INTERNATIONAL JOURNAL OF LEPROSY
and Other Mycobacterial Diseases

OFFICIAL ORGAN OF THE INTERNATIONAL LEPROSY ASSOCIATION

Publication Office: Leahi Hospital, 3675 Kilauea Avenue,
Honolulu, Hi. 96816

VOLUME 45, NUMBER 3 JULY-SEPT. 1977

EDITORIALS

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Transfer Factor as a Probe of the Immune Defect in Lepromatous Leprosy

In the last decade considerable interest and enthusiasm has been evident concerning the use of transfer factor (TF) in clinical immunotherapy.[1] "Transfer factor (TF) is an operational designation for non-immunoglobulin substances present in extracts of sensitive blood lymphocytes that can introduce specific cell-mediated immunity in nonsensitive humans".[2] This activity was discovered when experiments in humans showed that first viable intact leukocytes could transfer positive delayed-hypersensitivity skin tests from sensitive leukocyte donors to nonsensitive recipients and eventually that nonviable leukocyte extracts had the same property. This leukocyte extract was termed transfer factor.

TF is dialyzable, with a molecular weight less than 10,000, and is neither an antigen nor an immunoglobulin. It does not contain histocompatibility antigens and in normal recipients, TF induces sensitivity within hours and the sensitivity which is transferred lasts for years. Apparently TF exists as a preformed molecule within lymphocytes and is released when the cell comes in contact with antigen, functioning to convert additional lymphocytes *in situ* to an antigen responsive state.[3]

Transfer factor specificity. The chemical nature of the material in crude TF which is responsible for specific transfer of cell-mediated immunity is unknown. The dialyzable material from disrupted leukocytes contains a large number of substances and many of these such as uracil,[4] hypoxanthine,[5] ascorbic acid, serotonin, cholinergic materials,[6]

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[5] O'Dorisio, M. S., Neidhart, J. A., Daniel, F. B., Balcerzak, S. P., and LoBuglio, A. F. Identification of hypoxanthine as the major component of a chromatographic fraction of transfer factor. Cell. Immunol. **23** (1976) 191-202.

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simple polypeptides,[7] nicotinamide,[8] etc., may cause a variety of nonspecific stimulatory and suppressive effects *in vitro* and *in vivo* on immune and inflammatory reactions. Indeed some observers have questioned the existence of *de novo* transfer of antigen specific cell-mediated immunity with TF at all. The possibility has been raised that TF acts entirely nonspecifically by enhancing low levels of preexisting cell-mediated immunity in recipients.[9]

Unquestionably TF exerts nonspecific effects,[10] but a number of observations strongly suggest that the material also has antigen-specific effects and is also able to transfer delayed-hypersensitivity reactivity *de novo* to recipients. These studies have been recently reviewed by Valentine,[2] and essentially consist of observations of specific transfer of sensitivities to many microbial antigens, histocompatibility antigens, antigens of sarcomas, candida, and keyhole limpet hemocyanin.[11,12] A recent study has shown rather convincing transfers of antigen-specific cell-mediated immunity to gnotobiotic or germ-free nonhuman primates with human TF.[13] Because these animals had not been previously skin tested and had very limited microflora, the transferred delayed-hypersensitivity appeared to be clearly on the basis of a *de novo* informational effect of TF.

Rationale for TF in leprosy. Based usually on the presumption that the reagent may transfer specific cell-mediated immunity to recipients, TF has been used in a number of usually uncontrolled clinical trials. For some conditions, e.g., the Wiskott-Aldrich syndrome and chronic mucocutaneous candidiasis, TF has provided a hope for successful therapy where little or none previously existed. In contrast to these relatively rare and poorly treatable conditions, the use of TF in leprosy is rather different. The potential recipient population for TF in leprosy involves millions of individuals and available chemotherapy, while by no means ideal, is by and large effective. Why then should trials of TF in leprosy be conducted?

At least three reasons come to mind for studying TF in leprosy. Firstly, TF immunotherapy may benefit recipients with the disease, either by hastening clearance of bacilli in active patients or by preventing relapse of treated inactive patients.[14] Secondly, leprosy is perhaps the best understood model available of granulomatous infectious disease immunology and TF trials in leprosy might therefore shed light on the nature of TF itself and the mechanisms of cell-mediated immunity to intracellular parasites in general. Thirdly, and of perhaps most interest to leprosy workers, TF trials in leprosy may help clarify the nature of the fundamental defect in the immune response in lepromatous leprosy. More information about this defect may have a major impact on the feasibility of various approaches to the development of a vaccine for leprosy. The present discussion will be limited to speculations concerning the implications of the results of early TF trials for the immunology of leprosy.

