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Protection and Immunopathology in Tuberculosis: the Role of Immunotherapy

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One of the main reasons for the current failure to control tuberculosis is that, even using the best available chemotherapy, treatment must be continued for at least 6 months. This treatment regimen is not a realistic proposition in most developing countries, or even in the inner cities of rich ones, because patients feel well after a few weeks and stop taking the drugs. The solution, available only to the rich, is directly observed therapy (DOT), in which the patient is supervised while taking every dose.

There are two interrelated reasons for the need for 6-month regimens. The first is obvious and often discussed. Chemotherapy kills the vast majority of the bacteria within a few days, but there are so called persisters that are presumably not metabolizing (Grange 1992), and so are not killed by the drugs. It is not clear whether these are in true stationary phase (Siegele & Kolter 1992), or merely replicating extremely slowly in old lesions or sites of fibrosis or calcification, where oxygen availability may be low. The other reason for prolonged treatment is usually overlooked, but is in our opinion fundamental to the understanding of tuberculosis. Tuberculosis patients have a necrotizing pattern of response to *M. tuberculosis*, analogous to the phenomenon first noted by Koch (1891) in guinea-pigs. There is overwhelming evidence that the Koch phenomenon is not a correlate of optimal protective immunity to tuberculosis. Indeed pre-immunization of animals so that they exhibit the Koch phenomenon before challenging with virulent *M. tuberculosis* results in a clear and reproducible increase in susceptibility to the disease, compared with non-immunized controls (Wilson et al 1940; Rook & Hernandez-Pando 1996). This and other aspects of the Koch phenomenon are discussed in detail later. Its relevance at this point is that this inappropriate pattern of response does not correct itself during conventional treatment. Therefore, if chemotherapy is stopped at 3 months, relapse rates approach 20% (Balasubramanian et al 1990) even when optimal rifampicin chemotherapy is used, achieving sputum negativity well before 3 months, and in spite of the fact that there are very few live organisms in the patients' tissues at this time.

The task for the immunologist is therefore to learn to replace the pathological response with the protective one very early during treatment. We should be able to devise ultra-short-course chemotherapy regimens supplemented with immunotherapy, that would provide realistic tuberculosis control in the developing world.

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Protective Immunity to Intracellular Bacteria: the Type 1 Cytokine Profile

Immunity to tuberculosis requires a Th1 pattern of response accompanied by a range of cytotoxic cell types that can lyse phagocytes if they fail to destroy ingested organisms (Orme et al 1993a, b; Bloom et al 1994; Ladel et al 1995a, b; Tanaka et al 1995). This may allow the organisms to be taken up by fresh activated phagocytes.

The Protective Role of TNF-α

It has become clear that in addition to Type 1 cytokines, tumour necrosis factor-α (TNF-α) is also essential for immunity in mice. Treating mice with neutralizing anti-TNF-α antibodies has led to dissemination of BCG infection (Kindler et al 1989) and neutralizing antibodies or knockout of the 55-kDa TNF-α receptor led to rapid death from *M. tuberculosis* (Flynn et al 1995). Thus, TNF-α is necessary for protection, probably because it triggers killing mechanisms in γ-interferon-activated macrophages (Chan et al 1992; Hirsch et al 1994). Nevertheless, TNF-α has not been shown to be needed for immunity to tuberculosis in man.

The Immunopathological Response of Disease: the Koch Phenomenon

It has long been clear that many symptoms of tuberculosis, such as fever, weight loss and tissue damage, resemble the known pathological effects of TNF-α. Evidence that these symptoms may indeed be caused by TNF-α in man has come from experiments using thalidomide which decreases the half-life of the mRNA for this cytokine (Moreira et al 1993). Patients treated with thalidomide show rapid symptomatic relief and weight gain (Kaplan 1994). We are therefore faced with a paradox. TNF-α is essential for immunity, but may also be responsible for pathology. Recent work provides a probable resolution of this dilemma. TNF-α may become more toxic in the presence of a mixed Th1 and Th2 response pattern.

Although immunity to tuberculosis certainly requires a Type 1 response, there is clear evidence in tuberculous mice that Type 2 cytokines are also expressed (Orme et al 1993a), and although less obvious, the same is true in man. For instance the interleukin-4 gene is expressed in patients' peripheral blood mononuclear cells, while there is a deficit in interleukin-2 expression (Schauf et al 1993). The same two points have emerged from studies using 3-colour flow cytometry to look at actual cytokine production (Thapa et al, unpublished observations). This suggests the presence of a Th2 component in

disease, and is supported by the fact that tuberculosis patients often have high circulating levels of interleukin-10 (Cooper et al 1995) and IgE antibody (interleukin-4-dependent) (Yong et al 1989). When tuberculin test sites were studied using a laser doppler velicometer it was found that the extent to which blood flow was reduced in the centre of the site at both 6 and 48 h, was related to the level of specific IgE antibody to *M. tuberculosis* (Gibbs et al 1991). This appears to represent incipient necrosis (Koch phenomenon) which is characteristic of the disordered immune response accompanying the disease.

