Transplant Int (1992) 5 [Suppl 1]: S571–S577

TRANSPLANT
International

Is tolerance a prospective for clinical research?

John W. Fabre

Division of Cell and Molecular Biology, The Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, UK

Tolerance is an emotive issue in transplantation. It is the promised land for which we all strive and which we all hope we shall live to see. In such circumstances, tolerance must always be a prospective for clinical research! The question is, therefore, better posed in a more optimistic fashion and with a small act of faith: do we, in 1991, have that crucial combination of basic scientific knowledge and creative imagination to make it possible?

Key words: Tolerance induction – Non specific immunosuppression – T cells

Definitions and objectives

Tolerance can be most simply defined in operational terms as graft acceptance without the use of non-specific immunosuppressive agents. More precisely, it is donor-specific immunosuppression. The important thing to bear in mind is that the objective of introducing tolerance in the clinic is improved safety (e.g. fewer infections) and improved efficacy (i.e. fewer rejections). Of course, perfect safety and perfect efficacy is the ideal. But either improved safety alone or improved efficacy alone are honourable enough objectives in the shorter term. There are three broad possibilities with regard to clinical application:

1. Tolerance without any non-specific immunosuppression.
2. Tolerance with some non-specific immunosuppression, probably limited to the days, weeks or months after grafting. The objective here is to make a clinically useful reduction in the patient's burden of non-specific immunosuppression.
3. Tolerance with normal non-specific immunosuppression. The aim here is to improve efficacy.

Offprint requests to: John W. Fabre

Historical perspectives

The key discovery in the field was made in 1945 by Owen [1]. He observed that dizygotic cattle twins almost invariably have identical blood groups. Building on Lillie's earlier observation that cattle twins share vascular connections in utero by way of synchorial placentae [2], Owen proposed that the consequent exchange of haematopoietic precursors in foetal life resulted in the life-long acceptance of these foreign cells. He was, of course, absolutely correct and was able to demonstrate that the adult twins were stable haematopoietic chimaeras. It was on the foundation of Owen's observation that MacFarlane-Burnett proposed, in 1949, his brilliant clonal selection theory of immunity [3]. The seal was set on this phase of development of the field when Billingham et al. [4] reproduced in the laboratory what Owen had observed in nature. They induced the life-long acceptance of skin allografts in mice by the simple expedient of injecting donor haematopoietic cells during foetal life.

Although the experimental model of Billingham et al. [4] was of no immediate clinical relevance, the experiments were of great importance. The work of Owen and of Billingham et al. has demonstrated that the spectrum of antigens regarded as self is not a fixed and immutable characteristic, but an acquired characteristic subject to experimental and, therefore, potentially, therapeutic manipulation.

Preliminary points

There are three preliminary points which are useful to consider as a background to the more detailed discussion on tolerance.

1. The T-cell is the only important target

Although allograft rejection is a complex phenomenon involving many cell types and soluble factors, everything depends on the T-cell. If the T-cell is neutralised, nothing

happens. Therefore, the only target that needs to be considered for tolerance induction is the T-cell.

2. The thymic and peripheral compartments

A second and potentially very important point is that there exist two distinct T-cell compartments, and each needs to be considered separately for tolerance induction. These are the thymic and peripheral compartments. The peripheral compartment is the pool of mature T cells in the lymph nodes, blood, spleen and so on, which are present at the moment the vascular clamps are released and which are ready to attack the graft. Once sensitized, long-lived T cells with specificity for the graft can be generated. The thymic compartment continuously produces throughout our lives new T cells and seeds them into the peripheral compartment. There is no a priori reason why these new T cells should not have specificity for and be able to attack the graft. This generation of new T cells, although most marked in young persons, nevertheless persists throughout life.

3. Mechanisms of clonal T cell inactivation

There are believed to be three mechanisms:

(a) Clonal deletion. This involves destruction of the specifically reactive T-cell. Although clonal deletion is believed to occur mainly in the thymus [5], it has also been reported for peripheral T cells [6].

(b) Clonal anergy. This is a relatively new concept, and conveys the idea that the T cell is functionally inactivated, but not killed. It is believed to occur when T cells make contact with an antigen in the absence of costimulatory signals necessary for activation [7]. The anergic state is poorly understood. Although it is believed to occur mainly in the periphery, anergy might also be induced to some degree in the thymus [8].