Possibilities for the fundamental lepromatous defect.

a. *General considerations.* There is increasing agreement among leprosy workers that the fundamental defect in lepromatous leprosy is immunologic and is characterized by a lack of demonstrable T lymphocytes capable of responding to antigens of *M. leprae.*[15] Currently there is disagreement in the

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[8] Burger, D. R. Vandenbark, A. A., Daves, D., Anderson, W. A., Jr., Vetto, R. M., and Finke, P. Nicotinamide: Suppression of lymphocyte transformation with a component identified in human transfer factor. J. Immunol. **117** (1976) 797-801.

[9] Bloom, Barry R. Does transfer factor act specifically or as an immunologic adjuvant? N. Engl. J. Med. **288** (1973) 908-909.

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[13] Eichberg, J. W., Steele, R. W., Kalter, S. S., Kniker, W. T., Heberling, R. L., Eller, J. J. and Rodriguez, A. R. Cellular immunity in gnotobiotic primates induced by transfer factor. Cell. Immunol. **26** (1976) 114-119.

[14] Bullock, W. E., Fields, J. P., and Brandriss, M. W. An evaluation of transfer factor as immunotherapy for patients with lepromatous leprosy. N. Engl. J. Med. **287** (1972) 1053-1059.

[15] Godal, T., Myrvang, B., Stanford, J. L., and Samuel, D. R. Recent advances in the immunology of leprosy with special references to new approaches in immunoprophylaxis. Bull. Inst. Pasteur **72** (1974) 273-310.

area of how this deficiency comes about. Some of the possibilities for this condition are illustrated in the Figure. It may be assumed that there are certain steps involved in a normal or successful immune response to *M. leprae* which operate to successfully eliminate the pathogen before it can cause clinical disease. These processes which lead to a clone of T lymphocytes capable of recognizing and responding to antigens of *M. leprae* occur in most individuals exposed to viable bacilli[15] and must be defective in individuals who develop lepromatous leprosy.

Some of the possibilities for the lepromatous defect are clearly not likely. For example, lepromatous leprosy patients respond in a more or less normal fashion to a great variety of potential pathogens which are normally dealt with by cell-mediated immune mechanisms. This indicates that the lepromatous defect is not a general malfunction in stem cells, in the generation of lymphocyte precursors, in the general actions of the thymus to convert lymphocytes to T lymphocytes, in the generation of monocytes, or in the development of tissue macrophages from circulating monocytes or histiocytes.

The efferent limb of cell-mediated immune responses must function adequately for non-*M. leprae* antigens in lepromatous leprosy patients in view of generally normal resistance to pathogens normally dealt with by cell-mediated immune mechanisms. Thus the fundamental lepromatous defect does not lie in a general deficiency in the ability of sensitized T lymphocytes to undergo lymphocyte blast transformation, to generate lymphokines such as MIF (migration inhibitory factor), LIF (leukocyte inhibitory factor), SRF (skin reactive factor), or MAF (macrophage activating factor). Similarly the fundamental lepromatous defect does not lie in the general ability of macrophages to respond to MAF, etc., and become activated and destroy intracellular parasites.

There are a number of studies which show an usually modest, but significant, generalized decrease in cell-mediated immunity in leprosy, particularly in lepromatous leprosy, and particularly in untreated lepromatous leprosy.[16-26] In contrast to these modest and frequently variable expressions of a generalized deficiency in cell-mediated immunity, technics designed to measure specific cell-mediated immunity to *M. leprae* reveal strikingly and virtually uniformly the complete absence of responses in lepromatous leprosy patients.

Thus, the fundamental defect in lepromatous leprosy is highly specific for antigens of *M. leprae,* is characterized by a lack of T lymphocytes capable of being activated by antigens of *M. leprae*[15] and must lie at some point after the development of T lymphocytes and macrophages on the one hand, and the response of sensitized T lymphocytes to *M. leprae* on the other.

b. *Most likely possibilities.* The possibilities for the fundamental lepromatous defect (assuming that there is only one) therefore include 1) *M. leprae,* 2) the macrophage, 3) the "leprosy Ir gene," and 4) various control mechanisms on the activation of specifically sensitized T lymphocytes by *M. leprae* antigens. A brief consideration of these possibilities may be warranted.