This may be of significance, because the combination of Th1, Th2 and TNF-α is known to result in severe pathological damage in other systems. For instance, the granulomata formed in response to the ova of *Schistosoma* in mice are of this type (Grzych et al 1991), and depend on the simultaneous presence of all three elements. These granulomata are acutely sensitive to further tissue damage if systemic cytokine release is induced (Carswell et al 1975; Ferluga et al 1979). Moreover, if the Th2 component is reduced by pre-immunization with ova antigens plus interleukin-12, the granulomata are much smaller, and very significantly, the residual tissue damage and fibrosis are reduced (Wynn et al 1995).

Mixed Th1–Th2 Responses and TNF-α-mediated Toxicity in Murine Tuberculosis

The correlation between helper T cell type and the toxicity of TNF-α is readily demonstrated experimentally in mice using mycobacterial antigens. When mice were immunized with a low dose (10^7) of an intensely immunogenic killed mycobacterial preparation (autoclaved *Mycobacterium vaccae*), only Th1 cytokine production was primed (Hernandez-Pando & Rook 1994). If 1 μg of TNF-α was injected into delayed-type hypersensitivity (DTH) response sites elicited 24 h earlier in such animals, no necrosis was caused. However, the 100-fold larger dose (10^9), of the same killed *M. vaccae* preparation evoked a mixed pattern with priming for both Th1 and Th2 cytokine secretion. Injection of TNF-α into DTH response sites elicited in these animals resulted in necrosis (Hernandez-Pando & Rook 1994). Therefore, it can be postulated that TNF-α released into a relatively pure Th1-mediated inflammatory site, acts as a supplementary macrophage-activating molecule, but when released into a mixed Th1–Th2 site, it causes damage.

Confirmation of the relevance of the observations outlined above has emerged from a study of the TNF-α-sensitivity of DTH response sites elicited in mice with pulmonary tuberculosis (Hernandez-Pando et al 1995; Rook & Hernandez-Pando 1996). During the first 3 weeks, DTH sites are not sensitive to TNF-α. This is the period of Type 1 response (Orme et al 1993b). After 50 days, the animals enter the phase of progressive disease accompanied by Th2 cytokine production, and high IgG1 antibody titres – a Th2-associated murine subclass. In these animals, DTH sites become TNF-α-sensitive.

The argument is further strengthened by looking at the consequences of generating in normal mice, the immunological state seen at day 50 in infected mice. When mice were pre-immunized using the dose of 10^9 killed *M. vaccae*, they developed the mixed Th1–Th2 cytokine pattern with TNF-α-sensitive DTH responsiveness described earlier. Such animals were found to be more susceptible to intratracheal *M. tuberculosis* than were unimmunized control animals (Rook & Hernandez-Pando 1996). Moreover, adrenal atrophy occured within days of infection (Hernandez-Pando et al 1995), further illustrating the parallels between the immunological state evoked by 10^9 killed *M. vaccae*, and the state accompanying late progressive disease, when an apparently identical form of adrenal atrophy occurs (Rook & Hernandez-Pando 1996). These observations suggest that this mixed Th1–Th2 response is equivalent to the Koch phenomenon (Koch 1891). Interestingly, pre-immunized guinea-pigs that manifested the Koch phenomenon to tuberculin tests performed before deep intramuscular challenge with *M. tuberculosis*, proved to be more susceptible than unimmunized controls (Wilson et al 1940).

It is therefore our hypothesis, that the pathological immune response accompanying the disease (Koch phenomenon) is associated with the presence of an inappropriate Th2 component (or with a function that correlates with the presence of Th2-cytokine-secreting cells), leading to enhanced toxicity of cytokines, and to susceptibility to tissue damage. This understanding gives us several targets for immunotherapy, which clearly could be attempted with appropriately presented antigens, or with mediators (cytokines or hormones) that manipulate the Th1–Th2 ratio. We have opted for an antigen-driven approach.

