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SPECIAL ARTICLE

Suppressor cells of mouse and man. What is the evidence that they contribute to the aetiology of the mycobacterioses?

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The existence of subpopulations of lymphocytes which decrease rather than increase certain immune responses, was first suggested in 1970.[1] At first this idea faced considerable opposition, but a decade later most immunologists accept 'suppressor' cells as an essential negative feedback mechanism. All other biological pathways are subject to both positive and negative regulation, and there is no reason to suppose that the immune response is any different. Indeed it now seems reasonable to suppose that the immune response has particular need for negative feedback in order to stop uncontrolled proliferation of lymphocytes, excessively vigorous tissue-damaging responses and, perhaps, auto-immune responses.

Thus the 'regulator' or 'suppressor' T-cell is primarily a normal homeostatic mechanism, which accompanies and modulates all normal immune responses. However, if suppressor cells exist, they can presumably go wrong. Then malfunctioning suppressor mechanisms might inappropriately reduce a much needed response to a pathogen such as *Mycobacterium leprae*, or conversely, fail to suppress an inappropriate response. The advent of monoclonal antibodies has allowed rapid progress in delineating the suppressor cell subsets in lymphocyte populations. Three recent reviews have discussed this in relation to mouse[2,3] and man.[4]

Table 1 shows how these new reagents have allowed T-lymphocytes to be divided neatly into two (or three) subpopulations. It was hoped at first that this would make it possible to define the function of individual cells – unfortunately we are rapidly being disillusioned. As Table 1 shows, several different unrelated functions can be found in each subpopulation. The most important fact in the present context is the existence of suppressor cells within both of them. The most often studied in relation to the mycobacterioses, are the TH_2^+, $T5^+$, $T8^+$ (human) or Lyt $2,3^+$ (mouse) suppressors which include cells which, once triggered, will non-specifically suppress *in vitro* proliferative responses or antibody production (e.g. to mitogens). However, more recently defined are Lyt 1^+, 2, 3^- cells which specifically suppress delayed type hypersensitivity responses to several particulate antigens, including sheep and horse erythrocytes and *Leishmania tropica*.[3]

These experiments with *L. tropica*[5] in Balb/c mice are particularly exciting for leprologists, because they constitute the first clearcut demonstration that suppressor cells can be the direct cause of a failed host response to an intracellular parasite, leading to dissemination and death. It should therefore be noted that the suppressor cells involved are Lyt $1^+,2,3^-$, not the commonly studied Lyt $1^-,2,3^+$ type.

The way in which suppressor T-cells operate is complex and there are probably several mechanisms which cannot all be discussed in detail here. A well-studied example involves a Lyt $2,3^+$ cell[6] which secretes a suppressor factor with a molecular weight between 55 and 60 kd, which is antigen-specific and also carries determinants coded by the I-J subregion of

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Table 1.

	Antigens* expressed on the lymphocyte surface	
	TH_2, T5, T8, (Man) Lyt 2,3, (Mouse)	T4, (Man) Lyt 1, (Mouse)
Helper cells for antibody production	No	Yes
Suppressors of antibody production	Yes	No
Cytotoxic activity	Yes	No
Activation of macrophages via lymphokine release	?	Yes
Mediate delayed swelling reaction in skin	Yes (some viruses and contact sensitizers)	Yes (most antigens including listeria, leishmania and mycobacteria
Suppressor of delayed type hypersensitivity to particulate antigens (leishmania and erythrocytes)	No	Yes
Suppressors of *in vitro* proliferative responses	Yes	No

*These antigens are not alleles and both can be expressed simultaneously.

the Major Histocompatibility Complex (MHC) (the expression of I–J antigens is a characteristic of suppressor cells[2,6]). This factor then 'arms' 'acceptor' cells which, on encountering the relevant antigen, secrete a non-antigen-specific mediator, which suppresses the response by a mechanism which has not yet been elucidated. (This pathway can be considered to be analogous to the 'arming' of mast-cells by antigen-specific molecules (IgE), so that they release non-specific mediators, for example histamine, when they encounter antigen.) We must assume that each stage in this pathway is itself regulated – induction of suppressor cells, release of mediators, availability of 'acceptor' cells, triggering of 'acceptor' cells.

