REGULATION OF TRANSPLANTATION IMMUNE REACTIONS
BY ANTI-IDiotypic IMMUNITY: A NEW APPROACH TO
ACHIEVE SPECIFIC TRANSPLANTATION TOLERANCE IN
MAN?

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Certain diseases in man require, as treatment, organ grafting. Improved tissue
typing procedures have led to selection of suitable donor-recipient combina-
tions with significant increases in survival time of the grafted tissue. Yet,
complete matching between donor and recipient as to major histocompati-
bility antigens will continue to be a rare event for the foreseeable future.
Thus most recipients of kidney grafts for example, will have to undergo
at present heavy immunosuppressive treatment in order to prevent rejection
of the kidneys. Complications inflicted on the patients by this drug therapy
are manifold and serious and sometimes lead to the actual death of the recipi-
ent. It is thus quite clear that an approach allowing selective induction of
unresponsiveness (tolerance) of the prospective recipient with regard to immune
reactivity towards the antigens of the donor, would be of great value.

Previous attempts to evoke specific unresponsiveness to transplantation
antigens have encompassed such approaches as the administration of ‘block-
ing’ antibodies causing ‘enhancement’ (increased survival) of certain grafts,
or administration of large numbers of donor cells to achieve a state of specific
immune paralysis in the recipient. Whilst certain success using these approaches
has been achieved in animal experimental systems, in particular when trying
to achieve tolerance by antigenic administration into newborns, attempts
to transfer such experimental systems to clinical practice have so far not been
rewarding. We believe that this is in part due to the fact that ‘enhancement’
is in itself a very delicate phenomenon with a requirement for specific anti-
bodies of non-harmful nature in high titres, a thing difficult to achieve in
the human systems. Tolerance in humans, as achieved by the administration
of large numbers of foreign haemopoietic cells, has only been possible in
rare cases of bone marrow disorders, or during embryonic life and then with
a sizeable frequency of complications, in particular graft-versus-host disease.

Here we have used another approach in the attempt to achieve specific
immune unresponsiveness in adult, immunocompetent individuals. The
rationale behind our approach reads as follows: thymus-dependent, T, lymphocytes constitute the major effector cells involved in the rejection of foreign tissue. Thus, the principal aim of the study would be to cause the selective elimination of exactly those T lymphocytes in the individual which carry genetically predetermined receptors with specific reactivity towards the antigens of the potential donor. In order to be able to achieve such specific elimination of a minority sub-group of cells, they must carry some distinguishing marker; in this case the antigen-specific receptors.

Antibodies and antigen-binding receptors have as unique markers their antigen-binding sites, and these sites can themselves serve as antigens. Antigenic determinants on or close to the antigen-binding sites are called idiotypic (unique), and antibodies against such sites are called anti-idiotypic antibodies\(^1\). Now, it is also known that the immune system of a given individual is genetically equipped to produce antibodies that can react with a variety of self-structures if conditions are set properly. Amongst the self-components against which such auto-immunity can be induced are the idiotypic determinants present on the individual’s own antibodies or receptors for antigen\(^2,5\). It is important for understanding the following approach to know that T and B lymphocytes reacting against the same antigenic determinants use antigen-binding receptors (or produce antibodies in the case of B cells) with partly identical idiotypic determinants\(^4\). It is thus possible to use either B and/or T cell receptors as immunogen to induce anti-idiotypic immune reactions against both groups of lymphocytes with relevant idiotypic receptors.

The initial work leading to the conclusion that shared idiotypic determinants are present on T and B lymphocytes reactive against the same antigen, was carried out in systems using transplantation antigens. An important practical problem in this study was to analyse the relative proportion of antigen-reactive lymphocytes in an individual with reactivity towards e.g. major histocompatibility complex, MHC, of type X, that would carry easily identifiable idiotypes. If the heterogeneity of such idiotypes were large, it would be extremely unlikely that any anti-idiotypic immunity could be induced with enough eliminating power to be of practical value. However, the results were highly gratifying using anti-idiotypic antibodies produced in F\(_1\) hybrids between two inbred strains of rats against the antibodies produced in one parental strain, against the alloantigens of the other strain. Thus, in the presence of complement it was possible to treat the relevant parental T lymphocytes in vitro to achieve a near complete elimination of the relevant, idiotypic and immunocompetent cells\(^4\). Table I shows in summary the results obtained with such an approach.

