

An Overview of the Immune System with Specific Reference to Membrane Encapsulation and Islet Transplantation

DEREK W.R. GRAY

Nuffield Department of Surgery, University of Oxford, Oxford, United Kingdom

ABSTRACT: The concept of immunoisolation by use of a bioartificial membrane is discussed, concentrating on the immunological mechanisms that are likely to be operative in the light of recent information on the workings of the immune system. Special attention is given to the use of encapsulation for the purpose of treating autoimmune diabetes by implantation of xenogeneic islet tissue. It is argued that the term immunoisolation is misleading because the immune system is always activated by the indirect pathway of antigen presentation and that the term immunomodulation would be more appropriate.

KEYWORDS: overview; immune system; membrane encapsulation; islet transplantation

INTRODUCTION

For many years, the working hypothesis behind encapsulation technology and similar techniques was the concept termed *immunoisolation*, that is using a membrane prevented the immune system from detecting and reacting to the presence of foreign tissue within the encapsulation device. A large number of rodent and even large animal experiments have been performed with the aim of confirming this concept to be correct and producing functioning grafts of tissues, such as isolated islets. The author observes that each new conference on the topic brings a fresh batch of researchers claiming a new method of encapsulation with preliminary evidence of function in the rodent model. However, the techniques for encapsulation and similar approaches never seem to be reliably effective once an attempt is made to move up to large animal studies or clinical transplantation. In this article, the author briefly reviews some fundamental concepts that have recently changed or consolidated our knowledge of the immune system. Subsequently, the relevance to encapsulation techniques for islet transplantation is discussed. Before commencing it is worth clarifying the terminology used to describe various types of transplantation as presented in the GLOSSARY of this volume. However, it should be noted that the terms (allograft, autograft, syngeneic graft, xenograft, concordant, and discordant grafts) still hide major variations in severity of the barrier being crossed. For example, a graft between rat and mouse is designated as a xenograft, but there are

Address for correspondence: Prof. D.W.R. Gray, D.Phil., F.R.C.P., F.R.C.S., The Nuffield Department of Surgery, John Radcliffe Hospital, Headington, Oxford, OX3 9DU, U.K. Voice: 44 1865 220145; fax: 44 1865 768876.
derek.gray@nds.ox.ac.uk

relatively few differences in proteins other than the MHC, which is not the case when porcine tissue is transplanted to a human, for example. The crucial points to appreciate are, first, that rodent species are markedly less reactive to transplantation than humans, particularly with respect to the ability to wall off invaders, such as parasites, by production of dense fibrous tissue. Second, the immune barrier that is presented by transplantation between widely disparate xenografts is much greater than in an allograft, and still greater if the immune system has been preprimed, as is the case in autoimmune diabetes.

**THE IMMUNE SYSTEM:
GENERAL POINTS RELEVANT TO ENCAPSULATION TECHNOLOGY**

The mammalian immune system may be conveniently viewed as either a non-adaptive (innate) response that responds immediately to an invader, but with limited strength, or the adaptive immune system. The latter system separates further into antibody produced by the B cells and T-cell immunity which itself separates into CD8 T cells targeting peptides bound to MHC Class 1 and CD4 T cells targeting MHC Class II (see FIGURE 1). The major structural components of the immune system are outlined in FIGURE 2. From a point of view of encapsulation, without doubt it is the migratory dendritic cell that is the key player.^{1,2} Other than the structural components of the immune system, we must also consider the signaling and adhesion molecules, named in FIGURE 2. It has recently become clear that the entire immune system is largely derived from a quite limited number of primordial

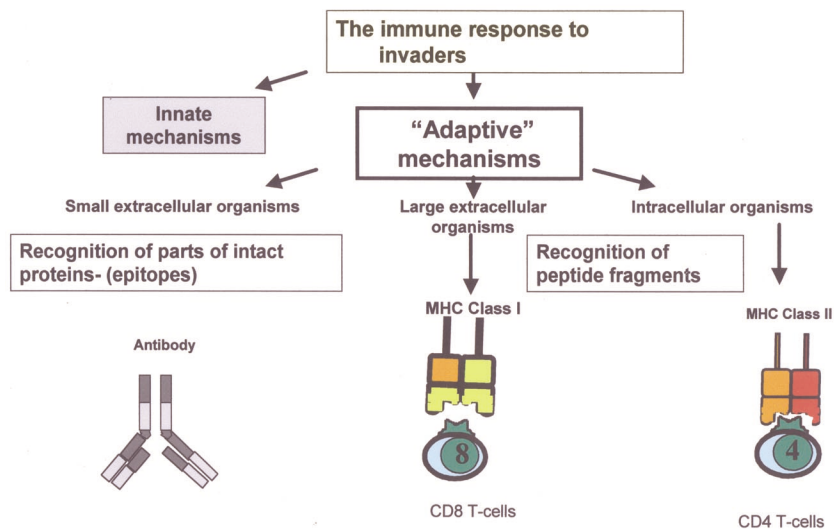


FIGURE 1. Two adaptive strategies for recognition of invaders.

