IMMUNOLOGICAL ENHANCEMENT: STRATEGY FOR CLINICAL RENAL TRANSPLANTATION

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Immunological enhancement is the enhanced survival of an incompatible graft caused initially by contact of the graft with antibody directed against it. In this article, I will be considering the mechanisms of enhancement of incompatible rat kidney grafts with the object of highlighting possible approaches for exploiting the phenomenon in human clinical practice. Before considering mechanisms, a number of basic facts need to be mentioned:-

1) Immunological enhancement can be induced in several ways, namely,
   (a) by injecting the graft recipient with antibodies directed against the relevant incompatibilities of the allograft\(^1\) - passive enhancement;
   (b) by injecting the graft recipient with cells or cell extracts carrying the same antigenic incompatibilities as those presented by the subsequent allograft\(^2\) - active enhancement;
   (c) treatment of graft recipient with both antibody (as in (a)) and antigenic inocula (as in (b))\(^3\)

2) The protection against rejection afforded to rat kidney grafts by immunological enhancement can vary from minimal extension of graft survival by a few days to permanent graft survival. The factors which influence this include:-
   (a) the amount of incompatibility. Two haplotype incompatibilities are less successfully protected against rejection than are single haplotype differences\(^4\).
   (b) Donor-recipient strain combination\(^5\).
   (c) Dose and specificity of antiserum used in passive enhancement\(^6\). In some donor-recipient combinations, very low doses are effective\(^7\); antisera against Immune Response associated (Ia) antigens are highly effective enhancing agents\(^8,9\), although they are probably not the exclusive enhancing antibodies\(^10\).
(d) Dose, timing, and route of antigen pretreatment in active enhancement\(^5\).

In view of the varying effectiveness of enhancement in the rat kidney allograft model, a similar variation is only to be expected in man. Studies to determine the immunological status of rats bearing permanently surviving, enhanced kidney allografts (one haplotype incompatible) show that passive enhancement goes through two stages - Induction and Maintenance. The induction stage occurs during the first 7-10 days after transplantation and injection of enhancing antiserum. During this period, there is a profound depression of the early lymphocytotoxic IgM alloantibody response, and a less marked depression of the subsequent IgG lymphocytotoxic response. There is also a delay of 2-4 days in the development of maximum cell-mediated immunity as measured by short term culture in vitro of killer lymphocytes with labelled target cells\(^1\). However the amplitude of this late response is not significantly lower than that occurring in allografted rats which have not received enhancing antibody\(^1\). The evidence of Strom et al\(^3\) shows that the depression of active antibody synthesis in rats injected with enhancing antiserum is associated with the absence of progressive necrosis of glomerular endothelial cells and necrotising arteritis; both of these features are prominent in non-enhanced kidney allografts undergoing rejection. It therefore appears that in the case of rat kidney allografts presenting a single haplotype incompatibility, the unaided activity of the cellular arm of the immune response is insufficient to cause complete graft rejection although variable amounts of damage are sustained. It seems probable that in the case of a double haplotype incompatible kidney, the cell-mediated response causes a greater degree of damage and this is sufficient per se to reduce graft function below a life sustaining level.

The maintenance stage of enhancement succeeds the induction stage, and is characterised by a gradually increasing specific non-reactivity against donor antigens\(^1\). If serum levels of lymphocytotoxic IgG antibody are detectable during the induction stage these gradually fall, and cell-mediated immunity (4 hour in vitro assay of killer T lymphocytes) also wanes to undetectable levels\(^6\). If long-term graft bearing rats are challenged with kidney donor genotype lymphocytes, they respond with weak, mainly IgM, lymphocytotoxic antibody and a weak killer T lymphocyte response. However, the responses do not produce rejection of the kidney allograft. Repeated challenges merely depress both cellular and humoral responses further.

Attempts to terminate the enhanced state by adoptive immunisation, or parabiosis with immune syngeneic partners have not led to graft rejection. We therefore concluded that during the maintenance stage of enhancement there is an active mechanism which frustrates graft rejection.

In order to explain these observations it is necessary to consider the functions of different lymphocyte subpopulations in immune responses against transplantation antigens of the major histocompatibility (MHC). Figure 1 illustrates
Figure 1. Simplified scheme of immune response against MHC antigens
an over-simplified scheme which however contains the elements necessary for developing the concept proposed. Cantor and Boyse\textsuperscript{17,18}, have demonstrated that T lymphocytes can be divided into at least two populations, one responding to Ia-type antigens and another which matures into clones of killer T lymphocytes with specificity for the classical Histocompatibility or H-type antigens. Both these T cell populations are shown in the Figure. In addition the population of B lymphocytes which develop into antibody producing cells with specificity for H-type antigens is shown. One known function of the Ia responsive T cell population is to act as helper cells for B cells synthesising IgG antibody against thymus dependent antigens, of which MHC antigens are examples.