In further experiments using double re-cycling of anti-Ig bead columns and anti-idiotypic antibodies to make Ig-negative T cells ‘Ig-positive’ if idiotypic, it was possible to purify selectively and show that all specific immune reactivity could be found in small groups of cells with predetermined, idioype-positive surface receptors\(^5\). When pure, such idiotypic T cells appear extremely restricted in immune reactivity against MHC antigens, only expressing signif-
# TABLE I. Elimination of specific immunocompetent T lymphocytes by anti-idiotypic antiserum and complement

### A  MLC reactivity

<table>
<thead>
<tr>
<th>Responder</th>
<th>Stimulator</th>
<th>Treatment with serum plus complement</th>
<th>$^3$H-TdR incorp., mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis</td>
<td>DA</td>
<td>Anti-Lewis-anti-DA</td>
<td>1,932 ± 29</td>
</tr>
<tr>
<td>Lewis</td>
<td>DA</td>
<td>Normal serum</td>
<td>32,112 ± 145</td>
</tr>
<tr>
<td>Lewis</td>
<td>BN</td>
<td>Anti-Lewis-anti-DA</td>
<td>22,851 ± 891</td>
</tr>
<tr>
<td>Lewis</td>
<td>BN</td>
<td>Normal serum</td>
<td>21,844 ± 1,091</td>
</tr>
</tbody>
</table>

### B  Graft-versus-Host reactivity

<table>
<thead>
<tr>
<th>Lymphoid cells</th>
<th>Treatment with serum plus complement</th>
<th>Recipients</th>
<th>Lymph Nodes Mean Weight (± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis T</td>
<td>Anti-Lewis-anti-DA</td>
<td>(Lewis x DA)$F_1$</td>
<td>11.2 ± 0.9</td>
</tr>
<tr>
<td>Lewis T</td>
<td>Normal serum</td>
<td>(Lewis x DA)$F_1$</td>
<td>70.7 ± 8.6</td>
</tr>
<tr>
<td>Lewis T</td>
<td>Anti-Lewis-anti-DA</td>
<td>(Lewis x BN)$F_1$</td>
<td>31.5 ± 3.7</td>
</tr>
<tr>
<td>Lewis T</td>
<td>Normal serum</td>
<td>(Lewis x BN)$F_1$</td>
<td>33.7 ± 5.3</td>
</tr>
</tbody>
</table>

For further details see Binz & Wigzell⁴
Lewis, DA and BN are inbred strains of rats differing at the MHC locus.

icant activity against the expected haplotype⁵.

These results proved one thing: anti-idiotypic immunity against receptors reactive for MHC antigens can produce such a complete covering activity against the idiotypic spectrum representing reactivity against that antigen, that close to complete elimination of immune reactivity can be achieved.

Our initial attempts to induce specific unresponsiveness towards transplantation antigens by auto-anti-idiotypic immunity were carried out in rats³, using alloantibodies as immunogen. Such alloantibodies produced in Lewis rats against DA alloantigens (Lewis and DA differ with regard to the AgB locus, the rat counterpart to the human HLA system) were purified using anti-idiotypic antibodies produced in (Lewis x DA)$F_1$ rats inoculated with Lewis T cells. Such anti-idiotypic antibodies were covalently coupled to Sepharose and served as an insoluble immunosorbent for the idiotype-positive Lewis-anti-DA alloantibody molecules when filtered through such a column. The bound idiotypic molecules could be recovered in a 'pure' form. They were subsequently polymerised using glutaraldehyde (to introduce new antigenic determinants to increase 'helper' T cell function), were mixed with Freund's complete adjuvant and inoculated back into normal Lewis rats.

As a consequence of this immunisation protocol we could soon demonstrate
that these allo-antibody immunised rats produced auto-anti-idiotypic antibodies as detected in a radioimmunoassay against normal Lewis lymphocytes in vitro. The control lymphocytes used were (Lewis x DA)F₁ lymphocytes, where anti-DA lymphocytes with corresponding idiotypes are known to be lacking for reasons of tolerance⁴. Furthermore, lymphocytes from such 'auto-immunised' rats displayed specific reduction in or unresponsiveness against the relevant antigen with normal reactivity towards third party strain antigens.