(c) Clonal suppression. This is a familiar concept and implies the active suppression of a T cell clone by other T cells. Although the phenomenon is a real and powerful one, it is poorly understood [9].

Advances in basic science

Over the past 5 years there have been huge advances in our knowledge of two areas of basic immunology of immediate relevance to transplantation. These are the physiology of self tolerance and the nature of T-cell allorecognition in transplantation. These advances provide us with crucially important guide-lines for our objective of achieving tolerance in the clinic.

1. The physiology of self tolerance

Throughout our lives, T cell precursors from the bone marrow enter the thymus. These cells express neither CD4 or CD8 antigens nor any chains of the T cell receptor. They give rise to two types of T-cell, distinguished by the type of antigen receptor expressed [10]. A minor component become γδ T cells, which emigrate from the thymus and accumulate preferentially in the skin and mucosal surfaces. Although it is not yet certain, the available data suggest that γδ T cells do not play an important role in allorecognition [11]. We can probably afford to ignore them in our discussion of tolerance, but we must not forget them entirely.

The major population of T cells has the αβT cell receptor, and in the course of its maturation it goes through two selection steps. Early in its development, the αβT cell co-expresses the CD4 and CD8 molecules and goes through a positive selection step by interacting with the epithelial cells of the thymus cortex. It is at this stage that the bias towards self (more precisely, towards the MHC molecules on the thymic epithelial cells), or the property of self MHC restriction, is acquired. Those T cells that have antigen receptors with too low an affinity for the MHC molecules on the thymic epithelium die by neglect. There then follows a negative selection step, where T cells with receptors having a high affinity for the MHC molecules on dendritic cells at the cortico-medullary junction die by a process called apoptosis. This is the clonal deletion which is responsible for much of self tolerance, and predicted in 1949 by MacFarlane Burnett [3]. Cells which survive this step become mature CD4 + or CD8 + positive αβT cells.

What is crucial to us as transplanters is that the dendritic cells responsible for the clonal deletion step are of bone marrow origin [12]. This was demonstrated many years before the role of these cells in clonal deletion had been established. It follows that in allogeneic haematopoietic chimaeras, thymic tolerance towards donor alloantigen will be brought about by the same powerful mechanisms which operate for self tolerance. This is the important message which the new knowledge on the physiology of self tolerance has to offer transplantation.

Donor bone marrow has been used in conjunction with ALS and other immunosuppressive agents for suppressing organ graft rejection experimentally for many years [13] and is currently being tested in a clinical trial in kidney graft recipients [14]. However, given the difficulty in establishing fully allogeneic haematopoietic chimaeras, it would seem very unlikely that the above protocols are in fact achieving chimaerism. The bone marrow might simply represent a source of donor antigen, such as that which would be present on any other source of donor cells.

2. T cell allorecognition

It is now well established that the T-cell does not recognise conventional antigens as intact 3-dimensional structures but only as peptides incorporated into self MHC molecules [15]. The outstanding exception to this general rule occurs in transplantation. T cells recognising allogeneic MHC molecules actually do recognise them as intact, 3-dimensional structures on the surface of the foreign cells. This is termed direct recognition. The precursor frequency of T cells responding in direct recognition is high, with estimates varying between 1% and 10% of all T cells in any individual being able to give proliferative responses to a foreign MHC haplotype [16]. Direct T cell recognition gives rise to strong primary immune responses, and it has been considered the major, if not the only, pathway for T cell recognition in transplantation.

Very little attention has been given to T cell recognition of graft antigens by the normal, physiological pathway, i.e. where the graft is treated simply as a source of foreign protein and the allogeneic MHC antigens (and other polymorphic proteins) are processed and presented on recipient antigen presenting cells (APC) [17–19]. Using synthetic peptides corresponding to polymorphic regions of donor MHC molecules [20] and isolated, denatured chains of donor MHC molecules [21] to prime graft recipients to indirect recognition without influencing direct recognition, we have recently obtained definitive proof that indirect allorecognition can play a significant role in allograft rejection.

