TOLERANCE AND SUPPRESSOR T CELLS

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Immunological tolerance was originally defined as 'a specific weakening or suppression of reactivity caused by the exposure of animals to antigenic stimuli before maturation of the faculty of immunological response'\(^1\). Whilst it remains true that tolerance to histocompatibility antigens can be induced with far greater ease in immunologically immature, or relatively immature, animals (for example, in neonatal mice or rats), many experimental models have since been described in which a donor-specific unresponsiveness to skin or kidney allografts can be established in fully mature, adult animals. Such procedures do, however, usually require the use of some form of immunosuppression such as sublethal whole-body X-irradiation, immunosuppressive drugs or antilymphocyte serum (ALS) as well as donor antigen, and whereas neonatally induced tolerance may often be due to the deletion or permanent inactivation of a specific lymphocyte clone\(^2\) virtually all experimental models based on adult animals appear to be mediated by active mechanisms of one kind or another\(^3\). For this reason the term 'tolerance' is often used in a far wider sense than was originally envisaged, to encompass any kind of antigen-induced unresponsiveness that can be shown to be highly specific for the antigens being used - irrespective of the mechanisms involved. For the purpose of this contribution I shall subscribe to this wider definition.

The great attraction of neonatally induced tolerance is that it can be induced simply by transfusing the recipient with an appropriate number of viable cells from the prospective donor or from other members of the donor strain. This exposure of the recipient's immature lymphoid system to the donor's antigens frequently prevents the animal from responding to the donor's skin or other tissues transplanted later in life: as predicted by Burnet and Fenner\(^4\), the animal is no longer able to distinguish between 'self' and 'non-self'. Most important of all, the recipient mouse, although unable to respond to the donor's histocompatibility antigens, is immunologically unaffected in every other sense and competent not only to fight off infectious
organisms in the normal way but to reject skin grafts from other strains of mice more or less unrelated to the donor strain. Furthermore, the lymphocytes of many tolerant mice seem to be genuinely unresponsive; in other words, they need not owe their unresponsiveness to agents such as antigen-antibody complexes which could secondarily bring about pathogenic changes in a transplanted, or in the patient's own, kidney. On the face of it this kind of tolerance would appear to be the answer to every transplant surgeon's prayer.

There are unfortunately, two major difficulties that it has not been possible to resolve. First, treatment of adult animals in this way nearly always leads to sensitisation, even when the dose of cells per gm of body weight is roughly maintained, unless high doses of immunosuppressive drugs or ALS are used or heroic numbers of cells are injected on numerous occasions; and second, by far the most suitable cells for intravenous injection and tolerance induction are lymphoid cells, i.e. cells which are themselves equipped to respond immunologically and which can, therefore, in theory and in practice, initiate an immunological response against the histocompatibility antigens of the recipient - thus producing graft-versus-host or runt disease. In mice and rats one can eliminate the danger of graft-versus-host reactions by the simple stratagem of using cells from a first generation cross between the donor and the recipient strains, for such cells - although carrying the donor strain's antigens - also carry the recipient's antigens and they cannot therefore react against the recipient's tissues. We can say that F₁ cells are good tolerogens but at the same time immunologically perfectly safe.

We have learnt a great deal from tolerance experiments of this kind, and they certainly opened our eyes, for the first time, to the possibility that survival of allografts might be attainable in adult animals and in man. But the practical difficulties of creating human cellular chimeras similar to those produced by the injection of F₁ hybrid cells into rodent neonates are probably too great to be overcome, and other strategies may have to be followed.

There are several active restraint mechanisms which are known to mediate specific tolerance to skin or other tissue grafts in experimental animals: they include enhancing antibodies, antigen-antibody complexes, suppressor cells and anti-idiotypic antibodies. Batchelor and Wigzell will be dealing with some of these (see also), and my brief is confined to suppressor T cells.

The separation of lymphocytes into subpopulations of thymus-derived (T) and bone marrow derived (B) cells is now well established. In the mouse one can identify T cells relatively easily by the presence of a specific antigen, theta, on the cell membrane; in man the presence of IgG on the surface of B cells helps us to distinguish between T and B cells. T cells can play a decisive part in the rejection of kidney allografts, and humoral antibodies produced by plasma cells, which are in turn derived from B cells, may cause hyperacute rejection or slow, attritional damage to the transplanted kidneys of immunosuppressed patients. Again in the mouse it is now possible to characterise T cell populations still further, thanks to the discovery of a
number of cell membrane markers such as the Ly series, and it is possible to distinguish between cells that provide B cells with ‘help’ in antibody formation, cells that are precursors of cytotoxic killer cells, the cytotoxic T cells themselves, or other T cells that can switch off the immunological function of other cells, both B and T, which might otherwise have been activated in the presence of antigen\textsuperscript{8-10}. I shall explain to you one experimental system, in the mouse, in which suppressor T cells are strongly implicated as a means of keeping other T cells quiescent, thus ensuring a highly specific unresponsiveness to skin allografts, and I shall then consider very briefly whether it is realistic to think that this model may every be applied in clinical transplantation. I am assuming, of course, that it is generally agreed that contemporary methods of non-specific immunosuppression are far from satisfactory.

When P. J. Kilshaw and I began to study the induction of tolerance in adult animals we decided to use only methods that were likely to be acceptable in clinical transplantation. We therefore avoided the use of viable lymphoid cells from the donor and used tissue extracts instead, and we turned away from long-term immunosuppression with drugs in favour of a brief course of ALS on days 2, 4 and 6 following skin grafting\textsuperscript{11}. Later, with M. Pinto, we added one further factor: \textit{Bordetella pertussis} vaccine given 4 days before the first dose of ALS was found to potentiate the unresponsiveness\textsuperscript{12}, presumably because it induces a marked lymphocytosis which is maximal at 4 days and thus enables the ALS to destroy a larger pool of normally recirculating lymphocytes. The donor extract, which can come from either spleen or liver, is given optimally 16 days prior to transplantation of skin grafts. This very limited and innocuous treatment leads to a very long-lasting unresponsiveness to donor strain skin allografts in 30-50% of the recipients. As in neonatally induced tolerance, the unresponsiveness proved to be exquisitely specific for the histocompatibility antigens of the donor strain.