Having shown the principle to be valid, that it is indeed possible to induce auto-anti-idiotypic immunity leading to selective abolition of the relevant immunocompetent cells, we now started to consider the practical problems to be encountered if the above described principle were to be applied to clinical situations. Admittedly it would be possible to obtain human anti-HLA antibodies for possible use as immunogen, but would anti-HLA antibodies directed against a given HLA specificity suffice irrespective of in which individual such antibodies were produced? The answer to this question is no, as we were able to show in the rat system that the pools of immunoglobulin genes for the heavy chain are crucial⁶. Thus, in the rat one could have two strains of rats with identical AgB loci but with different immunoglobulin gene pools. The idiotypes of receptors for a foreign AgB locus produced by these two strains of rats could be shown to differ. Accordingly, only antibodies or receptors from an individual with the same immunoglobulin gene pool could be used for the induction of successful 'auto-anti-idiotypic' immunity. In the human system this would mean that if one were to try to use immune alloantibodies as immunogen in the present system they would have to come from a human being with the same immunoglobulin gene pool as the human one wished to immunise with such purified immunoglobulin molecules. Although in theory feasible, this would essentially disqualify the use of immune anti-HLA antibodies in a pure form as immunogen in the anti-idiotypic approach. Thus, one would have to use idiotypic molecules from the very same human being that will later be immunised with pure idiotypic receptors.

Our second problem was then to analyse in the experimental animal system, what approach might be applied from analogous reasoning to the human system. The use of a potential recipient of a tissue graft as producer of the required immune alloantibodies we considered out of the question. However, we already know that there exist in normal individuals (true for man as well as for mice and rats) a large number of genetically precommitted T cells with specific receptors for any given foreign MHC antigen where the receptors carry idiotypes⁷. We started to look for the presence of such naturally occurring receptors in a shed form in the body fluids of normal, adult rats. It was found possible to detect and isolate (by anti-idiotypic immunoabsorbsents) such naturally occurring receptors in small quantities from serum and also, in partly degraded form, from the urine⁷. We next used such naturally occurring receptors in a pure, polymerised form with adjuvant to immunise the rats that actually produced the receptors. The results were again very clear cut; a significant production of auto-anti-idiotypic antibodies
was initiated and the lymphocytes from such individuals showed a significant decrease in reactivity towards the relevant, foreign AgB antigens. In Table II are shown some results demonstrating the specific reduction of GvH reactivity of lymphocytes from such individuals, as well as showing the resulting, highly significant prolongation of skin grafts when the relevant, AgB incompatible skin is transplanted to such auto-immunised rats. It should be noted that the rat strains used, Lewis and DA, not only differ with regard to the major locus, AgB antigens but also with regard to several minor loci. This may explain the eventual rejection without the need to implicate anti-AgB immune reactivity.

These findings show that it is indeed possible to use the potential recipient as the donor of immunogenic, idiotypic receptors without having to immunise him against the donor antigens. However, the procedure requires access to suitable anti-idiotypic antibodies to use as immunosorbents, a reagent hard to reproduce for potential clinical situations. It is possible to purify idiotypic, natural receptors using fixed donor cells as immunosorbents instead of antidiotypic antibodies, but it is difficult to get such cellular immunosorbents to function in a reproducible manner without cellular ingredients leaking too much and becoming mixed with the idiotypic receptors. For the present we have abandoned the use of such cellular immunosorbents but it should be remembered as a possible technique, merely requiring some improvement.

Our major reason for discontinuing the cellular immunosorbent technique at this stage has been the development of yet another approach for attempting the induction of auto-anti-idiotypic immunity in systems involving transplantation antigens. The rationale behind this new approach was based on three earlier known facts: a) immunocompetent T blasts express their idiotypic markers on their surface, b) blasts can be obtained in a pure form from mixed cell populations by sedimentation at I-g; c) idiotypic receptors have to be administered at a comparatively high local density in order to produce auto-anti-idiotypic immunity. Accordingly, we set up mixed leukocyte cultures, using the prospective recipient as cell donor for the responder cells and applying the potential donor cells as stimulator cells. Responder T blasts were then separated out from the MLCs shortly after the peak of proliferation had passed, mixed with Freund’s complete adjuvant and used as auto-immunogen in the recipient. The results obtained were again very clear-cut. Thus in these earlier experiments, the auto-blast immunised animals (tested now as functioning in mouse, rat, and guinea pig combinations) could be shown to produce auto-anti-idiotypic antibodies and their lymphocytes to express specific reduction or ablation of MLC or GvH reactivity against the relevant allo-antigens. Foreign tissue of ‘donor’ type will have a markedly prolonged survival in many animals. Examples of results obtained from auto-anti-idiotypic immunity induced by blast immunisation, are recorded in Table III, and the results in the different test systems appear directly analogous to the results obtained when using as immunogen soluble natural or immune anti-MHC binding receptors (See Table I or II.).