3. An important implication of the recent advances

It is potentially of fundamental importance in tolerance induction that there exist these two quite distinct pathways for T cell recognition. For example, it is clear that thymic tolerance for direct recognition can occur only if there are living donor dendritic cells in the thymus, i.e. if there is donor haematopoietic chimaerism. It is otherwise quite impossible for the T cells to interact with the intact 3-dimensional form of donor MHC antigens in the thymus. By complete contrast, exposure to intraperitoneally injected ovalbumin peptides can result in deletion of specifically reactive T-cell clones in the thymus [22]. This suggests that peripheral exposure to histocompatibility antigens might result in thymic tolerance by clonal deletion for indirect allorecognition (irrespective of the effect on the peripheral lymphocyte pool).

An additional important consideration

When we consider the various approaches to tolerance induction, one additional theoretical point becomes very important. It concerns the susceptibility of the allograft to direct T-cell recognition at various times after transplantation. The dendritic cell is the major cell type for direct stimulation of T cells in vitro [23]. Whether or not other cell types have the capacity to stimulate direct recognition of unprimed T cells is somewhat controversial, the major argument being with the vascular endothelial cell [for discussion see 24]. However, most groups have found that MHC class II positive vascular endothelial cells, but not other cell types, do have the capacity to stimulate direct recognition in unprimed T cells, although the stimulation might not be as strong as with the dendritic cell.

These points are important for transplantation because allografts contain a special type of bone marrow derived dendritic cell, the interstitial dendritic cell [25], which is almost certainly the immunogenic passenger leucocyte. These cells are migratory, and 1 or 2 weeks after transplantation the donor interstitial dendritic cells in the graft have emigrated and been replaced by interstitial dendritic cells of recipient type [26]. It follows that the capacity of the graft to stimulate direct recognition will be much diminished or absent within 1 or 2 weeks after grafting, if the interstitial dendritic cell is the major cell type for stimulating direct T-cell recognition. If, however, the vascular endothelial cell also has this capacity, the graft retains the capacity to stimulate direct recognition throughout its life. This is because the vascular endothelial cell is an intergral component of the allograft and remains of donor type unless there has been much damage [27].

One of the major and most curious anomalies in transplantation might be explained on the above basis. It concerns the ease with which allografts are accepted in rodents, especially after very brief treatment with immunosuppression. I postulated some years ago that the species difference in expression of class II MHC antigens on vascular endothelial cells could be the crucial factor, the rat not normally expressing class II whilst in man many vascular endothelial cells normally do express class II antigens [28]. If our thinking is correct, this would leave rat allografts without the capacity to stimulate direct recognition after 1 or 2 weeks in the new host, whereas this capacity would be maintained indefinitely by human allografts [28].

Possible approaches to tolerance induction (Table 1)

Approach 1. Antibodies to CD4 molecules, cytokine receptors etc., aim to interrupt necessary costimulatory signals and thereby induce anergy. It should not be forgotten that this approach induces powerful non-specific immunosuppression, a fact which, surprisingly, is often overlooked.

Approach 2. This corresponds to the old definition of "active enhancement" and is the oldest approach to tolerance induction. Water soluble MHC molecules have long been seen as a potentially powerful approach for tolerance induction with low risk of sensitisation. Cells carrying donor MHC antigens but without costimulatory capacity (e.g. transfected fibroblasts) have been considered recently for the induction of anergy.

Approach 3. Selective irradiation of the organised lymphatic tissues appears to produce powerful non-specific suppression and might allow graft recipients in the longer term to stop all immunosuppressive medication [29].

Table 1. Possible approaches for tolerance induction

1. Antibodies to CD4 molecules, adhesion molecules, cytokines and cytokine receptors. Soluble competitors of these systems, e.g. soluble cytokine receptors
2. Donor antigen treatment
   (a) Water soluble MHC antigens
   (b) Cells expressing donor MHC antigens but lacking costimulatory capacity
   (c) Other forms, e.g. blood cells, spleen cells, liver membranes
3. Total lymphoid irradiation (TLI)
4. Passive enhancement, i.e., antibodies to donor MHC antigens
5. Toxins conjugated to IL-2 or to antibodies to the IL-2 receptor
6. Donor thymus graft
7. Donor haematopoietic chimaerism

However, TLI is a cumbersome technique, it produces long-term effects on the immune system and the results are not consistent.