1. *M. leprae.* The initial inoculum of *M. leprae* itself could somehow differ in those individuals destined to develop lepromatous leprosy as opposed to those individuals destined to develop subclinical disease or one of

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[17] Bullock, W. E. Studies on immune mechanism in leprosy. N. Engl. J. Med. **278** (1968) 298-304.

[18] Dierks, R. E. and Shepard, C. C. Effect of phytohemagglutinin and various mycobacterial agents on lymphocyte cultures from leprosy patients (32698). Proc. Soc. Exp. Biol. Med. **127** (1968) 391-395.

[19] Sheagren, J. N., Block, J. B., Trautman, J. R., and Wolff, Sheldon, M. Immunologic reactivity in patients with leprosy. Ann. Intern. Med. **70** (1969) 295-298.

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[22] Han, S. H., Weiser, R. S., and Tseng, J. J. Lymphotoxin production by lymphocytes from leprosy patients. Int. J. Lepr. **39** (1971c) 719-725.

[23] Gajl-Paczalska, K. J., Lim, S. D., Jacobson, R. R., and Good, R. A. B lymphocytes in lepromatous leprosy. N. Engl. J. Med. **288** (1973) 1033-1035.

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[25] Nath, I., Curtis, J., Bhutani, L. K. and Talwar, G. P. Reduction of a subpopulation of T lymphocytes in lepromatous leprosy. Clin. Exp. Immunol. **18** (1974) 81-87.

[26] Lim, S. D., Kiszkiss, D. F., Choi, Y. S., Gajl-Peczalska, K., and Good, R. A. Immunodeficiency in leprosy. Birth Defects **11** (1975) 244-249.

the more localized forms of leprosy. Possibilities include inoculum size, route of inoculation,[27] timing or frequencies of inoculation, etc., which could induce tolerance in certain individuals. A possibility which is not likely but which cannot be entirely dismissed is that there could be subtle antigenic strain differences in *M. leprae* which conceivably could be sufficient to allow for differences in the ultimate clinical manifestations of the disease.

2. The macrophage. A site for the fundamental lepromatous defect which has recently received attention on these pages is the macrophage.[28] This hypothesis holds that the macrophages of lepromatous patients are congenitally deficient in an enzyme which normally digests a crucial component of *M. leprae*. The lack of demonstrable cell-mediated immunity in lepromatous patients is taken to be a consequence of faulty *M. leprae* antigen processing and presentation to lymphocytes (see Figure). The hypothesis logically leads to the prediction that lepromatous macrophages are inherently unable to dispose of *M. leprae* and that, regardless of the stimulus provided by lymphokines, *M. leprae* cannot be efficiently eliminated in lepromatous patients. The hypothesis also predicts that antigen processing and presentation would be defective both in the initial sensitization of T lymphocytes and also in the stimulation of T lymphocytes already sensitized to *M. leprae*. Thus, even if T lymphocytes which were sensitized to *M. leprae* were to be provided to a lepromatous individual the hypothesis predicts that the lepromatous macrophages could not process and present *M. leprae* antigens to the sensitized T lymphocytes (see Figure). The substrate specificity of the missing enzyme in this hypothesis is striking, inasmuch as lepromatous macrophages are able to clear various microorganisms, including other mycobacteria, in a normal fashion.[29]

Additionally, lepromatous patients produce abundant antibodies to antigens of *M. leprae*. While none of these antigen specificities may involve the antigens which are crucial for cell-mediated immunity, at least some of them are in all probability thymic-dependent antigens. In order for antibody production to occur in response to thymic-dependent antigens, macrophages must process these antigens and present them to helper T lymphocytes. Thus, if lepromatous patients produce antibodies to thymic-dependent antigens of *M. leprae*, it must be assumed that at least some processing and presentation of at least some antigens of *M. leprae* can be accomplished by lepromatous macrophages.