It seems clear that we have established with certainty that auto-anti-idiotypic
TABLE II. Demonstration of auto-anti-idiotypic immunity in Lewis rats immunised with their own, naturally occurring anti-DA receptors

A  Demonstration by radioimmunoassay using $^{125}$I-protein A as specific indicator for bound IgG anti-idiotypic molecules to idiotypic T cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Serum</th>
<th>$^{125}$I-protein A bound Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis T cells</td>
<td>Auto-anti-DA</td>
<td>4,366 ± 234</td>
</tr>
<tr>
<td>Lewis T cells</td>
<td>Normal Lewis</td>
<td>2,313 ± 53</td>
</tr>
<tr>
<td>Lewis x DA F₁ T cells</td>
<td>Auto-anti-DA</td>
<td>2,204 ± 192</td>
</tr>
<tr>
<td>Lewis x DA F₁ T cells</td>
<td>Normal Lewis</td>
<td>2,517 ± 250</td>
</tr>
</tbody>
</table>

B  Specific reduction in GvH ability by T lymphocytes from auto-anti-DA immunised Lewis rats

<table>
<thead>
<tr>
<th>Cells</th>
<th>Recipients</th>
<th>Mean lymph node weight ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Auto-immune'</td>
<td>Lewis x DA F₁</td>
<td>14.4 ± 0.8</td>
</tr>
<tr>
<td>'Auto-immune'</td>
<td>Lewis x BN F₁</td>
<td>26.4 ± 2.4</td>
</tr>
<tr>
<td>Normal Lewis T</td>
<td>Lewis x DA F₁</td>
<td>30.7 ± 4.2</td>
</tr>
<tr>
<td>Normal Lewis T</td>
<td>Lewis x BN F₁</td>
<td>25.8 ± 2.4</td>
</tr>
</tbody>
</table>

C  Prolonged survival of Lewis x DA F₁ skin on rats auto-immunised with naturally occurring anti-DA receptors

<table>
<thead>
<tr>
<th>Recipients</th>
<th>Lewis x DA F₁ skin (range in days)</th>
<th>Lewis x BN F₁ skin (range in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Auto-immune'</td>
<td>14 to 39 days</td>
<td>10 to 13 days</td>
</tr>
<tr>
<td>Normal Lewis rats</td>
<td>9 to 10 days</td>
<td>10 to 12 days</td>
</tr>
</tbody>
</table>

for details see Binz and Wigzell³
TABLE III. Demonstration of specific unresponsiveness in Lewis rats immunised with Lewis-anti-DA T lymphoblasts

A Specific reduction in MLC reactivity against DA alloantigens

<table>
<thead>
<tr>
<th>Responder cells</th>
<th>Stimulator cells</th>
<th>(^{3}\text{H-Tdr incorporation})</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Auto-immune' T</td>
<td>DA</td>
<td>2,155 ± 146</td>
</tr>
<tr>
<td>'Auto-immune' T</td>
<td>BN</td>
<td>17,984 ± 1,175</td>
</tr>
<tr>
<td>Normal Lewis T</td>
<td>DA</td>
<td>24,484 ± 1,709</td>
</tr>
<tr>
<td>Normal Lewis</td>
<td>BN</td>
<td>15,197 ± 817</td>
</tr>
</tbody>
</table>

B Specific reduction in Graft-versus-Host reactivity against DA allo-antigens

<table>
<thead>
<tr>
<th>Injected cells</th>
<th>Recipients</th>
<th>Mean lymph node weight ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Auto-immune'</td>
<td>Lewis x DA F(_1)</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>'Auto-immune'</td>
<td>Lewis x BN F(_1)</td>
<td>25.6 ± 0.8</td>
</tr>
<tr>
<td>Normal Lewis</td>
<td>Lewis x DA F(_1)</td>
<td>45.9 ± 4.9</td>
</tr>
<tr>
<td>Normal Lewis</td>
<td>Lewis x BN F(_1)</td>
<td>24.2 ± 1.2</td>
</tr>
</tbody>
</table>

for details see: Andersson, Binz & Wigzell\(^10\).