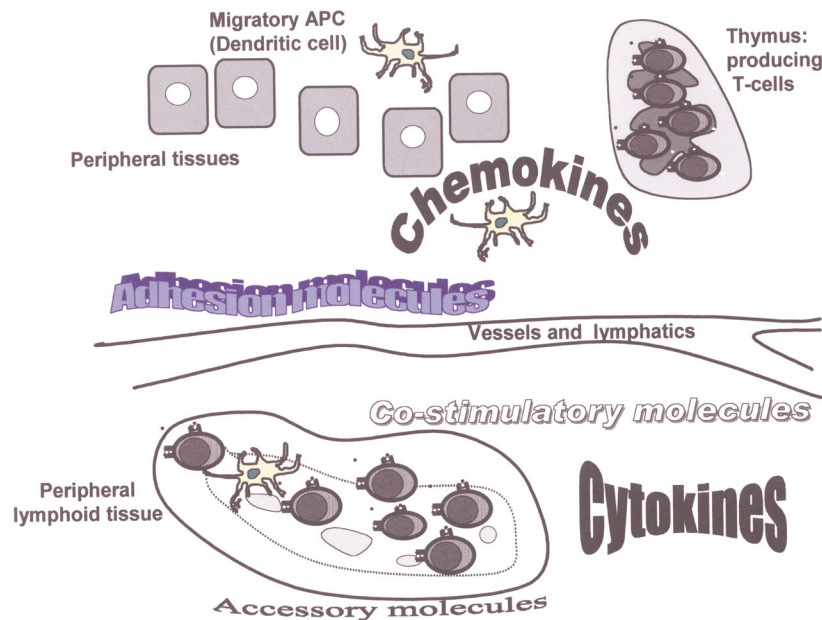


FIGURE 2. The components of the immune system summarized.

molecules that have mutated, duplicated, or become membrane-bound or soluble molecules (see TABLE 1). Some of these are large molecules, such as the derivatives of the Ig super-family, which are not likely to pass the pores of an encapsulation membrane, but other molecules, such as cytokines, are quite small.

A further basic concept to understand is that the strategy the immune system adopts for T-cell function is to have a highly variable set of T-cell receptors which will be able to identify virtually any peptide presented³ (see TABLE 2). However, this implies that the number of cells that will initially respond to a foreign peptide is normally very low, of the order of one in 10^5 – 10^6 .^{4,5} This means that rapid clonal expansion of the T cell once it has identified foreign peptide is crucial, and indeed this is precisely where most immunosuppression drugs are targeted, preventing rejection by stopping the necessary expansion. Despite the fact that the starting frequency of responding cells is so low, the response of the normal immune system to systemic infection is so rapid that within days a significant percentage of the total T cells in an individual may be targeted against a single virus.⁶ However, the low starting frequency of T cells responding to a single foreign peptide contrasts strongly with the remarkable response of T cells that are exposed to allogeneic MHC molecules, as happens when an allograft is transplanted (see FIGURE 3) and then the number of responding cells may be 1,000-fold higher (see TABLE 3).⁷ The exact mechanism by which this occurs is uncertain, since one might have expected a less good “fit” of the MHC molecule to result in non-recognition. The explanation put forward is that the combined allo-MHC + any peptide complex corresponds to a shape that

TABLE 1. Some basic immunology concepts relevant to encapsulation

Concept 1. The immune system has developed from relatively few “primordial molecules”, some of which are large molecules, but some quite small (e.g., chemokines)

- Molecules binding to peptides with a variable binding site: *immunoglobulin superfamily*
- Molecules binding to sugars: *lectins*
- Molecules binding to structural proteins: *fibronectins*
- Signalling peptides and receptors: *chemokines*
- Growth hormones and receptors: *interleukins* and *cytokines*

Concept 2. The strategy for detecting and responding to novel invading organisms depends on detecting peptides derived from shed proteins

- Huge number of variant TCRs
- Number of T-cells responding to any single peptide low (precursor frequency not less than 1:100,000)
- Strategy critically dependent upon ability to rapidly proliferate responding cells

Concept 3. An allograft is an unexpected aberration that “catches out” the immune system

- A very complex situation arises as a consequence of MHC molecules “almost but not quite fitting”
- Between graft and host, most components of the immune system are the same or similar molecules which function normally
- Note that for xenografts more or less the opposite is true