The Choice of Antigen Preparation for Antigen-specific Immunotherapy

In order to devise an antigen-driven protocol we need to select antigens that can be administered safely to tuberculosis patients. The antigens of *M. tuberculosis* itself cannot be used for this purpose because they evoke the Koch phenomenon (Anderson 1891). That is, injection of material derived from *M. tuberculosis* into tuberculosis patients will cause both local necrosis at the site of injection, and necrosis in the lesions themselves (Anderson 1891). Does this mean that we need to screen dozens of purified or recombinant antigens from *M. tuberculosis* in the hope that some will not have this property? There is a much simpler solution, which also avoids the problems of major histocompatibility complex diversity that arise in outbred populations when a few antigens are selected. Tuberculosis patients who still maintain necrotizing skin-test positivity to antigens of *M. tuberculosis* itself, have diminished or absent skin-test responses to environmental saprophytes (Kardjito et al 1986). Therefore the common epitopes can be administered safely. This is illustrated in Fig. 1, and constitutes a remarkable paradox, implying that the common epitopes are handled in a quite different way from the tuberculosis-specific components. There are at least three possible hypotheses to explain this. First, the common epitopes are by definition common, and are encountered from birth in a variety of mycobacteria (and related genera). Most of this exposure is by the oral route. Therefore it is possible that, as with other antigens encountered by this route, there has been priming of regulatory transforming growth factor-β-producing T cells (Chen et al 1995). These may switch off the response to the common epitopes during the infection. A second possibility is that since the common epitopes have been encountered at low dose over many years, the response to them has become locked into Th1 mode, and cannot be converted to Th2 or Th0 (Bretscher et al 1992).

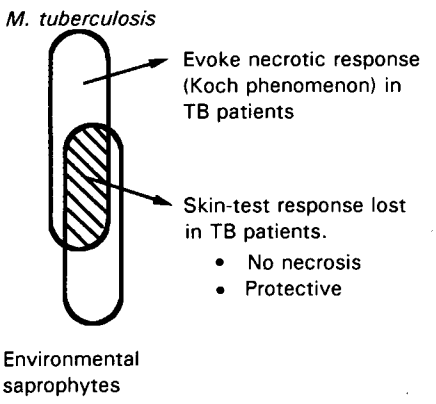

FIG. 1. Soluble antigen from *M. tuberculosis* evokes necrotic skin-test responses in tuberculosis patients, while responses to environmental saprophytic mycobacteria are lost in spite of the large proportion of shared epitopes. These shared epitopes can be targets of protective immunity.

Finally, a third possibility is that the common epitopes, are handled in an intrinsically different manner by the immune response because they are situated in such highly conserved proteins as heat shock proteins which are potentially targets of autoimmunity (Cohen & Young 1991). Whatever the explanation for a patients' lack of necrotic reaction to these epitopes and loss of skin-test responsiveness to them, they are clearly safe to administer. But is there any rationale for doing so?

The Protective Role of the Common Mycobacterial Epitopes

It is apparent from earlier sections that epitopes that are common to *M. tuberculosis* and to environmental saprophytes such as *M. vaccae*, are capable of acting as targets for a protective Type 1 response, or for a detrimental TNF-α-sensitive mixed Th1–Th2 response. This should not surprise immunologists, but it often does. We have known for many years that the Bacillus Calmette–Guérin (BCG) is as effective a vaccine against leprosy as it is against tuberculosis (Fine et al 1986). It must therefore be able to work through common epitopes. Similarly, there is evidence that contact with an environmental organism leading to mycobacterial skin-test positivity is protecting the population of Malawi from both tuberculosis and leprosy (Fine et al 1994). Indeed the 65-kDa heat shock protein of *M. leprae* can protect mice against *M. tuberculosis* (Silva & Lowrie 1994), and the awareness of the importance to protective immunity of conserved proteins such as heatshock proteins has been repeatedly emphasised by others (Young 1992). In spite of these facts there is a deep prejudice against the view that protection can be mediated via epitopes that are not species-specific. This prejudice dates from the era of the early antibody-mediated vaccines, since antibodies neutralize microbial components by binding conformational epitopes on toxins, enzymes or adhesion molecules. These substances are often species-restricted. The fact that T cells do not neutralize anything, but recognize short peptide sequences cleaved from microbial proteins, together with the fact that T cells are not taxonomists, should be sufficient to dispell the prejudice. The concept of bacterial species-specificity is irrelevant to T-cell function.

The importance of the common epitopes has several interesting consequences. Since mycobacteria are not part of the normal commensal flora of man, the nature, route and dose of mycobacterial contact is a variable that depends on where and how an individual lives. This variable priming of anti-mycobacterial responses by saprophytes, which as explained above, can protect, or predispose to infection, almost certainly explains the variable efficacy of BCG in different parts of the world (Fine 1993), and variable the protective effect of saprophytic species in some environments (Fine et al 1994).

