SPECIFIC SUPPRESSION OF REJECTION IN RENAL TRANSPLANTATION

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Specific suppression of rejection is defined as the direct or indirect suppression of the lymphocyte clones which are specifically reactive to donor histocompatibility antigens and are normally responsible for the rejection response. In other words the recipient of a renal allograft would not reject the graft, but would be immunologically normal in every other respect. This ultimate goal of transplantation immunology has been achieved in models of organ grafting in the rodent, but still seems a rather distant goal in clinical practice [see reference 1 for review and references]. Specific suppression may be induced by pretreatment of the recipient with donor histocompatibility antigen (antigen-induced suppression or active enhancement) or by passive administration of antibody specific for donor histocompatibility antigens to the recipient (antibody-induced suppression or passive enhancement).

Antigen-induced Suppression

Billingham, Brent and Medawar [2] first induced specific suppression in the laboratory in 1953 by injecting mouse fetuses in utero with tissue homogenates of a foreign strain. These mice, on reaching adulthood accepted skin grafts from the original same donor strain with no evidence of rejection; in other words they now recognised the foreign histocompatibility antigens as self. This phenomenon was known as tolerance and almost certainly represents true clonal deletion of the specific antigen-reactive lymphocyte clones. However true tolerance is very difficult to achieve in the immunologically mature adult recipient, and hence the term is best replaced by that of antigen-induced suppression.

The induction of specific suppression of the immune response to histocompatibility antigens some 26 years ago provided enormous impetus to the search for methods of achieving such a state in the recipient of an organ allograft. Early experiments were performed using skin allograft models, where antigen pretreatment in various forms could result in some modest prolongation of graft survival. However the advent of microsurgical techniques which allowed renal allografts to be performed in inbred strains of rats allowed this whole question of antigen pre-treatment in adult animals to be re-examined.

For example in 1972, Fabre and Morris [3] showed in the rat that the prior administration of donor histocompatibility antigens in the form of blood was able to suppress rejection of renal allografts, with some animals surviving indefinitely (Table I). However it is worth noting, in view of the current interest in the beneficial effects of blood transfusions before renal transplantation in man, that the effect of blood transfusions ranged from accelerated rejection to complete
### TABLE I. The effect of dosage and timing of intravenous donor blood transfusions on renal allograft survival in two rat strain combinations*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(DA X Lewis) F1 to DA</th>
<th>(DA X Lewis) F1 to Lewis</th>
</tr>
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<tbody>
<tr>
<td>Nil</td>
<td>8, 11, 14, 17, 21, 70, &gt; 100, &gt; 100</td>
<td>9, 10, 10, 11, 11, 11, 12, 13, 15</td>
</tr>
<tr>
<td>Single injection of 0.5ml of donor strain blood at</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( - 1 day)</td>
<td>7, 8, 9, 10, 20, &gt; 100</td>
<td>6, 6, 7, 8, 9</td>
</tr>
<tr>
<td>( - 7 days)</td>
<td>10, &gt; 100, &gt; 100, &gt; 100, &gt; 100, &gt; 100</td>
<td>10, 14, 14, 14, 20, 82</td>
</tr>
<tr>
<td>( - 28 days)</td>
<td>13, 15, &gt; 100, &gt; 100, &gt; 100, &gt; 100</td>
<td>7, 7, 8, 9, 9, 10, 10, 18, 16</td>
</tr>
<tr>
<td>Twice weekly rejection of 0.5ml donor strain blood for</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( - 4 weeks)</td>
<td>&gt; 100, &gt; 100, &gt; 100, &gt; 100, &gt; 100</td>
<td>13, 40, 63, 110, &gt; 200</td>
</tr>
<tr>
<td>( - 10 weeks)</td>
<td>not done</td>
<td>7, 17, 31, 36, &gt; 200, &gt; 200</td>
</tr>
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* Adapted from Fabre and Morris [3]

suppression of rejection, depending on dosage, timing, and the strength of the histocompatibility barrier. Subsequent experiments in the Rhesus monkey by Balner’s group [4] also showed that blood transfusions could significantly prolong the survival of renal allografts in recipients treated with azathioprine and prednisolone. But again some schedules were capable of producing accelerated rejection. Thus antigen pretreatment of a recipient can induce a state of specific suppression to a renal allograft, but even in an experimental model where the conditions of the experiment may be carefully controlled, the outcome is unpredictable.