Concept 4. Many phenomena in transplantation parallel those demonstrable in autoimmune disease/ regulation

- Susceptibility to immunosuppressive agents
- Role of peptides in immune response
- Th1 versus Th2 cytokines
- Development of anergy
- Role of regulatory cells

It is likely that the phenomenon of graft acceptance involves the mechanisms that normally control autoimmunity

TABLE 2. How the immune system is switched on is largely understood, but we still do not understand how it is turned off

Suggested mechanisms include:

- Th1 / Th2 hypothesis
- Microchimerism
- Anergy/apoptosis
- Danger hypothesis
- Regulatory cells
- Linked epitope suppression
- The authors hypothesis: MHC-based Suppression²⁷

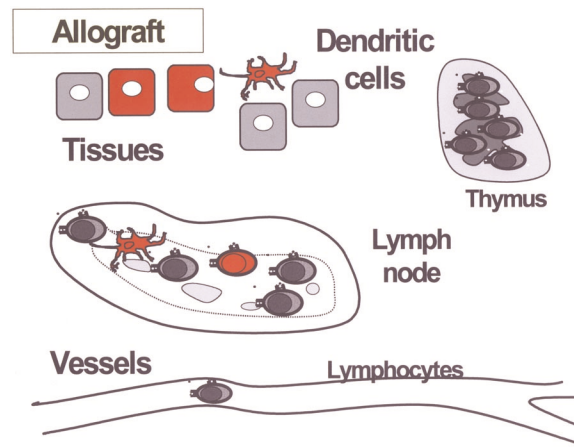


FIGURE 3. An allograft represents an expected and complex situation in which components of the immune system derived from the graft attempt to interact with the host. The result is an unexpectedly violent reaction, termed rejection.

is interpreted by the T cell as a self MHC molecule with a foreign peptide (see FIGURE 4).⁸ The two responses of the T cell to transplanted tissue described above form the basis of what is termed the *indirect presentation* pathway,⁹ corresponding to detection of peptide via what is essentially the normal route, and the so-called *direct presentation* pathway by which the mistaken recognition of the whole MHC occurs.⁸ Understanding these two ways in which T cells may be activated by transplanted tissue is crucial to interpreting the effect of inserting a membrane barrier, and it is particularly important to understand that the two pathways have something of a reciprocal arrangement depending on whether the graft is an allograft or xenograft (see FIGURES 5 and 6).

TABLE 3. Encapsulation technology and immunology: the author's advice

Stop trying to do what is probably not achievable (e.g., molecular cut-off)
Drop the term immunoisolation—it is not
Use the term immunoprotection if you must
You cannot ignore the immunobiology—instead work with it
Exploit current niches
Define the secrets of biocompatibility
When the secrets of tolerance are unravelled, encapsulation technology may come to the fore as the best way of delivering appropriate membrane-bound biological signals

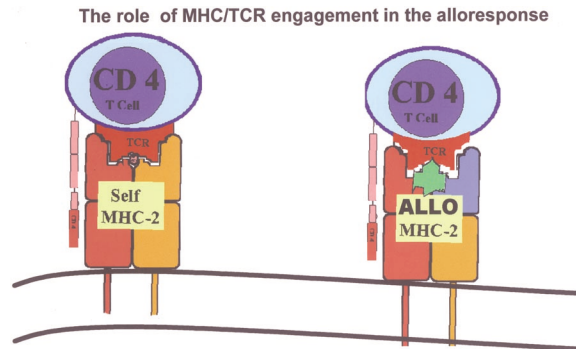


FIGURE 4. Allogeneic MHC molecules are reacted against by host T cells as though they all were self MHC molecules carrying an invader-derived peptide.

POINTS RELATED TO ISLET TRANSPLANTATION AND ENCAPSULATION

Moving from more general concepts to the issues that specifically relate to transplantation of islets, there are a number of experimental models routinely used to ascertain the function of islet transplants and the effect of encapsulation. These are outlined in FIGURE 7, which emphasizes that there is an exponential increase in difficulty of these models as one approaches clinical transplantation. The commonly favored site for islet transplantation is into the portal vein,¹⁰ where the islets have been shown to lodge in the terminal portal radicals¹¹ (see FIGURE 8).