immunisation using several different approaches, can be used to achieve selective unresponsiveness against major histocompatibility antigens by the induction of auto-immune responses in adult, immunocompetent individuals. Other known features of the present system should be mentioned. The induction of auto-anti-idiotypic immunity leads to a long-lasting state of unresponsiveness, which may extend throughout the life span of the individual\(^3\). We believe the reason for the prolonged state of immunosuppression against the relevant alloantigen to be the constant production of new, idiotype-positive lymphocytes from the stem cells in the bone marrow, thus serving as an internal, automatic boosting device. The seemingly irreversible state of elimination of a certain group of idiotypic, immunocompetent lymphocytes may, however, also have a negative side. As T cells may have more than one specialised immune function\(^11\) is it possible that such cells carry some indispensable function besides their reactivity towards the foreign alloantigens? So far we have not found the depressed immune reactivity in this group of auto-immunised animals to include any other, unexpected antigen. The suppression seems to be an extremely select immune depression. Another possible complication to be considered and thoroughly explored before considering the present approach ready for clinical application, is the creation of immune complexes. Such complexes might be created in large enough amounts through the constant new production of virgin, idio-
typic lymphocytes (see above) to serve as antigen, and lead to possible damage. However, we have so far found no evidence for such immune complex damage, but wish to carry out additional studies to exclude this possible side effect.

In conclusion, auto-anti-idiotypic immunisation can be used as a highly efficient means for achieving a long-wanted goal, that of selective immune suppression against certain antigens in an adult, immuno-competent individual. The resulting immune suppression is extremely specific, long-lasting and seems to carry no detectable side effects for the health of the individual. The principle involved seems to be of general application. It should be possible to apply not only to the induction of transplantation tolerance (in graft recipients of organs such as kidneys, although in bone marrow transplantation the donors should be 'tolerised'), but to delayed hypersensitivity disorders and even in certain auto-immune diseases12.

Acknowledgments

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References

5 Binz, H and Wigzell, H (1975) J. Exp. Med., 142, 1231

Open Discussion

BARTOLI (Sassari, Italy) First of all, not related to the kidney, I would like to know whether you tried to use the same system to favour tumour inoculum growth in experimental animals? Secondly I would like to ask you whether possible extension to the clinical situation could bring about graft vs. host reactions that are potentially harmful?

WIGZELL Tumours follow the laws of transplantation. We have not tested, in a specific way, whether the autoblast-immunised animals will be more susceptible to tumour, nor do we know whether there might be any increase in tumour frequency after autoblast-immunisation. Why do you ask the second question?
BARTOLI Well, you mentioned using the same system for marrow transplantation.

WIGZELL Yes, we never actually have, but the question is of course quite relevant because these autologous cells generated in the MLC are surely, in some systems at least, prone to carry over with them alloantigens from the stimulating cells, so one could believe that in various ways one could achieve immunity instead of tolerance. So far we have not noted that, but if for instance we transferred the autologous back, together with antigen in a certain form, we might achieve the opposite; that is immunity. Of course we do not inoculate the autologous back alive. They are killed. If you take autologous alive and inoculate, you will get immune memory.

GELIN (Gothenburg) Have you tried secondary grafts?

WIGZELL We have not done secondary grafts in vivo, but we have tested the reactivity of the lymphocytes from autologous immunised animals which have rejected one graft, in vitro, and they are still MLC negative. You should realise that we are working with a system which contains strong transplantation antigens, as well as many weak histocompatibility antigens. We believe that the eventual cause of the rejection of our tissue was conventional immuno-reactivity against minor loci and not against the major, because, of course, we gave no immunosuppressive therapy whatsoever to these animals.

TILIKAINE (Helsinki) Do you have any experience with anti-idiotypic antibodies in comparison with the suppressor T-cell extracts. Is it in any way the same material, or are they quite different?

WIGZELL Are you talking about antigen-specific suppressive factors solubilised from suppressor T-cells?

TILIKAINE Yes, the ones against the genetic region.

WIGZELL The biochemistry of these factors is not comparable with ours.

THORSBY What we would like to do now is to discuss with the three distinguished speakers how to transfer these experimental systems, some of which are being called ‘in vitro artefacts’, into clinical reality.

HAYRY (Helsinki) There is one point considering specific immunosuppression which has not been brought up, and perhaps should be introduced before you gentlemen continue this discussion. That is the possibility of reducing the immunogenicity of the allograft. There is fairly strong evidence that this can be done both experimentally and in clinical kidney transplantation. This possibility has especially been advocated by Dr Ronald Guttmann in Montreal. The idea is that you treat the allograft donor with cytotoxic drugs before removal of the graft. Guttmann has shown that with proper pretreatment the graft is not rejected promptly, or that the rejection episodes are weaker than without drug pretreatment. Dr Soors who also did the heart
transplants for Hans Wigzell, has explored the effectiveness of different anticancer chemotherapeutic agents to prolong allograft survival in the rat heart allograft systems. If you transplant the heart from one rat strain to another, the graft is rejected in seven days. If you transplant the heart from a donor that has been pretreated by any one of these anticancer drugs the rejection time is extended from seven to let’s say 15-26 days, and all the way up to nearly one month. This donor pretreatment is very simple to perform, and it is enough to give the drugs to the graft donor 6 hours before removal of the graft. There are two drugs which should be mentioned here, because they are ineffective: mercaptopurine and azathioprine. The vinca alkaloids, which affect the division of cells, are also ineffective. I don’t want to go into explanations of the possible mechanism of this prolongation of graft survival, but the conventional thinking regarding the ‘passenger’ leukocytes as being responsible is not necessarily true. As we just heard from Professor Brent and Dr Thorsby other cellular constituents than the passenger leukocytes of, for example kidney, contain the Ia antigens.