The mechanism by which antigen pretreatment induces suppression in the adult is unknown, and indeed in the case of pretreatment with blood it is not even certain that the initial suppression is specific. The mechanism by which the state of specific suppression is maintained has been explored in some detail [5], and as it is probably the same mechanism which is operative in the maintenance phase of antibody-induced suppression, this will be discussed later.

**Antibody-induced Suppression (Passive Enhancement)**

The renal allograft in the rat has been the basic experimental model in which antibody-induced suppressed rejection of an organ allograft has been accomplished. There is a considerable variation in the effect of passive enhancement on renal allografts in different strain combinations, and it is much more difficult to enhance a kidney from a homozygous donor than a F1 hybrid donor (Table II). In an unmodified recipient of a renal allograft both a humoral response and a cellular response to the graft can be measured following transplantation, and histologically
TABLE II. The Effectiveness of Passive Enhancement of Renal Allografts Across Different Major Histocompatibility Barriers in the Rat* [1]

<table>
<thead>
<tr>
<th>Strain Combination</th>
<th>Median Survival (days)</th>
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</thead>
<tbody>
<tr>
<td>AS2 to DA</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>(AS × August) F1 to AS</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>August to AS</td>
<td>43</td>
</tr>
<tr>
<td>(DA × Lewis) F1 to Lewis</td>
<td>23</td>
</tr>
<tr>
<td>DA to Lewis</td>
<td>15</td>
</tr>
</tbody>
</table>

* In all strain combinations, untreated recipients have severe acute rejection from which they die, almost invariably in the second week

by day seven the graft shows tubular and glomerular necrosis and arteriolar fibrinoid necrosis. In the enhanced animal the humoral response is ablated completely, while the cellular immune response is delayed by a day or two but its amplitude is not depressed. The histology of day seven shows areas of mononuclear cellular infiltration only. Thus passive enhancement may effect predominantly the humoral response to a renal allograft. It has been suggested that the action of passive enhancement is directed at the suppression of helper T cell activity [6]. This probably explains the marked synergism that has been demonstrated between passive enhancement and anti-lymphocyte serum, the latter influencing predominantly the cellular response [7, 8].

The specificity of enhancing antisera appears to be directed in the main against Ia antigens. Davies and Alkins [9] showed in the rat that if an antiserum was absorbed with donor erythrocytes (which in the rat express only SD antigens, the equivalent of HLA–A, B, C antigens, but not Ia antigens, the equivalent of HLA–DR) then the ability of the antiserum to enhance cardiac allografts was not diminished. This has been confirmed since and it does appear that most enhancing activity in rat antisera resides in the anti-Ia activity. Nevertheless it has been clearly shown that antisera with specificity against SD antigens alone can also enhance renal allografts [10, 11].

Attempts to induce enhancement of renal allografts in the dog and the monkey have proved largely unsuccessful. In the dog we found little evidence of suppression of rejection, and more alarming perhaps from the point of view of clinical application, 7 of 15 dogs showed evidence of severe antibody mediated damage following the administration of antiserum. In the Rhesus monkey, Balner’s group failed to show any marked effect of donor-specific antiserum and also of donor-specific anti-Ia on renal allograft survival [12].

A discussion of the mechanisms involved in passive enhancement of renal allografts must consider both the induction phase and the maintenance phase of this phenomenon. Little is known about the mechanism by which a state of specific suppression is induced by donor-specific alloantibody but any hypothesis must take into account the considerable amounts of Ia antigen present in the rat kidney and more recently confirmed in the human kidney, as well as the dominant role of anti-Ia antibody in the induction of enhancement. Furthermore in view of
the very small amounts of antiserum (as little as 10 μl) that have been shown capable of enhancement [15] a peripheral mechanism requiring masking of target antigens is not tenable, so pointing to a central mechanism for the induction of enhancement. As the pepsin-digest of IgG, namely F(ab′)₂ will not enhance renal allografts [16], suggesting that the FC portion of IgG is needed for enhancement, it is possible that the formation of Ia antigen-antibody complexes are presented to helper T cells, specific for the Ia antigen concerned, which in turn leads to opsonisation and phagocytosis of the T cells through the agency of the FC receptors in the Ia antigen-antibody complexes. Such a mechanism involving T helper cells would then lead to failure of a humoral response to the organ allograft, which is T cell dependent, while leaving the cellular response intact (assuming that a different population of helper T cells were involved in the development of cellular immunity). Another equally plausible hypothesis is that the complexes are responsible for the generation of suppressor T cells which suppress helper T cell activity in the humoral response.