Approach 4. The administration of antibodies to donor MHC antigens, known as passive enhancement, is probably effective by blocking donor interstitial dendritic cells [30], but is a weak form of immunosuppression with many problems in clinical application [31, 32].

Approach 5. The objective here is to destroy T cells which have been activated by graft antigens to express IL-2 receptors.

Approach 6. This is a superficially attractive idea since it implies that tolerance in the donor thymus will result in powerful tolerance to donor antigens. As we shall see this might not be the case.

Approach 7. The establishment of donor haematopoietic chimaerism is the most rigorous donor-specific treatment for organ transplantation.

Theoretical consideration of the various approaches to tolerance induction

In Table 2 and in the ensuing discussion I have tried to predict the effect on the thymic and peripheral T-cell compartments of some of the approaches listed in Table 1.

Table 2. Theoretical considerations concerning the effects of various protocols for tolerance induction

Treatment	Type of T-cell recognition	T-cell compartment	
		Thymus	Periphery
Cells without costimulatory capacity	Direct Indirect	No effect ? tolerance	Anergy ? sensitisation ? suppression
Antibodies to CD4 antigen	Direct Indirect	No effect (? tolerance)[a]	Anergy Anergy
Water soluble histocompatibility antigen	Direct Indirect	No effect ? tolerance	? No effect ? no effect ? sensitisation ? suppression
Donor lymphoid cells	Direct Indirect	No effect ? tolerance	? sensitisation ? suppression ? anergy ? sensitisation ? suppression
Donor thymus graft	Direct Indirect	? no effect ? anergy Tolerance	? sensitisation ? suppression ? sensitisation ? suppression
IL-2 toxin conjugates	Direct Indirect	No effect (? tolerance)[a]	Tolerance Tolerance
Donor haematopoietic chimaerism	Direct Indirect	Tolerance Tolerance[b]	(anergy)[b] (suppression)[b] (suppression)[b]

[a] The tolerance would be a consequence of antigen release from the accepted allograft, and not directly of the treatment

[b] Refers to situations with mixed haematopoietic chimaerism

1. Cells with donor MHC antigens but lacking costimulatory capacity

(a) Peripheral compartment. Assuming they work perfectly well (which is a major assumption) cells carrying donor MHC antigens but lacking costimulatory capacity should induce anergy for direct recognition in the peripheral T-cell compartment. However, as the antigens will be taken up and presented by recipient APC, the effect on indirect recognition in the peripheral pool is unpredictable. Either sensitisation or suppression could be generated, anergy not being possible since the antigen presentation is by professional APC. This approach, therefore is unlikely to be of value.

(b) Thymic compartment. From our preceding discussions, it is clear that there will be no effect at all on the production and release from the thymus of T cells with capacity for direct recognition of the graft. Therefore, once treatment is stopped, the newly released T cells with capacity for direct recognition should be able to attack the graft. Whether or not this will be a problem will depend on the capacity of the graft to stimulate direct recognition once the donor interstitial dendritic dells have emigrated, as previously discussed. In man, where grafts are likely to maintain the capacity for stimulating direct recognition in the long term, these newly emerging T cells are likely to represent a continuing and cumulative problem.

If there is sufficient access of administered donor antigen to the thymus, tolerance to the indirect recognition pathway is a possibility. Moreover, once the graft is accepted, release of antigen from the graft might by itself maintain tolerance for indirect recognition in the thymus. However, this is pure speculation at this stage.

2. Treatment with antibodies to CD4 antigen

(a) Peripheral compartment. Assuming antibodies to CD4 antigens work perfectly well, one would expect anergy for both direct and indirect recognition in the peripheral pool of T cells.

(b) Thymic compartment. There will not be any effect of antibodies to CD4 on direct allorecognition in the thymus, simply because there are no donor dendritic cells in the thymus. There will also probably not be any effect on tolerance to indirect recognition in the thymus. If anything, antibodies in CD4 antigens have been shown to interfere with self tolerance induction [33]. However, as discussed above, the presence of the graft and possibly access of donor antigens to the thymus might result in tolerance in the indirect pathway.

The problem in the clinical situation with the T cells for direct recognition newly emerging from the thymus would be as discussed in a preceding section. It is therefore unlikely that this approach will allow discontinuation of non-specific immunosuppression in man. It should really be seen as an adjunct to current therapy to improve efficacy.