BRUNNER (Basel) I think everybody would like to know what the panel thinks about the effect of blood transfusion. Which of the mechanisms presented do you think plays a role in the well-demonstrated effect of blood transfusion prior to kidney transplantation?

THORSBY Who would like to answer that, would you like to start off the discussion Leslie?

BRENT I certainly agree with the implication in this question that blood transfusion should be the starting point, because after all this is already being done clinically and has been shown to be effective clinically. This is the right approach and any other form of pretreatment will arise from variation and analysis of the blood transfusion regimen that is optimal. I do not think one can really answer the question until the patients who have received blood transfusions are very carefully analysed immunologically as to the nature of their reactivity to both the kidney donor’s antigens, and to other histocompatibility antigens. To my mind, the analysis of immunological reactivity in patients who have received blood is the next important development. We need to find out the best way in which blood can be given; the timing of the blood transfusion is probably very important and the nature of the blood is probably important.

BATCHelor I am prepared to stick my neck out a little bit further than Leslie Brent. Yes, I think the mechanism involved is very likely the one I was trying to expand in my presentation, in the sense that you are presenting to the recipient, material which may not be entirely free of Ia-like components. Obviously there may be some lymphocytes in the transfused blood, but I doubt if many of them are viable in the situation that you are using. Where blood is kept under normal blood transfusion conditions it is sitting in the refrigerator for about a week. My guess is that the vast majority of structurally-intact lymphocytes will not be viable, and you lose your immunising capacity very considerably if you kill the lymphocyte. So I think that you present a stimulus which is composed chiefly of HLA A, B and C types and is rather
weak on the HLA D factor, and that this is what tips the balance. It has a suppressive effect.

THORSBY Another approach to this problem is to ask the question, why are some grossly HLA mismatched kidney grafts so well accepted, with full immunological reactivity against any other antigen? What sort of immune status can we detect in a recipient of a grossly HLA mismatched kidney? Some studies have been carried out lately on that problem, and what seems to be the message so far is that there does not seem to be any tolerance in the MLC reactive cells, which most of us think are the T helper cells. The T helper cells are still there and they are able to react against the donor. Furthermore, these recipients are able to produce antibodies against the donor continuously and these antibodies can be detected by very sensitive tests, so there seems to be continuous antibody production going on in some of these recipients. What, however, seems to be rather difficult for these patients, is to create cytotoxic T-cells against their donor; at least in some studies this seems to be difficult. Most astonishing, in very recent studies, among others being carried out by Dr. Thomas in Richmond, Virginia, some of these patients have suppressor T-cells, which seem to inhibit rather specifically the generation of T-killer cells against the donor. So this might be the sort of immune status we would like to see in our patients, and it could be that status is what we achieve by giving some of these patients blood transfusions. I would recommend that some of us go home and study the effect of blood transfusions using some of these tests.

BATCHelor I think particularly promising in this respect would be to use something like platelets for which there is good evidence of non-immunogenicity in animal systems. In the human systems I think one has to be very careful before leaping up and saying they are immunogenic in humans. Most of the data that has been presented along those lines has been a question of using platelet preparations which are contaminated with lymphocytes, and we will have to work on getting very much better preparations.

THORSBY But perhaps, Richard, we should also say a word of caution here. Because there is a tendency now for the pendulum to swing to the direct opposite. Some years ago we were all very restrictive in giving blood transfusions to our patients; now some of our colleagues have said that to do kidney transplants without a previous blood transfusion is unethical, which is, I would say, a rather astonishing statement. Now truth usually lies in between somewhere and we should be aware of the side effects of uncritical blood transfusion. You can obviously create some sort of specific or non-specific unresponsiveness, based on all this kidney graft survival data. But we are all aware of those patients who really develop multi-specific antibodies and for whom we are not able to get any cross-match negative kidneys. So I think one should be a little bit careful in taking the message that we should all give blood transfusions; the question is, perhaps, that blood transfusion is good, but we do not know yet which cells, which antigens or which blood component we should give.