Once a stable state of immunological balance is achieved with acceptance of the graft (as mentioned earlier this maintenance phase after passive enhancement is probably no different from that seen after antigen-induced suppression), there are several possible mechanisms which might maintain this state (see reference 6 for review and full references).

1. Blocking factors — now considered an unlikely explanation as serum from long surviving animals will not produce immunosuppression in a syngeneic recipient (with one notable exception) nor will it produce inhibition of the MLR between donor and recipient.

2. Clonal deletion — also excluded as neither transfer of syngeneic lymphocytes to a recipient with a long surviving graft nor symbiosis of such a recipient with a normal syngeneic animal will lead to graft rejection.

3. Generation of suppressor cells — All the available evidence points to an active mechanism of suppression maintained by suppressor cells, but their presence has not yet been demonstrated in the rat.

4. Graft adaptation — some form of adaptation occurs, for long surviving grafts reimplanted into fresh syngeneic recipients show some prolongation of survival, due either to alteration in the passenger leukocyte population or to repopulation of the vasculature of the graft by host endothelium.

**Potential Problems Associated With the Induction of Specific Suppression**

In the case of antigen-induced suppression the major concern is the risk of recipient sensitisation against the histocompatibility antigens of the eventual kidney donor, and of course the indication that pretreatment needs to be carried out in most experimental models for some time before transplantation for successful immunosuppression to be achieved. The first risk is a considerable one and is also unpredictable in view of our ignorance about the mechanism by which antigen induces suppression. The second makes this approach impractical in the case of cadaver transplantation in the present state of practice of organ preservation.
The major potential problem of antibody-induced suppression is the hazard of producing antibody-mediated damage in the grafted kidney by the passive administration of donor-specific antiserum. Such damage is uncommonly seen in the rat, and is dose dependent, but is seen in the rabbit and dog [6]. Thus any future clinical application of passive enhancement will require techniques to be developed which will prevent damage while still allowing enhancement to occur. As mentioned earlier we have shown that F(ab')₂ which does not fix complement, unfortunately does not enhance [16]. However the prior administration of F(ab')₂ will prevent hyperacute rejection while still allowing enhancement of a renal allograft to occur in a model of hyperacute rejection of renal allografts in the rat [17]. Similarly in this model absorption of SD activity from an antiserum to leave anti-Ia activity does not result in hyperacute rejection (surprising in view of the amount of Ia on rat kidney), while still producing enhancement [17]. These experiments suggest two possible solutions to the problem of antibody-mediated damage in clinical practice, while another possibility might be temporary decompensation of the recipient with an agent such as cobra venom factor.

Clinical Instances of Specific Immunosuppression

The improvement in graft survival seen in transfused recipients in contrast to non-transfused recipients may represent in some instances an example of antigen-induced suppression. At present the transfusion effect and the mechanism by which it is produced in human renal transplantation is clouded in mystery. There may be some element of non-specific immunosuppression and the operation of a selection factor either in the donor or recipient as a result of blood transfusions, in addition to any specific immunosuppressive effect. There is unlikely to be a single explanation for the blood transfusion effect in man.

Two attempts have been made to induce passive enhancement of human renal allografts with F(ab')₂, but no evidence of improved graft function was found in recipients who were also receiving azathioprine and prednisolone [18, 19], not surprising in view of our later demonstration of the lack of efficacy of F(ab')₂ in producing enhancement of rat renal allografts [16].

One encouraging feature in human transplantation is the observation that renal transplants performed in the presence of a positive B-cell crossmatch (some of which will be due to donor-specific anti-Ia activity) do not undergo hyperacute rejection [20], although there is no firm data suggesting that such grafts show a better outcome.