3. Treatment with water soluble histocompatibility antigens

These have long been seen as a possible approach for potent induction of tolerance with minimal risk of sensitisation [34, 35]. In our hands, however, truly water soluble, monomeric class 1 MHC antigens have been without effect [36].

For an effective stimulatory interaction of a T-cell with an antigen, the antigen is required to be on a membrane, thereby allowing multiple interactions with the T-cell receptor, aided by accessory adhesion molecules. It is, therefore, hard to imagine that monomeric interactions with soluble, monomeric MHC molecules will occur to any significant degree. One would guess that water soluble MHC molecules will have no effect on direct recognition. With indirect recognition, suppression or sensitisation in the peripheral pool, as discussed for cells lacking costimulatory capacity, would be possible. The effects in the thymus would also be as for treatment with cells lacking costimulatory capacity.

Where water soluble MHC molecules have been shown to be effective for immunosuppression in rodent models or in in vitro systems, it is possible that contaminating aggregates might be responsible. Such aggregates are potentially dangerous in the clinical setting.

4. Treatment with allogeneic donor lymphoid cells

These have been shown to be a powerful approach for donor-specific immunosuppression in rodents. However, the results are never uniform, and the risk of unpredictable sensitisation makes this approach, and any other approach involving treatment with donor antigens (i.e. active enhancement), currently unacceptable for clinical application. However, from the theoretical point of view, the generation of powerful, donor specific suppression in the peripheral pool is very attractive. Not only would the peripheral pool be covered, but also any newly emerging T cells from the thymus. In the long-term this approach probably offers the only real hope of risk-free immunosuppression, but far too little is known about the factors that influence the immune response to antigen for it to be worth serious consideration at this stage.

Treatment with donor antigens sometimes induces alloantibodies to MHC antigens. These can be damaging but they can also suppress T cell immune responses, albeit indirectly. For example, in the rat, treatment with antibody to donor MHC antigens (passive enhancement) might be effective by inactivating donor interstitial dendritic cells [30].

5. Donor thymus grafts

This idea is superficially attractive and has been tried experimentally [37]. However, as the dendritic cells in the medulla of the donor thymus (once the dendritic cells resident at the time of grafting have emigrated) will be of recipient type, deletion of T cells directly reactive to donor MHC antigens will not occur. Nevertheless, as mentioned in a preceding section, the thymic epithelial cells probably can induce anergy [8] and this might be the mechanism operating in donor thymus grafts. The effect on peripheral T cells of donor thymus grafting might be that of any exposure to donor antigen.

6. IL-2 toxin conjugates

If optimally effective, i.e. if all of the potentially reactive T cells are activated during the course of treatment, this approach should result in deletion of all donor specific T cells in the peripheral pool. However, because there are no donor dendritic cells in the thymus, there will not be any effect on tolerance to direct recognition in the thymus.

7. Donor haematopoietic chimaerism

This is the only approach which will induce tolerance for direct recognition in the thymus, as it is the only approach which provides dendritic cells of donor type in the thymus medulla. If there is 100% donor haematopoietic chimaerism, this would have been achieved by a total destruction of the recipient's haematopoietic and immune systems. Questions of direct and indirect recognition of donor antigens in the periphery become irrelevant. However, the matter of indirect recognition in the thymus becomes both complicated and interesting. The developing donor T cells will have recipient MHC molecules as their restricting element since this is a characteristic acquired by interaction with and positive selection on the thymic epithelial cells. Therefore, indirect recognition should become irrelevant in this context.

With mixed donor and recipient haematopoietic chimaerism, some form of T cell inactivation must be operating in the periphery in both recipient-anti-donor and donor-anti-recipient directions. Since there would be abundant host and donor antigen in the thymus, tolerance for indirect recognition in both recipient-anti-donor and donor-anti-recipient directions would be likely.

Is tolerance, therefore, a prospective for clinical research? Let us look at the three clinical possibilities discussed at the beginning.

1. Tolerance without any recourse to non-specific immunosuppression

This is donor-specific immunosuppression that is as safe as a course of penicillin or vaccination against tetanus, i.e. achieved with minimal or no interference with the recipient's immune system. There is no known protocol whereby this could be achieved in the clinic at the present time. On theoretical grounds, as discussed in a preceding section, the induction of powerful peripheral suppression for direct and indirect recognition probably offers the only real hope of achieving this objective. A protocol involving treatment with donor antigen is probably how this

will be achieved, but reliable production of peripheral suppression without the risk of sensitisation will require better understanding of the factors that influence the host response to antigen.