What evidence is there for a role for suppressor cells in the pathogenesis of the mycobacterioses?

It has been known for several years that massive intravenous doses of mycobacteria can induce in mice a state of anergy[7] and an inability to respond to other antigens.[8] Similarly, *in vitro* proliferative responses to antigens or mitogens are lost during the late phase of dissemination which occurs during infection with lethal organisms such as *Mycobacterium ulcerans* or *M. lepraemurium*. Indeed, during this phase the antigens of the infecting organisms may inhibit rather than enhance *in vitro* lymphoproliferation.[9] Two types of suppressor cell have been implicated: the first is a T-cell, and can be found following large intravenous doses of BCG[10], *M. lepraemurium*,[11] or several *M. avium*-like organisms.[10] The Lyt phenotype is not known, but its effects, once triggered, are non-specific, and probably it will turn out to be Lyt $2,3^+$. There is at present no reason to believe that its appearance *causes* the disease to progress (unlike the Lyt 1^+ suppressors in *Leishmania tropica* infections of Balb/c mice[5]) and it seems more likely to be a consequence of dissemination. Thus it is readily induced by BCG, but the animals rapidly recover and the organisms are eliminated. Since we

know that BCG is pathogenic for mice with severe T-cell dysfunction,[12] this observation implies that this type of suppressor T-cell does not cause severe T-cell dysfunction.

The second kind of 'suppressor' cell found in the presence of heavy systemic loads of mycobacteria or mycobacterial products appears to be a macrophage,[11,13,14] associated with a chronic granulomatous response.[15] It is particularly prone to appear in C57Bl/6 mice and is under non-MHC-linked genetic control.[15] Again there is no evidence to implicate this cell as a cause rather than a consequence of susceptibility, though it may be relevant that C57Bl/6 mice are unusually susceptible to intravenous challenge.[16]

A problem with all this work is that no experiments have been performed to demonstrate whether transfer of the suppressor cells to normal recipients will increase their susceptibility to subsequent challenge. Moreover, we do not even know whether the intravenous challenge is relevant to the human mycobacterioses. Dissemination of mycobacterioses occurs, but the route of primary infection is obviously not intravascular.

One author has attempted to avoid both of these objections.[17] Mice (C57Bl/6 and Balb/c) were infected subcutaneously with *Mycobacterium lepraemurium.* It was found that a population of cells developed in the spleens of Balb/c mice (but not C57Bl/6), which, when transferred into irradiated Balb/c recipients, resulted in significantly decreased resistance to *M. lepraemurium.* This observation remains unique, and suggests a relevant type of suppression. Dissemination from subcutaneous infections is common in Balb/c mice, but not in the C57Bl/6 strain. However, the fact that the cell recipients had to be irradiated for the effect of the suppressors to be demonstrable, clearly detracts from its value. Moreover, the cell involved had quite different properties from the suppressor T-cells demonstrated following intravenous challenge (described above) and therefore this experiment does not provide independent support for the relevance of the latter.

Another group has studied the size of the granulomata developed in the tissues of mice following intravenous injections of killed BCG suspended in oil droplets.[18] They have found that some strains (such as C57Bl/6) develop very large granulomata. On the other hand in CBA mice, granuloma formation is rapidly 'switched off' by a suppressor cell population. It may therefore be relevant that C57Bl/6 mice are very good at localizing cutaneous challenges with virulent mycobacteria, whereas in CBA mice dissemination readily occurs. In contrast C57Bl/6 mice are very susceptible to intravenous challenge. Perhaps unsuppressed granuloma formation contributes to localization in the periphery, but also to pathology in deep tissues. This model seems interesting and relevant. It would be helpful to know how these 'granuloma-modulating' suppressor cells are related to those described in other experimental systems above.