Conclusions

The induction of specific suppression of rejection of renal allografts can be achieved in the rat by both donor-specific antigen-pretreatment or antibody-treatment. Mechanisms by which this state of unresponsiveness is induced and maintained remain subject to speculation, although indirect evidence points to an active suppressor cell mechanism being responsible for the maintenance state. However, it is a long way from rat to man, and in view of our ignorance concerning mechanisms it seems unlikely that the induction of specific suppression will be achieved in other than a few selected instances in the near future.
Acknowledgment

This work was supported by a grant from the Medical Research Council of the United Kingdom.

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Open Discussion

STRUVENBERG (Utrecht) I would like to ask you a general clinical question combining your presentation with that of Dr van Rood yesterday morning. We get the impression, hearing about the results of D related typing, that the graft survival in the first year depends on the topic you have been talking about, that is immunosuppression, and also very important is clinical care. The results after the second year, probably for the next years, will be highly influenced by the grade of histocompatibility one can get for the patients and then probably things like D related typing will come in. Could you comment on this general idea?

MORRIS Basically I think that DR matching looks quite exciting, but I think we must be very careful not to get carried away by it. Even in Oxford the data looks better than ever, but there is a tremendous ‘déjà vu’ about DR typing which many of us went through ten years ago with A and B matching, so I think there is still plenty of need for caution. However, accepting that DR matching may significantly influence the early course of the graft and therefore presumably the long term course, how does this influence the way we might use immunosuppression? Now I would hope, and we have always hoped, that the better the donor/recipient match, the lower the levels of immunosuppressant drugs we
might use. Perhaps with these patients one might be able to use Cyclosporin without steroid, because I would agree wholeheartedly with Professor Calne that I would personally be prepared to drop 10% in our graft survival figures if I could do without steroids.

CALNE I would certainly agree with the first part of your statement. I suspect that we do not have sufficient data at present to say that the better matched do significantly better with Cyclosporin A than the worst matched, and certainly in the experience of our pilot study most of the kidneys were badly matched, but there was no evidence that those with four mismatches did worse than those with two mismatches. In the animal work the results in completely crossbred mongrel dogs and in completely mismatched pigs seems to be ablated by the Cyclosporin A. Now it could well be that because they are a bad match, as the months or years go by, they are going to be the ones that are going to suffer and the other ones will not; but I do not think we have enough data to make that statement; it would seem likely.

MORRIS I think it is also important to remember that with better matching we should need less immunosuppression. As Professor Calne has pointed out, Cyclosporin is a very potent immunosuppressive agent in experimental animals and almost certainly so in humans and anything that is going to be a potent immunosuppressive agent is going to be potentially lethal to the patient. I would see, whether with Cyclosporin A or anything else, that the role of matching is to enable us to reduce the levels of immunosuppressive drugs.

HABERAL (Ankara) I would like to learn what is the mechanism of action of Cyclosporin A.

CALNE Well a little bit is known about the mechanism of Cyclosporin A. It seems to affect the early stage of T lymphocyte proliferation more than any other biological model that has been looked at; and there is a recent report in Nature, about two weeks ago by Gordon and Singer from Seattle, showing that the drug was preferentially effective against the T cells that float in sedimentation gradient. So it looks as if it may have a selective action against this subpopulation of T cells. Whether it is only that population that gets affected or whether it affects all lymphocytes I would suspect depends on dosage, and if you give enough of it you probably damage all cells. It is probably a general cell poison, but if you give therapeutic doses it probably just affects this subpopulation. But it could well be this sub-population that is also very important in the control of immunity to virus diseases, and we may have to do something about raising the patient’s level of immunity to virus diseases while they are being treated with Cyclosporin A, or certainly not to lower it by adding other drugs, which seems to be terrible.

MORRIS The other interesting thing is that it produces results very similar to those of passive enhancement. In the rat, for example, in our own laboratory’s work, all it does is completely suppress the humoral cytotoxic antibody response to a renal allograft, but again it has no effect on the cell-mediated immune response. It delays it by a few days but does not suppress it, and I think this would support Professor Calne’s suggestion that it must be acting on a subpopulation of T cells. It does not appear to have a direct effect on the killer
cytotoxic T cells, so it may be, perhaps, working on T helper cells or some such sub-population. We have no evidence; that is pure speculation, but it does look as if it may have quite a selective action.