BRENT Could I just suggest that we ask Barry Hulme to say a few words
about the St Mary’s patients, because one of the most striking things about them is that they have tended to make very little antibody in response to their transfusions. And at St Mary’s as far as I know - Barry will correct me - there has been no difficulty finding kidneys for any of the patients. Barry is that correct?

HULME (London) I think one of the important points is how much blood you give before you do the kidney transplant. Our own data was based on 120 consecutive first transplants. Forty patients had never received any blood, 40 patients had received between 1 and 6 units of blood and 40 patients had received more than 6 units of blood. Our own data demonstrate that the survival rate increased from 17% to 60% at one year when between 1 unit and 6 units were given. The data from Leiden is that if you give between 1 and 4 units of blood you can significantly increase the chances of survival of the cadaveric graft. We have been deliberately giving 1 unit of whole blood at a time, at one month intervals, screening the patients for cytotoxic antibodies and transplanting them when they had received at least 2 units of blood. In the last 15 months, following this through and going up to 4 units of blood, of the 15 consecutive patients who have now been studied, none developed any cytotoxic antibodies with 3 to 4 units of blood, and of the 12 patients that have been transplanted, all have functioning grafts. On the other hand, earlier, when we had stopped giving blood transfusions, we had 40 patients who had never received any blood at all, and of these, only seven had a functioning graft at one year. So clinically we are glad that the pendulum is swinging again.

GOLBY (Exeter) A simple clinical question: are we talking now about giving old transfusion blood, not dextran-washed, not fresh-frozen blood, but the standard one-week old blood?

BRENT The answer is yes.

CARDELLA (Toronto) I would like to ask Dr Wigzell if he would amplify his remarks with regard to the stable renal transplant recipient. Do you think that these patients have anti-idiotypic antibody, and can this be measured? Secondly, with relation to blood transfusion, do you have any experimental data to show that you can induce this type of antibody response by giving blood, and thirdly, could you elaborate on your transfer experiments where serum containing anti-idiotypic antibody is given to non-immunised animals?

WIGZELL To the second question first: it has been quite well established, in animal systems again, that administration of histocompatibility antigen in certain forms of the antigen will lead after an initial period of antibody production against antigen, to induction of anti-idiotypic antibodies directed against antibodies against transplantation antigens. That means then, directly, giving blood containing foreign histocompatibility antigen. At this point I would like to emphasise that it would be wise to introduce a distinction between talking about units of blood and talking about HLA type of the donor blood. Because it might very well be relevant that if you have used
6 units of blood, you have happened to use an HLA donor corresponding with the kidney donor. If one is going to do any kind of systematic analysis of this, surely one would like to know the HLA type of the blood donors, because this would be a specific thing, that might reduce the required number of transfusions and would be a more directed effort for the clinician. Antigen as such and hyperimmunisation as such, under various conditions can lead to the induction of the kind of auto-anti-idiotypic antibodies I was referring to, and might also have certain consequences for a subsequent graft in exactly the same way as it would lead to increased survival of the graft. With regard to human patients having an acceptable graft, it is very hard to test the possibility that these now have as a running mechanism for specific unresponsiveness, anti-idiotypic immunity. Why? For the very simple reason that the genetic set-up of T-cell receptors with regard to the antigen-binding site follows exactly the same immunogenetic rules as that for the B-cell; that is, each individual inherits a different set of genes, coded in the germ line and coded for the variable regions of the polypeptide chains which make the antigen combining site. Take for instance, Leslie Brent and I here, and assume that we have the same HLA antigens. It is very likely despite this, that if you make, let us say a reaction against Erik Thorsby, that auto-anti-idiotypic antibodies made in Leslie Brent and myself would have different specificity, because we inherit different B genes from our parents, if they are not inbred. That means that when you have to test in human beings, unless you work with inbred populations, you have to use a family member who is homozygous for the allotypes of the heavy chain immunoglobulin classes. The only way of testing this to see if this is the running mechanism, is to take blood, lymphocytes, that is, from the recipient before you put in the graft, and freeze the lymphocytes. That is the only way of subsequently being able to test whether that individual will make auto-anti-idiotypic antibodies, using the frozen down lymphocytes in a specific MLC.

FJELLSTRÖM (Sweden) I would mention a case report published, I think a year ago, in the American Journal of Surgery of a transplanted woman, who 5 years after the transplant, on her own risk terminated all immunosuppressive therapy. The graft survived for one and a half years after that and I think it is still surviving. My question is to the panel, are you able to test what mechanisms are operating on such occasions, is it induced tolerance, or is it a sort of auto-immunity such as Hans Wigzell talked about?