It is also known that interactions between different T cell populations are necessary in order to generate killer T lymphocytes\textsuperscript{19}. It has not yet been formally proved that the Ia responsive T cell which provides help for B cells is also a member of the same T cell population which interacts with the precursors of killer T lymphocytes, but we assume this to be true. The consequence of this scheme (which is also proposed by Wagner & Nossal\textsuperscript{19}) is that without activation of the Ia-type antigen responsive T cells, there is no ‘help’ for potentially reactive B cell precursors and no ‘amplification’ of killer T lymphocyte clones.

Because long-surviving kidney allografts contain little or no Ia-type antigen\textsuperscript{20}, the recipient is exposed to a continuous presence of only H-type antigen.\textsuperscript{21} We postulate that the continuous presence of H-type antigen without Ia-type antigen not only fails to provoke immunity but also induces cumulatively a specific non-reactivity extending to both humoral and cellular components, and this is responsible for the specific immunosuppression observed during the maintenance stage of enhancement.

In order to explore this idea further, a series of experiments were carried out using platelets as a matrix of H-type antigen which lacked Ia-type factors.\textsuperscript{22} The following points were established using Wistar or August strain rat platelets and AS strain recipients.

1) Platelets specifically absorb H-type but not Ia-type antibodies.

2) The density of H-type antigens on platelets is similar to that on lymphocytes, calculated by absorption tests and allowing for size differences.

3) Allogeneic platelets fail to induce primary antibody responses despite repeated injections.

4) Repeated injection of allogeneic platelets into non-primed rats leads to a temporary state of partial non-reactivity; cytotoxic antibody responses are very depressed, but with the protocols we used there was normal cell-mediated immunity after challenge with viable, allogeneic lymphoid cells.

5) Primed rats develop secondary responses after challenge with allogeneic platelets.
6) The secondary responses induced wane rather than increase on repeated injections of platelets.

These results are consistent with the hypothesis described earlier - that H-type antigens are by themselves insufficient to activate precursor B and T cells into antibody producers and killer lymphocytes respectively without the participation of the Ia-type antigen responsive T cells which perform an essential amplification function. In primed rats, memory B and T cells can be activated by H-type antigen without invoking the amplifier T cells, hence cellular and humoral responses are observed; but there is no further recruitment of precursor cells to the memory cell stage so the responses tend to wane on further challenge with H-type antigen only. In our experiments on the effect of platelet injections into non-primed recipients, inhibition of their capacity to produce humoral antibody was readily achieved. but consistent depression of killer lymphocyte production was not observed. However other workers have demonstrated that killer lymphocyte generation in vitro can be specifically inhibited by alloantigen-bearing cell membranes, but that dosage is a critical variable. We intend to examine further the effect of different doses of platelets in this context.

Turning to the important question of what strategy should be adopted for clinical practice, these experiments suggest that we should aim to achieve the specific non-reactivity which characterises the maintenance stage. Presumably this occurs spontaneously in the case of successful grafts where the requirements for immunosuppressive drugs falls steadily with time. However, ideally, our aim should be to achieve non-reactivity or at least partial depression of reactivity before rather than after transplantation. The rat experimental model suggests that this can be achieved by administering H-type antigens in a way that does not activate the Ia-type antigen responsive T cells. It seems possible that the protective effect which sometimes follows multiple blood transfusions may be an example of the same phenomenon; the variable results of transfusion could be attributed to such factors as the storage time of the blood which influences the number of viable lymphocytes it contains, and whether the recipient had been immunologically primed.

References

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Open Discussion

KNAPP (Nottingham) In the clinical situation transplantation is nearly always followed by drug therapy with steroids and azathioprine. In the experiments you have described did you give the animals steroids and azathioprine?

BATCHelor In the experimental system that I showed you, none of the rats had received any immunosuppressive therapy at all. We have however from time to time done experiments with immunosuppressive drugs. The question of how much synergistic action they have in enhancement will vary according to protocol, the drugs used etc., but so far I haven’t found any drugs which have an inhibitory effect upon enhancement. But I can think of situations in which you might expect that to occur.

HABERAL (Ankara) How many serum doses do you recommend. Did you do experiments using Con-A and immunosera? I made one experimental study with Con-A and immunosera. I transplanted a Fisher rat’s heart to Lewis rat’s abdomen, then I injected immunosera and Con-A to separate groups and in combination. In each of the separate groups, graft survival increased. The combined group did no better than the separate groups.

BATCHelor I’m slightly confused; what is the point of adding Con-A to the system? I don’t understand the rationale of that.