Antigen-driven Immunotherapy in Murine Tuberculosis

The facts that the common epitopes are important for protection, do not induce necrosis, and evoke subnormal skin-test responses in tuberculosis patients, lead inevitably to the suggestion that they could be used for immunotherapy. In other words, if a Th1 response to these crucial epitopes were re-established, would the immune system be enabled to deal with the infection, and would the inappropriate Th2 component be downregulated? We have tested the mycobacterial strain with strong Th1 immunogencity (even when used killed), discussed in relation to mouse experiments above. The optimal Th1-inducing dose will partially treat tuberculosis in mice in the absence of chemotherapy, causing significantly prolonged survival when given as a single injection 60 days after intratracheal infection (Rook & Hernandez-Pando 1996). Others have similar data with this preparation (Baldwin & Orme, personal communication). It is probably significant that recombinant *M. vaccae* expressing an allergen can downregulate a pre-existing Th2 response to that allergen, and bias it towards Th1 (Thole, personal communication). Therefore unaltered *M. vaccae* naturally expressing the common epitopes, is likely to be able to downregulate the inappropriate Th2 component of the response to *M. tuberculsosis.*

Antigen-driven Immunotherapy in Man

Pilot studies in tuberculosis and leprosy in man with a similar preparation of *M. vaccae* have been undertaken. These pilot studies indicate that a single injection of killed *M. vaccae* given in addition to chemotherapy can induce T-cell recognition of common mycobacterial antigens (Stanford et al 1990). Using 3-colour flow cytometry to measure intracellular cytokines, it has now been shown that in man, injections of SRL172, a preparation derived from *M. vaccae* NCTC 11659, will result in the presence of large numbers of Th1 cytokine-secreting cells in the peripheral blood, but no Th2 cells (Baban et al, unpublished data). Moreover, this immunotherapy causes bacilli to be cleared more quickly from the sputum of tuberculosis patients and from the tissues of leprosy patients, and clinical improvement is faster and more complete. These points are illustrated by data obtained in several studies, as shown in Table 1. It is noted that immunotherapy has given rather consistent results, whereas there is wide variation in the control groups. Clearly, superimposition of immunotherapy on a full course of optimal chemotherapy in patients infected with drug-sensitive organisms, will have little effect on cure rates, which reach over 90% even without immunotherapy (Romanian data, Table 1). On the other hand, when chemotherapy is largely absent, intermittent, or with out-of-date or false drugs (or both), the role of immunotherapy is striking (Nigerian data, Table 1) (Onyebujoh et al 1995), and this is the reality of the situation in most of the world.

Table 1. Results from pilot studies in tuberculosis where patients were injected with *M. vaccae*.

	Immunotherapy	Controls
Sputum smear positivity		
Day 20, Kano (Nigeria)	20/75 (27%)	53/65 (82%)
Day 28, Rosario (Argentina)	3/21 (14%)	8/16 (50%)
Day 56, Romania	15/135 (4%)	35/143 (25%)
1 Year, Kano	11/33 (33%)	22/26 (85%)
1 Year, Romania	4/128 (3%)	9/121 (7%)
Fall in erythrocyte sedimentation rate ($mm\ h^{-1}$)		
Day 20, Kano (Nigeria)	25	4
Day 56, Rosario (Argentina)	32	7
Day 56, Romania	29	28
1 Year, Kano	42	15
1 Year, Romania	43	35
Weight gain (kg)		
Day 20, Kano (Nigeria)	2·9	0·6
Day 28, Rosario (Argentina)	3·6	2·1
Day 56, Romania	2·5	2·4
1 Year, Kano	7·9	2·0
1 Year, Romania	6·8	3·7

Conclusions

To make progress in the worldwide fight against tuberculosis we must find a way of reducing the period of treatment necessary to achieve high cure-rates. However, it unlikely that this will be achieved by any conventional chemotherapeutic approaches. Effective therapy will require a combination of chemotherapy effective in early bactericidal activity (EBA), leading to the death of most actively metabolizing bacilli in the first few days of treatment (Mitchison 1985), plus immunotherapy to convert the Koch phenomenon back to non-necrotizing bactericidal activity capable of sterilizing the tissues of persisting bacilli.

Relatively little has been published about the application of this approach other than reviews of pilot studies such as those shown in Table 1 (Etemadi et al 1992; Onyebujoh et al 1995), but these have been sufficiently encouraging to warrant the set up of Phase 3 trials performed to Good Clinical Practice with the participation of an independent monitor and a Clinical Trial Organization. The study will be decoded in early 1997. If successful, tuberculosis will have pointed the way towards a new approach to the treatment of other chronic infections. The therapeutic vaccine will be important.

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