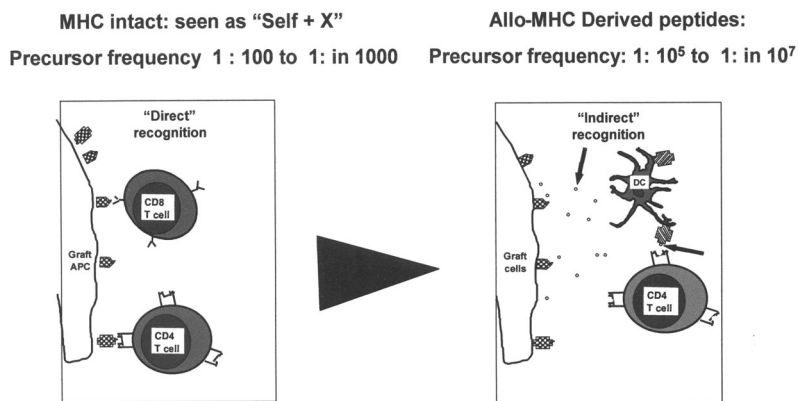


FIGURE 5. In an allograft situation, the major difference at the molecular level is in the MHC molecules and, therefore, the T cell response. Two ways in which this results in the graft being seen as “foreign” and attacked: the so-called *direct* and *indirect* pathways. The direct response predominates, at least in the early phase of vascularized graft rejection.

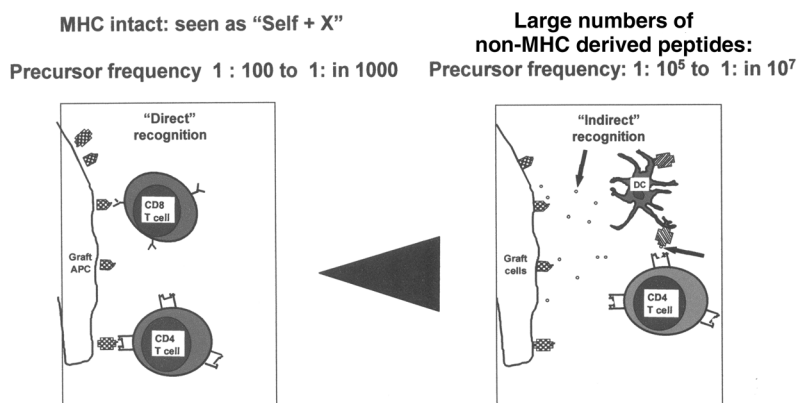


FIGURE 6. In the xenogeneic situation the MHC molecules may be so different that there is little direct T-cell response, but large numbers of different peptides derived from virtually every protein drive the indirect response.

There are three notable routes by which such transplanted islets may activate mechanisms that are harmful. FIGURE 9 relates to the first of these and describes the early, largely non-specific, inflammatory response to an islet transplant, part of which may in theory be modified by the presence of an encapsulation membrane barrier. However, there is little data specifically relating to the effect of encapsulation on these events, although there is an abundance of data concerning the effect of various individual cytokines on encapsulated islets.¹²⁻¹⁵ Since many of the molecules involved have a molecular size close to the cut-off for membranes in common use, the exact effect achieved is likely to vary depending on the pore size of the membrane. The effect is also likely to be most striking for xenografts, particularly in

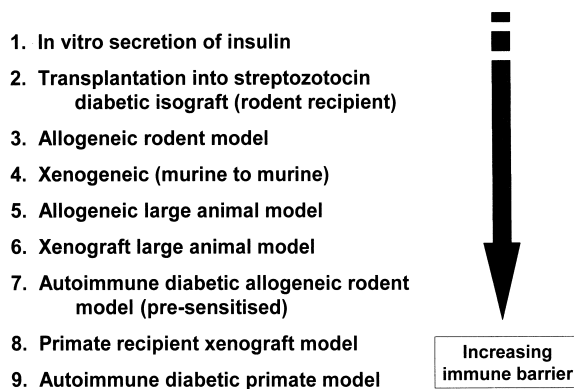


FIGURE 7. Models used for testing encapsulation devices containing insulin secreting tissue.

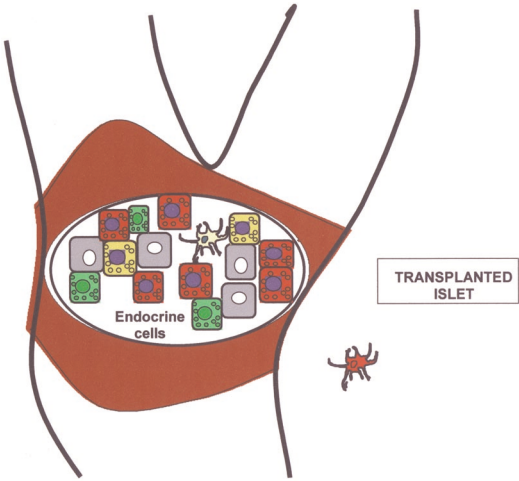


FIGURE 8. Cartoon representation of an islet transplanted into the liver, embolizing the terminal radicals of the portal vein, when it initially rests within a blind clot.

