The lymphocyte transformation test in leprosy with special reference to its use in epidemiology

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Ideally the lymphocyte transformation test (LTT) is an in vitro test for cell mediated immunity (CMI) to foreign antigens, as suggested when the test was introduced about 20 years ago (1). It is based on the observation that an early event in the immune response is a selective multiplication of lymphocytes with receptors for a given antigen. However, the situation for a low number of peripheral blood lymphocytes caught by chance at an arbitrary time and grown in vitro is quite different from the situation in vivo where lymphocytes are exposed to the antigens in a "natural" environment which is an integral part of an intact immune apparatus (2). In addition, the in vitro test is highly sensitive to many "irrelevant" influences from pH, drugs, foreign serum etc. (3). Results thus have to be interpreted with maximum caution.

Biological events in the LTT:

1) The major phases:
   a) Macrophages phagocytose and digest the mycobacteria. To stimulate lymphocytes specifically, they often have to "develop" the antigen(s) and present them as an integral part of their membrane.
   b) The result of such antigen presentation is partly dependent upon the anatomical location of the antigen-presenting cell and the tissue antigens on the collaborating macrophage/lymphocyte.
   c) Lymphocytes stimulated by the macrophages then increase RNA- and protein synthesis followed by DNA synthesis, increase in size to the characteristic "blastoid lymphocytes" (from which the test got its name) and finally go into mitosis. The outcome of stimulation can be recorded in different ways. The number of large "blastoid transformed" cells can be counted directly as a percentage of the total number of lymphocytes, the size distribution in the culture can be recorded electronically, or radioactively labeled metabolites can be added in the final
stage of culture and the incorporation counted to quantitate the protein, RNA or DNA synthesis.

Disturbances at any of these phases can lead to a "negative result" in the LTT (no significant difference between cultures with and without antigen present). This is often interpreted, without further analysis, as a lack of specifically responsive lymphocytes. Obviously this is dangerous.

2) Macrophage handling of M. leprae:
Macrophages are necessary for lymphocyte stimulation by antigen (4). No major phagocytosis defect has been found in monocyte derived macrophages from leprosy patients. A reported chemotactic defect of lepromatous granulocytes (5) seems irrelevant unless the passage through the granulocytes could be found essential for macrophage handling.

Lepromatous macrophages in vitro seem to digest cell walls of M. leprae poorly (6), and claims have been made that they are unable to present M. leprae antigens in their membrane (7).

The same antigen can give rise to different types of immune response depending upon the site of antigen presentation (8). In this connection the observation of a disturbance in the normal recirculation of lymphocytes in lepromatous rats is of interest (9). A hypothesis has been formulated which says that M. leprae presented to peripheral lymphoid tissues results in a protective immune response or tuberculoid leprosy, while direct presentation to the central lymphoid compartment (mainly the spleen) causes suppression of the immune response and lepromatous leprosy (10).

3) Macrophage/lymphocyte collaboration;
Collaboration between lymphocytes and macrophages is dependent upon their compatibility with regard to a special family of tissue antigens, the HLA-D/DR antigens (11). These antigens are closely related to the Ia antigens of the mouse (12), which again are related to the Ir genes that code for specific antigen responsiveness/unresponsiveness (13).

The specific unresponsiveness seen in lepromatous leprosy patients could thus be explained if they shared a common HLA-D/DR antigen which interfered with the macrophage/lymphocyte collaboration. No such common antigen has been found (14). It has also been shown that lepromatous macrophages can stimulate lymphocytes from HLA-D/DR identical siblings to DNA-synthesis (15).

4) The responding lymphocytes:
While B-lymphocytes can respond by cell division to a non-specific mitogenic stimulus in the LTT (16), it is generally accepted that antigen specific responses are due to T lymphocytes (17). T lymphocytes, however, are responsible for several types of reactions partly reflected in different subtypes of T lymphocytes (18).
Table 1. T-lymphocyte subclasses. DTH: delayed-type hypersensitivity; Aggressor MHC: T cells cytotoxic to cells with major foreign histocompatibility antigens; Aggressor non-MHC: T cells cytotoxic to cells with other foreign antigens, e.g. microbial antigens; Suppressor spec.: T cells which suppress responses to specific antigens; Suppressor non-spec.: T cells suppressing immune responses independent of antigen specificity; 1-J: a special type of Ia antigens.