The situation is no clearer when we consider man. There are a number of cellular mechanisms which regulate the *in vitro* proliferative responses of peripheral blood leukocytes from normal individuals.[19] These include:

1 T-cells which no longer suppress if precultured without stimulus for 24 or 48 hours.[19]
2 Indomethacin-sensitive inhibition by 'adherent' cells, probably monocytes (mediated by prostaglandins).[19]
3 Indomethacin-insensitive inhibition by adherent cells.[19]
4 Suppression triggered by lipid-rich components, common to all mycobacteria and acting on cells from all normal individuals.[20,21]
5 Cells which, when precultured with antigen and then treated with mitomycin-C, will inhibit the response to the same or different antigens, of fresh cells from the same donor.[22]

Mechanisms 1[23], 2[24] and 4[21] are not increased in cell populations from any part of the leprosy spectrum, or in tuberculosis.

Mechanism 3 may be increased because 'suppressor monocytes' have been reported in both leprosy[25,26] and tuberculosis.[27] It is not clear what relationship these cells bear to the

partially activated monocytes demonstrable in the blood of tuberculosis patients,[28] or to the 'suppressor' macrophages in the spleens of C57Bl/6 mice.[13,15]

Mechanism 5 is demonstrable using PPD, SKSD, or Candida antigen, and peripheral blood mononuclear cells from normal donors. It has recently been shown that this assay becomes positive with *M. leprae* antigen, using cells from normal people after prolonged exposure to leprosy patients.[29] It will be interesting to know whether it works with cells from patients.

Clearly none of these findings supports the idea that inappropriate suppressor cell activity contributes to the pathogenesis of leprosy. However, several groups have looked for triggering of suppressor cells by leprosy bacilli, hoping to find an effect using cells from patients, not demonstrable using cells from normal donors, and their results are suggestive, although conflicting and controversial. One problem has been the ability of all mycobacteria to inhibit *in vitro* proliferative responses of cells from all donors[18,19] (mechanism 4 above). However, Mehra and colleagues have found that Dharmendra lepromin does not have this property. It is probable that the extensive extractions with chloroform and ether which are involved in its preparation, remove the lipid-rich components responsible for the non-specific effect,[20] and perhaps reveal determinants which are not normally exposed. It is reported that this antigen will suppress the mitogenic response to Concanavalin A of mononuclear cells from lepromatous and borderline leprosy, but not from tuberculoid cases, or normal donors.[26,30] In further experiments[30] involving, unfortunately, mixtures of cells from Non-HLA-matched donors, the suppressors were said to carry an antigen (TH_2) which defines a subset of T-cells which appears identical to that defined by T5 and T8 and therefore analogous to the Lyt $2,3^+$ cells of the mouse (Table 1).

However, other groups using mixtures of cells from HLA-matched siblings have been unable[25,31] to confirm this finding, and Nath and her colleagues have reported that lepromin triggered suppression by cells from tuberculoid, but not from lepromatous cases.[25] It may be important that these authors did not use Dharmendra lepromin. However, this is not the only system in which cells from lepromatous cases appear to have less rather than more regulatory activity. Susan Watson (personal communication) has found that the $T8^+$ T-cells from these patients are defective in their capacity to suppress the response to Pokeweed mitogen, when compared to normal donors. (It is possible that Nath and her colleagues are detecting $T4^+$ suppressor cells, analogous to the Lyt 1^+ suppressors in the murine Leishmania model,[5] and if so they could, by analogy, turn out to be important.)

These findings are not necessarily incompatible with those of Mehra and her colleagues. Nevertheless this author's work is open to two types of interpretation. The optimistic view is that she has demonstrated the existence of one or more 'suppressor determinants' specific to *M. leprae*, which trigger an unbalanced suppressor cell proliferation. These cells could then suppress the response to other components of any organism which contained the 'suppressor determinant'. Such a mechanism could explain the ability of lepromatous patients to give strong skin-test responses to soluble antigens prepared from other mycobacterial species, while failing to respond to *M. leprae*, although it is rich in common antigens. An experimental model of this type has been described in relation to the responses to lysozyme in mice.[32] If this is correct, then removal of TH^+_2 or $T8^+$ cells, could be therapeutically useful.