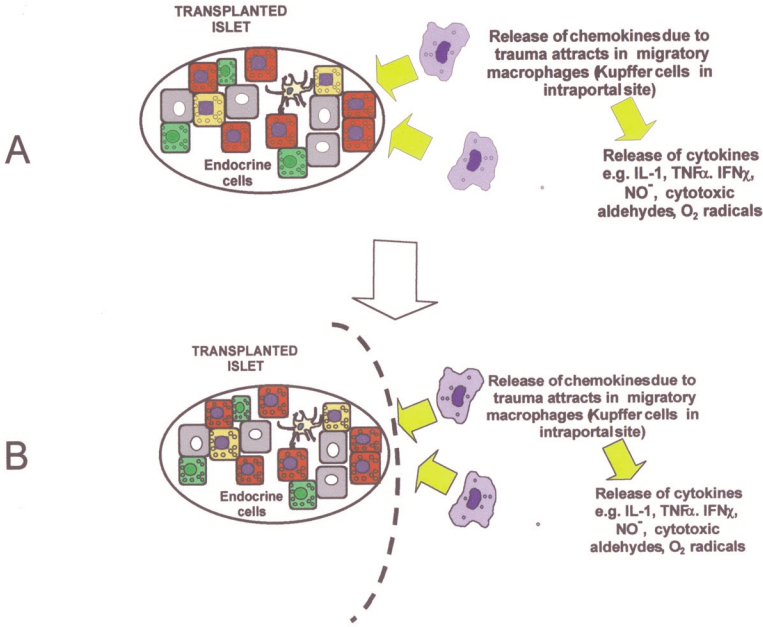


FIGURE 9. A representation of the early events that may influence islet graft implantation in the allogeneic situation (A) and the likely effect of introducing a membrane barrier as used in encapsulation of islets (B).

primate recipients, where the exposure of freshly isolated xenogeneic islets to human blood has been shown to result in rapid destruction of the graft. The ability of an encapsulation membrane to prevent this gross reaction is speculated as likely to be effective (see FIGURE 10), although the experiment has not been performed, to the author's knowledge.

The second route by which transplanted islets may activate mechanisms that are harmful is the now familiar *direct* pathway.⁸ As shown in FIGURE 11, this depends critically on the migration of dendritic cells from within the graft to the recipient lymphoid tissue, where they present the allogeneic MHC molecules to the recipient T cells. The insertion of an encapsulation membrane prevents the dendritic cell migration (FIG. 11) and is therefore highly effective in preventing allograft rejection, in which the direct pathway is the most important mechanism.

The third route by which transplanted islets may activate mechanisms that are harmful is via the indirect pathway⁹ (FIG. 11), and here the insertion of an encapsulation membrane is likely to be ineffective if the peptide and proteins shed by the graft are smaller than the pore size of the membrane.¹⁶ The role of the indirect

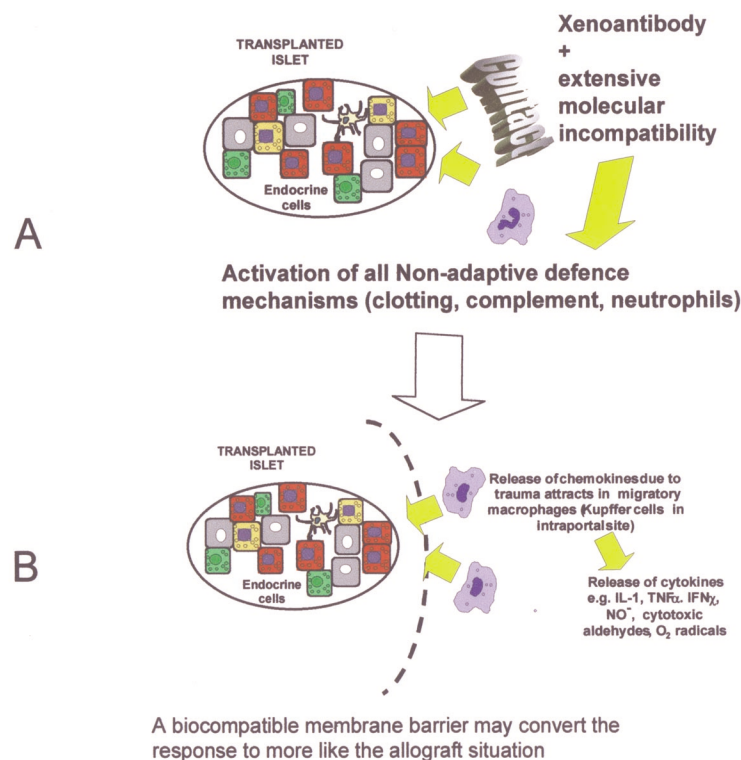


FIGURE 10. A representation of the early events that may influence islet graft implantation in the xenogeneic situation (A) and the likely effect of introducing a membrane barrier as used in encapsulation of islets (B).