<table>
<thead>
<tr>
<th>Cell/function</th>
<th>Symbol</th>
<th>Thy-1</th>
<th>Ly-1</th>
<th>Ly-2,3</th>
<th>Ia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initiator</td>
<td>T_I</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Helper B cell</td>
<td>T_HB</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Helper T cell</td>
<td>T_HT</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>DTH</td>
<td>T_D</td>
<td>?</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Aggressor MCH</td>
<td>T_C</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Aggressor non-MCH</td>
<td>T_E</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Suppressor spec.</td>
<td>T_S</td>
<td>?</td>
<td>+</td>
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<td>1-J</td>
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<td>Suppressor non-spec.</td>
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Both cells which actively synthesize DNA in LTT and the T-lymphocytes responsible for the delayed type hypersensitivity are Ly-1<sup>+</sup> cells (19,20). The antigen characteristics which might distinguish between helper T lymphocytes and the T cells responsible for delayed-type hypersensitivity, the Ia antigens (21), have not been studied on the responding cells in LTT. Such a distinction is important as the LTT responses correlate well with signs of chronic mononuclear inflammation in the patients' lesions and, thus, with the degree of delayed-type hypersensitivity (22). We do not know at present what type of CMI combats bacillary multiplication or is responsible for immune protection. Neither do we know what influences aggressor T-lymphocytes and suppressor T lymphocytes could have in the cultures (23).
5) Other factors which influence the LTT responses:
Mycobacteria contain both unspecific mitogenic factors (24) and factors suppressing a mitotic response (23). Plasma from lepromatous leprosy patients (25) and some plasmas from healthy individuals (26) also suppress mitotic responses, and some 10% of patients with tuberculoid leprosy have plasma factors which selectively suppress the responses to M. leprae antigens (27). In addition experimentally infected animals seem to pass through a phase of suppressed responses to a phase of positive responses to end up with clinical signs of mycobacterial infection and profound suppression (28).

Relevance of a positive LTT response to M. leprae in leprosy patients:

When LTT was introduced in leprosy, it was soon found that tuberculoid leprosy patients had positive lymphocyte responses to M. leprae antigens and lepromatous patients were almost universally negative (29). This was in agreement with previous experience with the early lepromin reaction (30). For groups of patients belonging to the same histopathological classification, average responses described a spectrum (31) well in concordance with the immune spectrum postulated by Ridley and Jopling (32).

Within each histopathological group, however, there is a substantial variation in strength of the individual responses. High responses correlate with active clinical and histopathological inflammation of the lesions, while many borderline tuberculoid patients, who control bacillary multiplication well, are non-responsive in the test. The reasons for negative responses in tuberculoid leprosy patients can be many: (22)

1) Wrong antigens.
CMI to leprosy bacilli can have many different antigen specificities. One M. leprae preparation can give positive responses in one patient while another preparation gives a negative response. In a second patient it can be the other way around.

2) Wrong lymphocytes.
In a culture containing 250,000 mononuclear cells, less than 100,000 lymphocytes of the right T cell subclass are left with the task of reflecting more than $10^6$ different antigen specificities (one lymphocyte can only have one antigen specificity) which the entire organism is expected to cover. Specifically sensitized lymphocytes in an infected individual are to a large extent assumingly trapped in local lesions and regional lymph nodes.

3) Wrong timing.
Borderline leprosy patients who experience acute reversal reactions have concomitantly a very significant rise in their LTT responses, even if responses before and after the reaction are entirely negative.
4) Wrong test.

DTH, which is the CMI function which is most closely reflected in LTT, might be largely irrelevant to the patient's ability to control bacillary multiplication.

In conclusion: There is no reason to believe that LTT responses to M. leprae antigens give valid information of the patient's ability to resist progression of the infection.