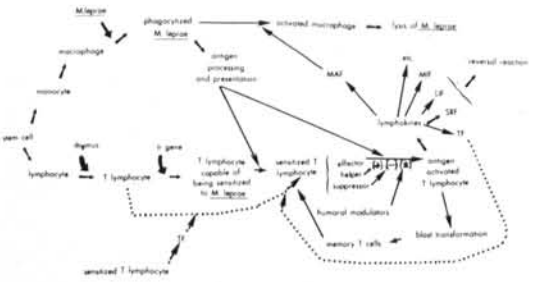

FIGURE. Model of the action of transfer factor on the immunology of leprosy.

3. The leprosy Ir gene. The next possibility for the fundamental defect in lepromatous leprosy is the immune response (Ir) gene. There is a considerable body of evidence which suggests that the fundamental lepromatous defect is genetically determined.[30-33] The major points which support this genetic hypothesis are 1) the well known familial clustering of leprosy patients, 2) striking differences among separate populations living in the same area as to the prevalence of leprosy and the type of leprosy,[30] 3) studies which show high concordance rates of leprosy in identical twins,[34] 4) the characteristic persistence of anergy to *M. leprae* after

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[33] Beiguelman, B. Leprosy and genetics. A review of past research with remarks concerning future investigations. Bull. WHO **37** (1967) 461-476.

[34] Mohammed Ali, P., and Ramanujam, K. Leprosy in twins. Int. J. Lepr. **34** (1966) 405-407.

lepromatous patients have had their antigen loads markedly reduced with chemotherapy, [30, 35] and 5) the classic study of Dharmendra and Chatterjee,[36] indicating that the immunologic defect of lepromatous leprosy seems to exist in individuals prior to exposure to *M. leprae.*

In the mouse, on chromosome 17, there exists a region of genes, the major histocompatibility of H-2 complex. This complex has a number of identifiable areas, designated K, I, S, D, and TL. Genes controlling the classical transplantation antigens are found in the K and D regions of the H-2 complex. In the I region there are genes which code for the ability of the animals to respond immunologically to certain antigens, the so-called Ir genes.[37] More than 30 immune response or Ir genes have been identified in guinea pigs, mice, rats, and Rhesus monkeys.[38]

In humans, the analogous HLA complex also probably contains an area comparable to the I region in animals, which codes for the ability of humans to respond immunologically to specific antigens. In some diseases, e.g., ankylosing spondylitis and its association with the B-27 histocompatibility antigen, the evidence for a disease susceptibility in man is clear,[38] i.e., there is a clear genetic predisposition for ankylosing spondylitis, the susceptible individuals being identifiable by the disease susceptibility gene (presence of an abnormal Ir gene or lack of a normal Ir gene) being closely linked to the serologically identifiable B-27 histocompatibility antigen. Data from families with diseases other than ankylosing spondylitis, such as psoriasis and celiac disease, also indicate disease susceptibility linked with classic HLA loci.[39]

In the I region of the H-2 complex in mice, there are genes which code for a series of proteins called Ia antigens.[40] In humans the area of the HLA complex comparable to the I region probably codes for Ia-like antigens on B lymphocytes.[41] Recently these Ia-like specificities have been somewhat defined in humans and in at least three diseases, multiple sclerosis, leukemia, and asthma, there appears to be a greater association between disease susceptibility and these Ia-like genes than with the classic HLA or transplantation antigens.[39]

A number of studies have attempted to find correlations between leprosy and various available genetic markers[33] including HLA antigens.[42] In general there have not been convincing associations between susceptibility to leprosy and these markers although a recent study of families with leprosy indicates that there may be such an association with HLA antigens.[43] Studies in leprosy involving the newer Ia-like B lymphocyte markers would be of considerable interest in this regard since these may more closely reflect associations between Ir gene loci and disease susceptibility than classic HLA or transplantation antigens.

Considering the enormous number of genes in the total complement of human chromosomes, it is obvious that the demonstration of an association between a given disease susceptibility gene and any of the relatively few available genetic markers is indeed fortuitous. The inability to demonstrate convincing correlations to date between leprosy and the limited markers

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[35] Dharmendra and Mukerjee, N. Lepromin test in cases of lepromatous leprosy treated with sulfones. Lepr. India **22** (1950) 128-130.

[36] Dharmendra and Chatterjee, K. R. Prognostic value of the lepromin test in contacts of leprosy cases. Lepr. India 27 (1955) 149-158.