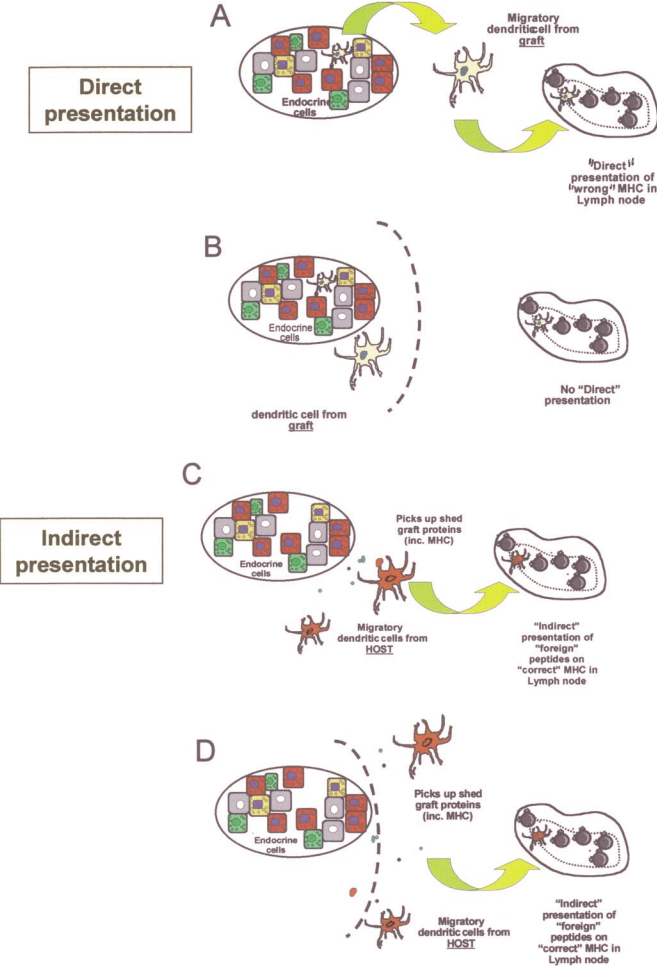


FIGURE 11. Series of diagrams to show how the migratory dendritic cell is crucial to the process of cellular rejection by direct presentation (A) and this is prevented by insertion of a membrane which prevents cell migration (B). However, the indirect pathway is driven by proteins and peptides diffusing out of the graft and picked up by host dendritic cells (depicted as *dark grey*) as in (C). A membrane will likely be largely ineffective against this pathway.

pathway in allograft rejection is relatively minor (at least in acute rejection, although chronic rejection may be a different matter). However, in xenotransplantation between disparate species the indirect pathway becomes dominant. In the hope that it may be possible to exclude such shed proteins it is, therefore, pertinent to ask what is their likely size, and unfortunately there is no data on this point. However, it is certain that sensitization does occur, as evidenced by antibody production following encapsulated islet xenotransplantation. It should also be pointed out that every cell inside an encapsulation membrane has the internal machinery for digesting proteins to peptides of 8–22 amino acids in length for the sole purpose of displaying them on the cell surface MHC molecules^{17,18} (see FIGURE 12). It is known that these MHC/peptide complexes cycle, but what is not known is what eventually happens to the peptides. It would seem unlikely that they would be internalized and broken down further. Rather, it is likely they are lost into a surrounding tissue fluid and indeed this may be the route by which dendritic cells normally pick up antigen. If this were to be the case it would of course make the concept of a molecular cut-off untenable.

The final concept discussed here in relation to encapsulation technology is the question of induction of tolerance or, as it is less controversially described, donor-specific unresponsiveness. That such a phenomenon exists is clear from the clinical experience of reducing immunosuppression several weeks after transplantation, eventually reaching levels at which there is a return of relatively normal immune responses yet no graft rejection (see FIGURE 13). Interestingly, the effect appears to be more powerful the larger the graft. The parallels between many of the phenomena in transplantation and autoimmune disease leads to the conclusion that the same mechanisms are involved (TABLE 1), and extensive recent data has pointed to the critical role of regulatory (suppressor) cells.^{19,20} The exact molecular mechanism by which T-cells are switched off remains uncertain. Some of the suggested mechanisms^{21–27} are listed in TABLE 2. What is becoming certain is that the mechanism involves cell–cell contact and is, therefore, likely to be prevented by insertion of a membrane. However, once the mechanism is understood it should prove possible to produce membranes with the necessary molecules immobilized on the membrane surface.