LTT responses to M. leprae antigens in healthy persons:

Healthy persons with known exposure to leprosy patients often have a positive response to M. leprae in vitro (33). The same occurred with the early lepromin test (34) and the leukocyte migration inhibition test (35). The early lepromin test, however, failed to give a clearcut difference between populations of high and low endemcity (36). With LTT a positive correlation between the responses to M. leprae and the degree of exposure to contagious patients has been obtained (37). The number of positive responders increase with increasing age. The lack of sexual difference in the number of responders gives no clue to the much higher incidence of leprosy in males. Consanguinity with a lepromatous case within the household was not correlated to a lower LTT response than was found among persons unrelated to the index case and living in the same household. This indicates that a hereditary lack of responsiveness is not the explanation for increased susceptibility to lepromatous leprosy in such families (38).

The problem of crossreactivity:

Cross reactions between mycobacteria have been debated for decades. The term has often been vaguely defined, resulting in much confusion (39). In leprosy the concept has been applied to:

1) ...the ability of antisera produced against other mycobacteria to react with M. leprae antigens (40),

2) ...the ability of other mycobacteria to evoke CMI responses leading to "false positive" responses to M. leprae antigens in persons not exposed to leprosy (41),

3) ...the ability of other mycobacteria to cause protective immunity to leprosy (42),

4) ...the ability of M. leprae to cause non-responsiveness to other mycobacteria in CMI tests with lepromatous leprosy patients (43),

5) ... and the ability of other mycobacteria to cause non-responsiveness to M. leprae in healthy persons and thus increased susceptibility to leprosy (44).

Based on different definitions of crossreactions, conclusions have varied widely. Since neither the mechanism for protective immunity nor the cause of unresponsiveness in lepromatous patients are known, crossreactivity in LTT will be defined according to 2). No
extrapolations to other aspects of crossreactivity can be done safely. Early studies with the LTT indicated a 6-15% crossreactivity between M. leprae and BCG (33). Later studies utilizing the incorporation of radioactive thymidine in dividing lymphocytes have indicated a crossreactivity in the order of 20-50% in healthy individuals not exposed to leprosy (41). This degree of crossreactivity does not apply to borderline tuberculoid leprosy patients in whom there is no correlation between lymphocyte responses to M. leprae and BCG (45). The crossreactivity is of the same order of magnitude whether whole bacilli or soluble antigens from sonicated bacilli are used in the test. The influence of sensitization to mycobacteria other than BCG and M. tuberculosis on the test has been much debated. Environmental mycobacteria are now regarded as a cause of desensitization leading to negative response and increased susceptibility (44) rather than as a source of false positive responses to M. leprae (46). Valid studies based on LTT are still lacking.

Applicability of LTT to epidemiological studies in leprosy:

LTT is cumbersome, expensive and prone to a lot of technical pitfalls and variance. Great variance according to stage of clinical/subclinical infection (10, 22), concurrent diseases (47), nutritional status (48), phase of menstrual cycle (26) and even the time of day (49) must be kept in mind. The optimal dose of antigen and the time for the responses to peak show individual variation (27). The lymphocytes should be prepared on the spot as soon as possible after blood collection, or carefully shipped as whole blood at ambient temperature for no longer than 12 hours (50). Acceptability in study populations is dependent upon local attitudes to blood sampling. The test is clearly not a good one for screening a large number of individuals.

The most severe drawback in the present situation is, however, that the relevance of a positive response in a healthy individual is not established. The explanations for a positive LTT response to M. leprae antigens could be:

1) crossreaction due to contact with other mycobacteria,
2) specific sensitization to M. leprae causing immune protection,
3) contact with M. leprae causing a subclinical infection which in the future will become overt leprosy,
4) contact with M. leprae leading to an immune response entirely irrelevant for protection or later disease,
5) "non specific" effect of factors in the test serum, antigen preparation or test conditions.

Some of these shortcomings can only be met by being fully aware of all the pitfalls while applying the test. Other problems must be solved by the purification and characterization of the M. leprae antigens for use in the test.
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