>
<td>Studies so far have revealed no natural sensitization to ICRC.</td>
</tr>
<tr>
<td>Use of BCG in sensitized population may produce severe local reaction.</td>
<td>Readily acceptable. Only one shot is required.</td>
</tr>
<tr>
<td>Acceptability: May face problems because of multiple sites of injections. Multiple doses are needed in patients.</td>
<td></td>
</tr>
<tr>
<td><strong>Cost (per dose)</strong></td>
<td><strong>£ 0.01 or Rs. 0.15</strong></td>
</tr>
<tr>
<td>£ 1 or Rs. 18/-</td>
<td><strong>£ 1 or Rs. 18/-</strong></td>
</tr>
</tbody>
</table>

**Notes:**

SPA — Skin Reaction to Soluble Protein Antigen. Relation to protective immunity NOT established.

Mitsuda: Excellent correlation with protective immunity.

* Figures in Parentheses denote number of patients/contacts who participated in the study.
these patients, although lepromin negative, are potentially at a higher level of immunity as compared to the LL. Similar observations have been made by Convit et al.\textsuperscript{45}

The mechanism of action of the ICRC antileprosy vaccine needs to be fully investigated. The vaccine could provide strong cross reacting antigens which could activate appropriate clones breaking the 'tolerance'. Alternatively the vaccine could act by enhancing the 'helper' cells, or by suppressing the 'suppressor' cells.

About 40\% of the LL patients given ICRC antileprosy vaccine, could not be converted to lepromin positivity\textsuperscript{46}. Convit et al.\textsuperscript{45} have found a similar group even after repeated administration of their vaccine. Genetic mechanisms have been incriminated in the pathogenesis of leprosy\textsuperscript{10}. It would be interesting to investigate the genetic make-up, including HLA-pattern, of the 'non-responders' to the two vaccines to find out if they represent genetically distinct groups.

**CONCLUDING REMARKS**

Although leprosy was shown to be the first human disorder caused by a microbe, the disease still remains unconquered. Effective drugs are now available but drug resistance is occurring at an alarmingly high rate. Moreover, drugs could hardly be used in mass prophylaxis. Global attempts are therefore, being made to develop anti-leprosy vaccine which would act by enhancing the host-defence mechanisms. Although *M. lepraee* has not been cultivated so far, large quantities could be obtained for vaccine preparation, from infected armadillos which permit profuse growth of the organisms. Alternatively, cultivable microbes, cross reacting with *M. lepraee* with reference to cMI-antigens, could also be used. So far, three vaccines containing (i) BCG alone, (ii) *M. lepraee* A + BCG and (iii) ICRC have been tried on man. Field trials with BCG have given mixed results. The last two vaccines, which have given promising results both in patients and susceptible subjects, are now ready for field trials.