2. Tolerance with reduced non-specific immunosuppression

In practice, this means non-specific immunosuppression restricted to the weeks or months around the time of grafting. There are probably two approaches to achieve this: the use of total lymphoid irradiation (TLI) and the induction of donor haematopoietic chimaerism. TLI can induce powerful peripheral suppression, but is a cumbersome approach and has long-term effects on the immune system. The induction of donor haematopoietic chimaerism is currently a dangerous procedure and has long-term risks of malfunction of the immune system. However, if these problems can be solved or minimised, the implications for organ transplantation would be incalculable.

3. Tolerance to improve safety and/or efficacy in patients treated with normal courses of non-specific immunosuppression

The hopes here probably rest mainly with the use of humanised antibodies to CD4 and other adhesion and accessory molecules, and humanised antibodies to cytokines and cytokine receptors. While antibodies to CD4 antigens can result in tolerance without long-term immunosuppression in rodents [34] this is unlikely to be the case in man, as discussed earlier in this paper. Depleting donor organs of interstitial dendritic cells might also make an important contribution in reducing the strength of the rejection response that must be dealt with, but this depends on how important a contribution they make to the immunogenicity of human organs, as discussed in a preceding section.

Conclusion

The promised land is still beyond the horizon, so nobody knows how quickly or how slowly we shall reach it. I hope that this review has clarified some issues and focussed attention on the more promising paths, so that the wait might not be too long. Certainly, I think that today we have a clearer idea of where we stand and the magnitude of the problems that face us.

References

1. Owen RD (1945) Immunogenetic consequence of vascular anastomoses between bovine twins. Science 102: 400–401
2. Lillie FR (1916) The theory of the freemartin state. Science 43: 611–613
3. Burnet FM, Fenner F (1949) The production of antibodies, 2nd edn. Macmillan, p 76
4. Billingham RE, Brent L, Medawar PB (1953) Actively acquired tolerance of foreign cells. Nature 4379: 603–606
5. Marrack P, Lo D, Brinster R, Palmiter R, Burkly L, Flavell RH, Kapler J (1988) The effect of thymus environment on T cell development and tolerance. Cell 53: 627–634
6. Webb S, Morris C, Sprent J (1990) Extrathymic tolerance of mature T cells: clonal elimination as a consequence of immunity. Cell 63: 1249–1256
7. Mueller DL, Jenkins MK, Schwartz RH (1989) Clonal expansion versus functional clonal inactivation: a costimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy. Annu Rev Immunol 7: 445–480
8. Ramsdell F, Lantz T, Fowlkes BJ (1989) A nondeletional mechanism of thymic self tolerance. Science 246: 1038–1041
9. Batchelor JR, Lombardi G, Lechler RI (1989) Speculations on the specificity of suppression. Immunol Today 10: 37–40
10. Nikolic-Zugic J (1991) Phenotypic and functional stages in the intrathymic development of αβT cells. Immunol Today 12: 65–69
11. Hunig T, Tiefenthaler G, Lawetzky A, Kubo R, Schlipkoter E (1989) T-cell subpopulations expressing distinct forms of the TCR in normal, athymic and neonatally TCR αβ-suppressed rats. Cold Spring Harbour Symposia on Quantitative Biology 54: 61–68
12. Barclay AN, Mayohoffer G (1981) Bone marrow origin of Ia positive cells in the medullar of rat thymus. J Exp Med 153: 1666–1671
13. Monaco P, Wood ML (1970) Studies on heterologous antilymphocyte serum in mice. VII. Optimal cellular antigen for induction of immunologic tolerance with antilymphocyte serum. Transplant Proc 2: 489–496
14. Barber WH, Laskow DA, Deierhoi MH, Juilian BA, Curtis JJ, Diethelm AG (1989) Use of cryopreserved donor bone marrow in cadover kidney allograft recipient. Transplant Proc 21: 1787–1789
15. Berzofksy JA, Brett SJ, Streicher GZ, Takahashi H (1988) Antigen processing for presentation of T lymphocytes: function, mechanisms and implications for the T-cell repertoire. Immunol Rev 106: 5–31
16. Bevan MJ (1984) High determinant density may explain the phenomenon of alloreactivity. Immunol Today 5: 128–131
17. Butcher GW, Howard JC (1982) Genetic control of transplant rejection. Transplantation 34: 161–166
18. Lechler RI, Batchelor JR (1982) Restoration of immunogenicity to passenger cell-depleted kidney allografts by the addition of donor strain dendritic cells. J Exp Med 155: 31–41
19. Sherwood RA, Brent L, Rayfield LS (1986) Presentation of alloantigens by host cells. Eur J Immunol 16: 569–574
20. Fangmann J, Dalchau R, Fabre JW. The rejection of skin allografts by indirect recognition of donor class I MHC peptides. J Exp Med (Submitted)
21. Dalchau R, Fangmann J, Fabre JW. Allorecognition of isolated denatured chains of class I and class II MHC molecules. Eur J Immunol (In Press)
22. Murphy KM, Heimberger AB, Loh DY (1990) Induction by antigen of intrathymic apotosis of $CD4^+$, $CD8^+$, TCR^{10} thymocytes in vivo. Science 250: 1720–1723
23. Steinman RM, Witman MD (1978) Lymphoid dendritic cells are potent stimulators of the primary mixed leucocyte reaction in mice. Proc Natl Acad Sci USA 75: 5132–5136
24. Halttunen J (1990) Failure of rat kidney nephron components to induce allogeneic lymphocytes to proliferate in mixed lymphocyte kidney cell culture. Transplantation 50: 481–487
25. Hart DNJ, Fabre JW (1981) Demonstration and characterization of Ia positive dendritic cells in the interstitial connective tissues of rat heart and other tissues, but not brain. J Exp Med 154: 347–361
26. Milton AD, Spencer SC, Fabre JW (1986) The effects of cyclosporin A on the induction of donor class I and class II MHC antigens in heart and kidney allografts in the rat. Transplantation 42: 337–347