However, the fact that many leprosy patients will give negative skin-test responses to leprosy antigen, while responding strongly to antigen preparations from other cross-reactive species, has now been explained simply, without any need for 'suppressor determinants'. Leprosy patients simply do *not* respond to the common antigens either *in vitro* or *in vivo*.[33] Thus their ability to respond to other mycobacterial species is due to their response to the species-specific components rather than to a lack of suppression of responses to the shared ones.

It is also possible that the determinant(s) which trigger the suppression in the work of Mehra and her colleagues are not specific to *M. leprae* and are merely exposed by the treat-

ment with organic solvents which is involved in the preparation of Dharmendra lepromin. Controls with other organisms, similarly treated, do not appear to have been performed, but are an obvious prerequisite for the suppressor determinant hypothesis.

The pessimistic view is that the suppressor cells which Mehra *et al.*[30] have demonstrated, like those seen following deliberate intravenous overload of mice with BCG,[10] are the consequence rather than the cause of dissemination. Indeed, these may be examples of a more general phenomenon. Thus at a workshop which took place during the spring (1982) meeting of the British Society for Immunology (chaired by Professor J H L Playfair), it was agreed that suppressor cells, which can be activated by specific antigen to exert non-specific suppressor effects, are commonly found in late disseminated infections with protozoa (malaria, leishmania), worms and bacteria, but that removal of such cells, when it has been achieved, does not alter the course of the disease. Thus the majority view[34] was that cells such as those described in disseminated murine mycobacterioses, or in blood of BL/LL[26,30] patients are consequences, not causes, of progressive disease.

Another approach to the study of suppressor cells in the mycobacterioses is to count the absolute numbers or percentage of cells with the suppressor phenotype (e.g. TH^{+}_{2}, $T5^{+}$ or $T8^{+}$) in peripheral blood lymphocyte populations from diseased individuals. It has, for instance, been observed that during ENL episodes there is a decrease in the number of cells carrying the suppressor phenotype, relative to the number of helpers.[35] But what does this mean? Presumably the blood carries lymphocytes from one site to another. Thus a transient decrease in suppressor cells in the peripheral blood could be due to decreased production of suppressor cells, or to increased sequestration of suppressor cells in the tissues. These two explanations have precisely opposite implications in terms of the role of suppressor T-cells in ENL.

Another possibility is the analysis by immunohistological techniques of the lymphocyte subpopulations infiltrating mycobacterial lesions. Thus it has been shown that a PPD skin-test site in a normal individual contains lymphocytes of both major phenotypes ($T4^{+}$ and $T8^{+}$) in the same ratio as in the blood (L Poulter, unpublished observations).

In conclusion, there is no doubt that suppressor cells are one of the most exciting areas of contemporary immunology, and it is now clear that in mouse and man, suppressor cells can be triggered by mycobacterial antigens. In both species suppressor cells with non-specific suppressor effects accompany disseminated disease, but it will be extremely difficult to prove that they are important for its pathogenesis. All immune responses are regulated and the demonstration of regulatory mechanisms in the laboratory does not prove that they were behaving in an abnormal manner in the donor. At present most workers are studying cells with non-specific suppressor effects. That is to say, cells which, when activated by mycobacterial antigen, will suppress responses not only to that antigen, but also to other stimuli.

Moreover, some authors study suppression of lymphoproliferative responses (the significance of which in terms of effector function is unclear) while others study suppression of totally unrelated antibody or cytotoxic T-cell responses. It may be that relevant suppressor cells will be found only when we study specific suppression of the relevant effector systems. The recent work of Liew *et al.*[5] is a hopeful pointer in this direction.

It is also important to remember that the initial defect which leads to the susceptibility of a lepromatous leprosy patient may not be over-active suppression. We can equally well hypothesize that over-activity of an inappropriate effector system leads to a failure to destroy bacilli and that the increasing bacterial load secondarily activates a normal suppressor response.

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