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[43] DeVries, R. R. P., Fat, R. F. M. Lai A., Nijenhuis, L. E., and Van Rood, J. J. HLA-linked genetic control of host response to *Mycobacterium leprae.* Lancet **2** (1976) 1328-1330.

available attests perhaps more to the incompleteness of currently available methodologies than to the lack of the existence of a disease susceptibility or Ir gene for leprosy. Thus, there is considerable evidence that man possesses Ir genes which function in a manner analogous to those in experimental animals, and a very attractive hypothesis for the fundamental lepromatous defect is that prelepromatous individuals have Ir genes which do not permit their T lymphocytes to respond to crucial antigens of *M. leprae.*

4. Control mechanisms. Finally, the fundamental lepromatous defect could reside in one or more of the various control mechanisms on T lymphocyte activation. This hypothesis presumes that T lymphocytes, specifically sensitized to the relevant crucial antigens of *M. leprae,* do in fact exist in lepromatous patients but that they are not detectable because of derangements in mechanisms which function to control the intensity of expression of cell-mediated immunity.

There are a number of nonspecific mechanisms which could conceivably prevent the activation of sensitized T lymphocytes. Such mechanisms as enhanced endogeneous glucocorticoid levels, metabolic abnormalities or autonomic imbalances resulting in changes in intracellular cylic nucleotides,[44,45] prostaglandins,[46] human alpha-feto protein,[47] iron deficiency,[48] T lymphocyte depletion by entrapment in the course of the disease,[49] replacement of paracortical areas of lymph nodes with lepromatous granuloma,[50] protein-calorie malnutrition or concommitant viral infection,[27] etc., are all not likely possibilities in view of the striking specificity of the lepromatous defect. In other words, such nonspecific mechanisms would be expected to create profound generalized T cell anergy in lepromatous patients if they alone were the site of the fundamental lepromatous defect, and this is clearly not the case clinically.

There are control mechanisms, aside from Ir gene products, which are known to be antigen specific and would therefore be more likely sites for the fundamental lepromatous defect. Human T lymphocytes are heterogeneous,[51] and different T cells are presumed to function as effectors, helpers or suppressors of cell-mediated immune responses. Thus, a functional deficiency of specifically sensitized helper T cells or a functional excess of specifically sensitized suppressor T cells could account for the lepromatous defect. There is recent evidence for suppressor cells in the spleens of mice infected with *M. lepraemurium.*[52] On the other hand, the observations that lepromatous patients can clear both *M. leprae* and *M. lepraemurium* (or BCG) when the two mycobacteria are administered intradermally as a mixture but that they can clear only *M. lepraemurium* or BCG but not *M. leprae* when the mycobacteria are administered separately,[53] would suggest that the fundamental lepromatous defect is neither in macrophages nor in suppressor T lymphocytes. If the inability of lepromatous patients to clear *M. leprae* were only on the basis that *M. leprae* activated suppressor T lymphocytes which then prevented lymphokine production by existing sensitized effector T cells, then the mixed mycobacterial inoculations should

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have been similarly protected from being cleared. Similar reasoning would apply to the possibility that the fundamental lepromatous defect is due to specifically sensitized B lymphocytes which function as suppressor cells on T lymphocyte responsiveness.[54]

There are antigen-specific humoral factors which may act as control mechanisms of cell-mediated immunity. Factors which are perhaps better known in cancer immunology; such as free antigen, "enhancing" antibody, and antigen-antibody complexes,[55, 56] could account for the fundamental lepromatous defect. The well-known bacillemia of untreated lepromatous leprosy,[57] the evidence for high titers of antimycobacterial antibodies, and evidence for circulating antigen-antibody complexes in lepromatous leprosy,[58] all points of these factors as potentially playing a major role in the pathogenesis of the disease. On the other hand, there are two points which tend to rule out these antigen-specific humoral factors as being the sites for the fundamental lepromatous defect. Firstly, the life-long persistence of anergy in lepromatous patients despite successful chemotherapy and secondly, the uniform absence of response to *M. leprae* in *in vitro* lymphocyte transformation tests, etc., of washed leukocytes from lepromatous patients whether they be cultured in autologous sera or in sera from healthy individuals. It should be pointed out that antigen-specific (and nonspecific) humoral factors, while not likely sites for the fundamental lepromatous defect, are very attractive as significant mechanisms for the dynamic changes occurring in borderline leprosy.[59, 60]

Results of TF in leprosy. From the preceding discussion it is evident that there are a number of reasonable possibilities for the fundamental lepromatous defect, some more attractive than others. The results of trials of TF in leprosy have provided additional information as to the most probable site for this defect.