27. Hart DNJ, Fabre JW (1981) Localisation of MHC antigens in long-surviving rat renal allografts: probable implication of the passenger leucocyte in graft adaptation. Transplant Proc 13: 95–99
28. Fabre JW (1982) The rat kidney allograft model: was it all too good to be true? Transplantation 34: 223–224
29. Slavin S (1987) Total lymphoid irradiation. Immunol Today 3: 88–92
30. Hart DNJ, Fabre JW (1982) The mechanism of induction of passive enhancement: evidence for an interaction of enhancing antibody with donor interstitial dendritic cells. Transplantation 33: 319–322
31. French ME, Batchelor JR (1972) Enhancement of renal allograft in rats and man. Transplant Rev 13: 115–141
32. Fabre JW (1982) Specific immunosuppression. In: PJ Morris (ed) Tissue Transplantation, Clinical Surgery International, vol. 8. Churchill Livingstone, Edinburgh, pp 80–94
33. MacDonald HR, Hengartner H, Pedrazzini T (1988) Intrathymic deletion of self-reactive cells prevented by neonatal anti-CD4 antibody treatment. Nature 335: 174–176
34. Medawar PB (1963) The use of antigenic tissue extracts to weaken the immune reaction against skin homografts in mice. Transplantation 1: 21–38
35. Reisfeld RA, Kahan BD (1971) Extraction and purification of soluble histocompatibility antigens. Transplant Rev 6: 81–112
36. Priestley CA, Dalchau R, Sawyer GJ, Fabre JW (1989) A detailed analysis of the potential of water soluble classical class I MHC molecules for the suppression of kidney allograft rejection and in vitro cytotoxic T cell responses. Transplantation 48: 1031–1038
37. Salaun J, Bandeira A, Khazaal I, Calman F, Coltbey M, Coutinho A, Le Douarin NM (1990) Thymic epithelium tolerizes for histocompatibility antigens. Science 247: 1471–1474
38. Cobbold SP, Martin G, Waldmann H (1990) The induction of skin graft tolerance in MHC mismatched or primed recipients: primed T cells can be tolerised in the periphery with anti CD4 and anti CD8 antibodies. Eur J Immunol 20: 2747–2755