Our notion that we were dealing here with a mechanism akin to that of neonatally induced tolerance had soon to be abandoned, for the lymphocytes from these animals were found to be almost normally reactive to donor strain antigens when they were removed from the milieu of the unresponsive mouse and tested in mixed lymphocyte culture, cell-mediated lysis, or in graft-versus-host assays\textsuperscript{13}. Clearly some factor(s) inhibited or suppressed these cells in the tolerant animals, and various experiments made it most unlikely that these were antibodies or complexes. The problem was at least in part resolved when attempts were made to transfer the unresponsiveness to ALS-treated mice belonging to the recipient strain by injecting into them, 10 days after skin transplantation, lymphoid cells from well established tolerant donors. These experiments provided evidence for the presence of suppressor T cells in the spleens and lymph nodes of tolerant animals. Thus, although unfractionated spleen cells were highly effective in transferring unresponsiveness, removal of T cells with the aid of anti-theta serum and
complement prevented transfer. On the other hand, highly purified splenic T cells, from which B cells had been excluded by filtration through a column of nylon wool, transferred unresponsiveness as effectively as unfractionated cells. Without doubt the presence of thymus-dependent lymphocytes is an essential component in the mediation of this unresponsiveness, and the most likely explanation is that suppressor T cells, which have been demonstrated and studied in many other experimental systems involving a variety of antigens, either directly or indirectly through factors released by them, specifically suppress another subpopulation of T cells which would normally have been recruited in response to the histocompatibility of the skin graft. A consideration of how this may happen is beyond the scope of this contribution: although various possibilities have been put forward we do not as yet know the precise mechanism.

The most serious drawbacks of this approach, from a clinical point of view, are that a) the graft recipient requires pretreatment with donor material and b) the proportion of long-surviving grafts is not as high as one would like it to be. Both these points are under study. Concerning the need for pretreatment, our experiments show that it may be possible to avoid reliance on the donor’s tissues by using a ‘cocktail’ extract containing many of the known mouse histocompatibility specificities, and it might be possible to follow this approach for the most common HLA specificities. So far as the reliability of the system is concerned, recent experiments with S.C. Opara indicate that three doses of procarbazine hydrochloride given in the first week following transplantation act highly synergistically with ALS so that the proportion of long-surviving grafts is of the order of 80%, even if B. pertussis is omitted from the treatment.

I am not claiming that it is practicable at the present time to use this approach in clinical transplantation; clearly far more work remains to be done experimentally. The points I do, however, want to make are these: a) administered in the appropriate manner, ALS or ALG is helpful in generating suppressor T cells; b) suppressor T cells, which do not have any known harmful effects for either the graft or the recipient, are endowed with the desirable specificity; and c) correctly prepared ALG can be administered to patients in relative safety, and a number of ALG preparations have now been shown to be beneficial in clinical renal transplantation (see). Finally, numerous reports, including those from the U.K National Organ Matching Service and from St Mary’s Hospital, indicate that transfusion of blood prior to renal transplantation improves kidney graft survival dramatically. Whilst other mechanisms may well account for this most interesting finding it is possible that this form of pretreatment is analogous to the pretreatment we give to our mice, and that it leads to the generation of suppressor lymphocytes.

References

11 Brent, L, Hansen, JA and Kilshaw, PJ (1973) Transplantation, 15, 160
12 Pinto, M, Brent, L and Thomas, AV (1974) Transplantation, 17, 477
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15 Brent, L and Kilshaw, PJ (in preparation)

Open Discussion

HABERAL (Ankara) Are you using whole blood, buffy coat, fresh or frozen blood?

BRENT These are Dr Hulme’s data. But so far as I know the blood came from a standard transfusion service, so that it will have been stored on average for about a week in normal conditions. It was not blood from which white cells had been removed.

BOULTON-JONES (Glasgow) In one of the first groups of experiments on the mice which you showed us, using B. pertussis and then the ALS, you had about 60% survival at 120 days and using the same technique in a subsequent series you had about 20% survival. Now that seems an awful big difference, is the method reproducible?

BRENT Yes, the method is extremely reproducible but much depends on the quality of the antilymphocyte serum. If you use an antilymphocyte serum which is weak, then the induction of specific unresponsiveness will be less marked compared with a high quality antilymphocyte serum. In these experiments many pools of antilymphocyte serum have been used and they have varied somewhat in their efficacy. The use of an effective antilymphocyte serum is absolutely essential if optimal results are to be achieved.

ROWINSKI (Warsaw) When did you take the spleen cells from your conditioned recipient? When is the best time? The second question is when should one give the transferred cells to the next recipient; at the time of transplant-
To answer your second question first, we give the cells ten days after skin grafting. This is a fairly arbitrary decision. The cells are given as early as possible after skin grafting, but after the effect of the antilymphocyte serum has worn off. If the cells were to be injected during the period in which antilymphocyte serum is given, many of the transferred cells might be killed and clearly this is highly undesirable. For that reason we give them at 10 days, i.e. 4 days after the last injection of antilymphocyte serum. The age of the donors has varied quite a lot, but to ensure that we used donors in the stable phase they had carried their grafts for at least three months. We have some evidence suggesting that the capacity for transfer develops with time.

We will return to possible applications of these interesting data later. The next speaker is Professor Richard Batchelor.