A number of studies have been performed attempting immunotherapy of leprosy with viable allogeneic leukocyte infusions,[14, 61-65] with generally encouraging results. The results of these trials of leukocyte infusions are difficult to interpret in the present context due to the multiplicity of cell types involved, i.e., a number of possibilities exists for the mechanism of any benefits obtained in recipients. Thus infused monocytes could have corrected a fundamental macrophage defect; infused T lymphocytes could have corrected a basic Ir gene defect; host vs. graft reactions induced by the infusions could have caused nonspecific adjuvant effects, etc.

Other studies involving the immunotherapy of leprosy have utilized dialyzable TF,[14, 64, 66, 67] in which there are more limited

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possibilities for a mechanism of action. To briefly review these findings, Bullock, *et al*[14] treated four lepromatous patients with TF in a single dose with material derived from approximately 4×10^8 leukocytes from donors sensitive to *M. leprae*. In three of four patients, there was Dharmendra skin test conversion; none of the four showed evidence of acquired cell-mediated immunity to *M. leprae* by *in vitro* immunologic testing; three of the four developed "flares" or reversal reactions[68] and none showed evidence of enhanced bacterial clearing due to TF. Silva *et al*,[66] also treated four lepromatous patients with TF prepared from the blood of lepromin sensitive donors. The material from 1×10^9 leukocytes was administered as a single dose and no changes were noted in lepromin skin tests, no reversal reactions were noted, and there was no evidence of enhanced bacterial clearing. Saha *et al*,[64] treated four lepromatous patients with TF prepared from a total of 1.2×10^9 leukocytes from lepromin sensitive donors. TF was administered in three doses at monthly intervals and two of the four recipients converted lepromin skin tests from negative to positive. No reversal reactions or enhanced rates of bacterial clearing were indicated. Hastings *et al*,[67] treated four lepromatous and one borderline patient with TF from lepromin sensitive donors. TF from approximately 7.4×10^9 lymphocytes was given in 36 divided doses over a 12 week period. In one of the four lepromatous recipients and in the one borderline patient, there was modest evidence of skin test conversion using integral lepromin. During the trial none of the four lepromatous patients showed evidence of cell-mediated immunity to *M. leprae* by *in vitro* immunologic testing, while the borderline patient did transiently acquire positive responses to *M. leprae in vitro* by lymphocyte blast transformation and indirect MIF assays. "Flares" or reversal reactions occurred in all four of the lepromatous recipients but did not appear in the borderline patient. There was clear evidence for enhanced bacterial clearing as measured by skin scrapings in all four of the lepromatous patients but not in the borderline recipient. Of note is that one of the lepromatous recipients had sulfone-resistant disease, received no concomitant chemotherapy, and cleared bacilli in an accelerated fashion, but his bacilli remained viable by serial mouse foot pad inoculations throughout the 12 weeks of the trial. This would be expected with an effect on the host's response to clear bacilli rather than a direct effect on the bacilli. Also of interest were the observations that multi-focal reversal reactions continued after TF injections were stopped in all four lepromatous recipients for a minimum of five months and a maximum of over three years suggesting that whatever was induced by TF to cause reversal reactions either persisted for a long period or was self-replicating. Two possibilities exist for the mechanism of this phenomenon, the induction of memory T cells (not shown in the Figure) or the serial passage of TF to uncommitted T lymphocytes (see Figure). On the other hand, rates of bacterial clearing appeared to slow to that expected with chemotherapy alone after the TF injections were stopped.

To summarize four widely different trials, 16 patients with active lepromatous leprosy were recipients of TF. In six there was evidence of lepromin skin test conversion and in seven "flares" or reversal reactions were seen. None of the lepromatous recipients showed conversion of *in vitro* immunologic reactivity to *M. leprae*, but four showed evidence of enhanced rates of bacterial clearing. These four subjects were all recipients of the largest amounts of TF given for the longest period of time.