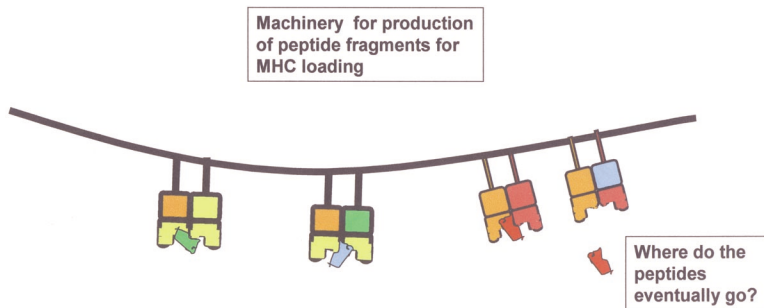


FIGURE 12. Question: Is the concept of a molecular cut-off realistic? How small are immunogenic peptides coming from the graft? Answer: Probably very small. Perhaps 8–15 amino acids.

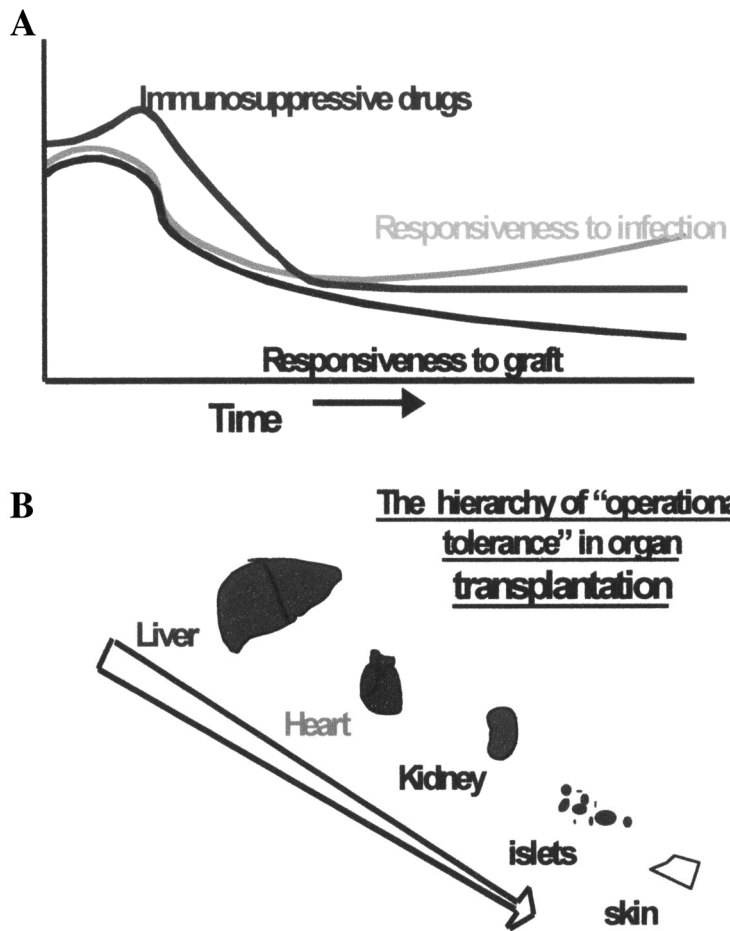


FIGURE 13. Two concepts that come from observation of clinical organ transplantation. **A.** The development of relative graft unresponsiveness while responsiveness to invaders returns almost to normal is crucial because it provides the therapeutic gap necessary for successful transplantation. **B.** The mechanism of graft acceptance appears to be easier to induce with increasing graft size.

CONCLUSIONS

The author has looked at immune processes in islet transplantation from the point of view of encapsulation technology, and has a possible advantage of an "outside" point of view with no particular bias since he has never made a capsule of any form. For what they are worth, some suggestions that specialists in the field might like to consider are listed in TABLE 3.