HABERAL You know Con-A is used in prevention of rejection.

BATCHelor One of the points which I hoped came out was that the mechanisms involved in the induction stage are different from those in the maintenance stage of enhancement. I think that the most promising hope from the clinical standpoint is to try and mimic what is going on during the maintenance stage. Therefore I was not really dealing with the problems of the induction stage in any detail. Whether you are going to get a synergistic action between Con-A and antiserum depends upon what cell population Con-A is working on, and precisely what the immunosuppressive action of the antiserum is during that initial stage. I cannot see my way round those complexities to making use of that clinically; nor do I know of any experiments in which Con-A has been used as an in vivo immunosuppressive agent.
Certainly I haven’t used it myself.

THORSBY Perhaps we could return to the complexities in the general discussion. Are there any more specific questions about the concept of immunological enhancement?

ROWINSKI (Warsaw) I would like to ask Professor Batchelor what is the specificity of his enhancing alloserum? Have you ever checked this serum against your recipient strain?

BATCHELOR Yes, I think not only our evidence, but the evidence of others makes it very clear that the anti-la element in enhancing alloantisera is an extremely effective enhancing antibody. I think the evidence also suggests that antibodies against the rat equivalent of the HLA A, B and C antigens can have an enhancing action; probably not as strong as that produced by the anti-la. I think the important thing is to prevent the amplification step by the helper cell population during the early stage. If you get through with an undamaged kidney for about a week or two, you then expect to have mostly H-antigens, without la being presented to the recipient.

BARNES (Birmingham) Has the administration of passive la antibody been shown to produce enhancement in any species other than rodents? As far as I know it has been tried, but unsuccessfully; have you any explanation for that? And secondly, have you any evidence for passive enhancement with platelets in the sort of system that you are suggesting?

BATCHELOR The only data I know in animals other than rodents is the work that you know about, I am sure, in rhesus monkeys, from the Dutch group. Their attempts have failed so far as I understand. What the explanation is, I don’t know at the moment. The action of the antibody really is quite a temporary thing; it just gives the kidney breathing space to survive the initial immune response until you get to the stage where you are presenting the animal with nothing but H antigen without la. Perhaps the conditions are not quite right to achieve that stage in the monkey.

BARNES So it is rather premature to go ahead clinically?

THORSBY We will discuss the clinical approach later in the general discussion.

BARNES You have not answered my second question: have you used active enhancement with platelets in the rat?

BATCHELOR We have done a few experiments and a proportion of the animals treated with platelets survived; but the trouble is that we have not really done enough experiments using a variety of platelet doses. I would rather not talk about it until we have done more.

BRENT Richard, the one thing that I find puzzling is that kidneys are thought not to have la antigens. What is the target in the kidney on which anti-la
antibodies act, accepting the idea that anti-Ia antibodies mediate enhancement? Perhaps there is Ia on the vascular endothelium of kidneys, and I have heard it rumoured that some Australian workers have shown this to be so.

BATCHelor The point about the Ia stimulus is that it excites the amplification of a response against the H antigens. The kidneys have H antigens so they provide perfectly vulnerable targets, but normally speaking you don’t excite a powerful immune response against those H antigens unless you also stimulate the animal with Ia differences as well, and that Ia difference is almost certainly present on circulating lymphocytes.

Thorsby Perhaps I may answer Professor Brent’s question. You don’t even have to go to Australia for the answer, because there are transplant immunologists in Oslo who seem to have shown that human endothelial cells are able to stimulate in MLC due to their expression of HLA D; also measured by human B cell alloantibodies. Even without the passenger leukocyte concept there should be Ia or HLA antigens in the kidney. Thank you very much, Richard.

I hope it is clear to everyone that all this is rather confusing. The point which should be clear is the distinction between immunological tolerance and enhancement, in its induction. You can induce immunological tolerance by injecting antigen and you can induce immunological enhancement by injecting antibodies. However, these stages of specific unresponsiveness are interrelated because they may be mediated by similar or identical mechanisms. In some animals which have been made tolerant by injection of antigen, one can detect blocking or enhancing antibodies. And in some animals in which you have induced enhanced survival by injecting alloantibodies, you may to your surprise detect lack of the histocompatibility antigen-reactive cell clones. They have been eliminated, as may be the case in immunological tolerance. So the distinction between induction of immunological tolerance and enhancement may lie in the way you do it, by using antigen or antibodies, whilst its maintenance may be dependent upon very similar mechanisms.

Now we shall return to the third and very fascinating possibility, which has been proposed by the group in Uppsala, headed by Professor Hans Wigzell for a couple of years: the elimination of the histocompatibility antigen-reactive cell clones by inducing autoimmunity against them.