The lack of conversion of *in vitro* immunologic tests after TF is by no means conclusive evidence that specific cell-mediated immunity to *M. leprae* was not induced by TF. In general it appears that skin test conversion, MIF production, and lymphocyte blast transformation test conversions, in that order of frequency, may be seen after TF[69] and some TF recipients with lepromatous leprosy showed lepromin skin test conversions. For an active leprosy patient to show reactivity against *M. leprae* in *in vitro* immunologic testing, the patient must have tuberculoid to borderline tuberculoid disease.[15] Thus a given polar lepromatous (LL) recipient of TF could undergo a reversal reaction

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to as far as midborderline (BB) without any expected change in his lack of response in these *in vitro* tests of specific cell-mediated immunity. Indeed *in vitro* immunologic responsiveness, even in borderline-tuberculoid (BT) and polar tuberculoid (TT) disease, is not uniformly positive with present methodologies.

The creation of reversal reactions in polar lepromatous patients is a strikingly unusual phenomenon. This would not be expected to occur secondary to nonspecific adjuvant effects since, firstly, the recipients of the TF showed virtually no nonspecific toxic effects,[67] and secondly, nonspecific immunotherapy in the past with such agents as Marianum antigen and diphtheria toxoid have been clearly unsuccessful in leprosy.[70]

Thus, there is rather convincing evidence that some of these lepromatous recipients of TF successfully developed activated T lymphocytes after TF which then generated lymphokines responsible for reversal reactions, lepromin skin test conversions, and macrophage activation and lysis of *M. leprae* (see Figure). The observations in lepromatous recipients of TF are exactly in keeping with what one would expect if TF operated by inducing individual T lymphocytes to become antigen responsive *in vivo* to relevant antigens of *M. leprae*. The observations that the intensity of reversal reactions induced by TF in lepromatous recipients tended to wane after approximately the 10th week of a 12 week trial and a total lack of clinical responsiveness in the single borderline patient treated by Hastings *et al*,[67] are in keeping with the transfer of specific antigen responsiveness to suppressor T cells[71] when more than an optimum dose of TF was administered to the lepromatous patients and the natural existence of these suppressor T cells in the borderline patient.

Implication of TF results for the site of the lepromatous defect. As discussed above, the likely overall possibilities for the fundamental lepromatous defect are a) *M. leprae*, b) the macrophage, c) the leprosy Ir gene, and d) antigen-specific control mechanisms on the activation of *M. leprae* sensitized T lymphocytes. TF trials in leprosy have provided additional information relevant to these possibilities.

If the effects of TF in lepromatous leprosy are on the basis of antigen-specific transfer of information to naive, previously unresponsive cells in recipients, then the basic lepromatous defect does not reside in any strain differences in *M. leprae* since the bacilli cleared by the TF recipients would be the same strain as those responsible for the disease in that recipient in the first place and must carry similar antigenic moieties as the strains which sensitized the donors of the TF. It should be realized that the TF results do not shed light on the possibilities of differences in the initial inoculum of *M. leprae* as possible sites for the fundamental lepromatous defect.

If the function of macrophages to process and present relevant *M. leprae* antigens (see Figure) is fundamentally similar in a) initial presentation to T lymphocytes in the normal induction of cell-mediated immunity (primary immunization), b) presentation to TF-sensitized T lymphocytes in the elicitation of cell-mediated immunity or delayed hypersensitivity to *M. leprae* (to elicit "flares" or reversal reactions) and c) to destroy intracellular *M. leprae* in response to lymphokines as a final effector mechanism of cell-mediated immunity in TF treated lepromatous patients, then the observations of reversal reactions and enhanced bacterial clearing in lepromatous subjects treated with TF means that their fundamental defect does not reside in the macrophage. In other words, if lepromatous macrophages function normally to process and present relevant *M. leprae* antigens to T lymphocytes sensitized by TF and if they also function normally to lyse *M. leprae* when influenced by lymphokines from activated T lymphocytes after they have been sensitized with TF, then it is most likely that they are capable of normal function in primary immunization with *M. leprae*.

If TF sensitized T lymphocytes function normally *in vivo* in lepromatous patients and if TF operates only to create T lymphocytes sensitized to *M. leprae*, then antigen-specific humoral factors are not responsible for the fundamental defect. These humoral factors would be expected to remain operative on T lymphocytes newly sensitized by TF just as

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they would be on pre-existing sensitized T lymphocytes. Thus, the observation of reversal reactions in TF treated lepromatous recipients speaks against any of the possible humoral modulators of cell-mediated immunity as being the site of the fundamental lepromatous defect.