REFERENCES

1. LAFFERTY, K.J. & J. WOOLNOUGH. 1977. The origin and mechanism of the allograft reaction. *Immunol. Rev.* **35**: 231–262.
2. STEINMAN, R.M. & M.C. NUSSENZWEIG. 1980. Dendritic cells: features and functions. *Immunol. Rev.* **53**: 127–147.
3. FREMONT, D.H., W.A. REES & H. KOZONO. 1996. Biophysical studies of T-cell receptors and their ligands. *Curr. Opin. Immunol.* **8**(1): 93–100.
4. FORD, D. & D. BURGER. 1983. Precursor frequency of antigen-specific T cells: effects of sensitization *in vivo* and *in vitro*. *Cell Immunol.* **79**(2): 334–344.
5. JINGWU, Z., R. MEDAER, G.A. HASHIM, *et al.* 1992. Myelin basic protein-specific T lymphocytes in multiple sclerosis and controls: precursor frequency, fine specificity, and cytotoxicity. *Ann. Neurol.* **32**(3): 330–338.
6. MONGKOLSAPAYA, J., A. JAYE, M.F. CALLAN, *et al.* 1999. Antigen-specific expansion of cytotoxic T lymphocytes in acute measles virus infection. *J. Virol.* **73**(1): 67–71.
7. LOMBARDI, G. & R. LECHLER. 1991. The molecular basis of allorecognition of major histocompatibility complex molecules by T lymphocytes. *Ann. Ist. Super Sanita* **27**(1): 7–14.
8. LECHLER, R., R. BATCHELOR & G. LOMBARDI. 1991. The relationship between MHC restricted and allospecific T cell recognition. *Immunol. Lett.* **29**(1–2): 41–50.
9. AUCHINCLOSS, JR., H. & H. SULTAN. 1996. Antigen processing and presentation in transplantation. *Curr. Opin. Immunol.* **8**(5): 681–687.
10. KEMP, C.B., M.J. KNIGHT, D.W. SCHARP, *et al.* 1973. Effect of transplantation site on the results of pancreatic islet isografts in diabetic rats. *Diabetologia* **9**: 486–491.
11. GRIFFITH, R.C., D.W. SCHARP, B.K. HARTMAN, *et al.* 1977. A morphologic study of intrahepatic portal-vein islet isografts. *Diabetes* **26**(3): 201–214.
12. CAMPBELL, I.L., A. ISCARO & L.C. HARRISON. 1988. IFN-gamma and tumor necrosis factor-alpha: cytotoxicity to murine islets of Langerhans. *J. Immunol.* **141**: 2325–2329.
13. RABINOVITCH, A., W. SUMOSKI, R.V. RAJOTTE & G.L. WARNOCK. 1990. Cytotoxic effects of cytokines on human pancreatic islet cells in monolayer culture. *J. Clin. Endocrinol. Metab.* **71**: 152–156.
14. PUKEL, C., H. BAQUERIZO & A. RABINOVITCH. 1988. Destruction of rat islet cell monolayers by cytokines: synergistic interactions of interferon- γ , tumor necrosis factor, lymphotoxin, and interleukin 1. *Diabetes* **37**: 133–136.
15. POULSEN, T.M., K. BENDTZEN, J. NERUP, *et al.* 1986. Affinity-purified human interleukin I is cytotoxic to isolated islets of Langerhans. *Diabetologia* **29**: 63–67.
16. GRAY, D.W.R. 1997. Encapsulated islet cells: the role of direct and indirect presentation and the relevance to xenotransplantation and autoimmune recurrence. *Brit. Med. Bull.* **54**: 777–788.
17. PAMER, E. & P. CRESSWELL. 1998. Mechanisms of MHC class I—restricted antigen processing. *Annu. Rev. Immunol.* **16**: 323–358.
18. CHAPMAN, H.A. 1998. Endosomal proteolysis and MHC class II function. *Curr. Opin. Immunol.* **10**(1): 93–102.
19. SAKAGUCHI, S. 2000. Regulatory T cells: key controllers of immunologic self-tolerance. *Cell* **101**(5): 455–458.
20. ZHAI, Y. & J.W. KUPIEC-WEGLINSKI. 1999. What is the role of regulatory T cells in transplantation tolerance? *Curr. Opin. Immunol.* **11**(5): 497–503.
21. FRASCA, L., P. CARMICHAEL, R. LECHLER & G. LOMBARDI. 1997. Anergic T cells effect linked suppression. *Eur. J. Immunol.* **27**(12): 3191–3197.
22. MOSSMANN, T.R. 1987. Two types of mouse helper T cell clone: Implications for immune regulation. *Immunol. Today* **8**: 223–227.
23. LOMBARDI, G., S. SIDHU, R. BATCHELOR & R. LECHLER. 1994. Anergic T cells as suppressor cells *in vitro* [see comments]. *Science* **264**(5165): 1587–1589.
24. QIN, S., S.P. COBBOLD, H. POPE, *et al.* 1993. “Infectious” transplantation tolerance. *Science* **259**(5097): 974–977.
25. STARZL, T.E. & A.J. DEMETRIS. 1998. Transplantation tolerance, microchimerism, and the two-way paradigm [see comments]. *Theor. Med. Bioeth.* **19**(5): 441–455.

26. MATZINGER, P. 1994. Tolerance, danger, and the extended family. *Annu. Rev. Immunol.* **12**(991): 991–1045.
27. GRAY, D.W.R. 1998. Major histocompatibility complex-based suppression: a mechanism for T-cell control. *Med. Hypotheses* **50**(4): 289–302.