Thus, by elimination, the most probable sites of the fundamental lepromatous defect are the Ir gene and antigen-specific T lymphocyte control mechanisms on cell-mediated immunity to *M. leprae*. If the Ir gene codes for products of regulatory T lymphocytes (helper and/or suppressor) which in turn regulate effector T lymphocytes in cell-mediated immunity[37] and if TF acts to transfer antigen-responsiveness to helper and suppressor T lymphocytes as well as effector T lymphocytes, then both the Ir gene and regulatory T lymphocytes may be the sites of the fundamental lepromatous defect. Indeed it has been suggested that the Ir gene product may be expressed on a molecular complex which is both specific for antigen and has immunoregulatory function.[37] To explain the action of TF in lepromatous recipients in this case requires the hypothesis that TF transfers *M. leprae* antigen-responsiveness selectively to helper T lymphocytes and not, at least to the same degree, to suppressor T lymphocytes. This hypothesis leads to the conclusion that the fundamental lepromatous defect functionally lies in an overactivity of *M. leprae* antigen-specific suppressor T lymphocytes and a deficiency of *M. leprae* antigen-specific helper T lymphocytes. As mentioned above, the ability of lepromatous patients to clear mycobacterial mixtures of *M. leprae* and *M. lepraemurium* (or BCG),[53] would suggest that the fundamental lepromatous defect, whether it involves Ir genes or not, does not lie only in a functional overactivity of *M. leprae* antigen responsive suppressor T cells.

On the other hand, it appears that genetic immunologic nonresponsiveness in animals may not always be on the basis of suppressor T lymphocytes.[37] This leaves open the possibility that some Ir genes may simply function to provide the capability to T lymphocytes of becoming sensitized to specific antigens. In view of the above discussion, if the leprosy Ir gene codes functionally for the ability of clones of effector T lymphocytes to become sensitized to relevant *M. leprae* antigens, then, if TF acts to convert uncommitted effector T lymphocytes to sensitized effector T lymphocytes, the fundamental lepromatous defect lies with the leprosy Ir gene. This view, which will in all probability prove to be quite simplistic as more knowledge is accumulated in this area, nonetheless appears most logical at present. This Ir gene site for the fundamental lepromatous defect, resulting in an inability of otherwise competent T lymphocytes to develop sensitivity to relevant antigens of *M. leprae* is compatible with the evidence for a genetic predisposition to leprosy, would be analogous to other disease susceptibility genes in man, and is compatible with the results of TF in lepromatous recipients.

Implications for a leprosy vaccine. If the results of TF trials in leprosy are an indication that the fundamental defect in lepromatous leprosy resides in a genetic location, then there is little hope in attempting immunization of prelepromatous individuals with *M. leprae*. Attempts to find a potential vaccine from a cultivatible mycobacterial species which is serologically identical to *M. leprae* could be criticized on a similar basis, i.e., prelepromatous individuals would not be expected to respond to an antigenically identical organism. Additionally, serologic identity may prove not to be as relevant to a vaccine as relatedness in cell-mediated immune systems. The most likely candidate for an effective vaccine to induce paraimmunity to *M. leprae* in a target population of genetically determined prelepromatous individuals would appear to be the most closely related mycobacterial species in cell-mediated immune systems which is capable of sensitizing patients with inactive lepromatous leprosy.

There is little doubt that our present concepts are only rudimentary concerning the nature of the fundamental defect in lepromatous leprosy and the implications of that defect for vaccine development. On the other hand, the understanding that came to leprologists when the dual compartment immune system model was enunciated was enormous. It may well be that the next major awakening in the nature of the defect in lepromatous leprosy may ultimately come about only after such seemingly esoteric immunologic questions are answered as the role of antibody-dependent cell-mediated cytoxicity in the pathogenesis of intracellular parasitism,

the molecular basis for the induction of cell-mediated immunity, the Ir gene product relationship to cell-mediated immunity, the full clinical significance and functional details of subtypes of human T lymphocytes, the specificity and molecular mechanism of the TF phenomenon, etc., all as they relate to our particular organism, *M. leprae,* and the intriguing but elusive nature of the defect in the defenses of its victims.

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