BRENT Of course there have been other patients who have for one reason or another, either taken themselves off immunosuppression or have been taken off immunosuppression on medical grounds, in whom rejection crises were observed fairly soon after cessation of immunosuppressive treatment. Unless one actually studies patients like these very carefully and logically, there is simply no knowing what mechanism is responsible. It is conceivable that that patient might be analogous to a neo-natally injected mouse or that she may have lost her antigen-reactive cell clones, for the sort of reason that Hans Wigzell has propounded or for other reasons. Such patients provide invaluable material, I would have thought, and we really have to subject them to modern immunological methods by which the reactivity of the patient can be ascertained.
LUKE (Lexington, USA) Speaking as a clinical nephrologist I would like to underscore some of the remarks you made Mr Chairman. I think there is danger if the message goes out from this august body that transfusion is ‘good’ for transplant candidates, that dialysers will transfuse freely and will sensitise a lot of patients. We have shown as have others that uraemic patients are in no way protected from being sensitised, and after more than 10 units of blood 50% of the population was sensitised broadly, and may be effectively excluded from transplant. What the St Mary’s group has been doing is a scientific study in which they are watching to see if sensitisation is occurring. I really think we have to be very careful clinically at the present stage of our knowledge.

THORSBY I quite agree.

TOYRKANTONIS (Salonica) Concerning kidney transplantation in children, some people start immunosuppressive drugs 10 days before transplantation and believe they have evidence that this therapy reduces the recurrence of glomerulonephritis in the kidney grafts after successful kidney transplantation. I would like to ask the members of the panel their opinion about this point.

THORSBY Can anybody reply to that very important question with which I think most of us are quite often confronted by the clinicians - when to start with immunosuppressive therapy?

BOULTON-JONES (Glasgow) Could I make one observation that may add to that and perhaps link with the blood transfusion data. Is it possible that the natural immunosuppression of patients with uraemia varies and that the patients who require most transfusion are also the patients who are most immunosuppressed, being the illest? Therefore by having prior immunosuppression you more easily get to the stage which maintains a functioning transplant. There are some preliminary data of Dr Briggs (Glasgow) which suggests that patients who have negative skin tests have their first rejection episode later, and have improved figures for transplant survival if they are immunosuppressed at the beginning.

THORSBY That is an explanation which is almost always mentioned in the discussion of any factor of blood transfusion. I don’t know whether there exists any good data to answer to that question.

BRENT I think it is a very persuasive argument but I do not think it could possibly apply to a series of patients who have been deliberately transfused as a matter of policy, without exception; so that I do not think this could account for the St Mary’s data.

HULME The data I presented was a retrospective survey and then the last 15 patients were a prospective survey. To go into a little more detail of the patients who were given blood or not given blood, these patients came to one transplant unit from two dialysis units, and one dialysis unit had a deliberate policy of never giving blood and the other unit had a policy of giving blood when the haemoglobin fell to a certain level, and this is how they were divided.
None of them were just given blood for an acute bleed, for instance, but I fully agree that the first lot of data was a retrospective study.

KNAPP (Nottingham) We presented data to the British Transplantation Society recently on our own relatively small series who had been transplanted and showed a big difference between those who had received blood transfusion and those who had not, to the extent of 90% to 20% one-year survival figures, respectively, mainly due to differences in acute rejection episodes. This was also retrospective information, but we analysed, as other groups could of course, in terms of whether their mean haemoglobin over the year was the same, whether their clinical state was the same, as assessed by the clinicians, by rehabilitation and other factors. We were able to show that there was no apparent difference between the two groups, and that they received blood transfusions usually long before they came to the unit, in relation to reasons largely unrelated to their renal failure. I think that a more detailed analysis of the retrospective data and possibly even a retrospective analysis of the blood donors and their HLA typing, as they may presumably still be alive and traceable, might provide some of the clues we have been looking for this afternoon.

THORSBY Yes, I think that is very appropriate.

KEMP (Odense, Denmark) I shall change the theme a little. Would you like to tell us whether prolonged kidney preservation is necessary if you want to apply your three different methods clinically?

THORSBY The answer to that is absolutely yes, but with the methods that we have been discussing here, I see great difficulties in constructing a machine that would be able to keep alive a kidney for those couple of weeks at least which would be necessary to either inject antigen and induce specific unresponsiveness by that method or, as Hans Wigzell is going, generating MLC-reactive cells against the donor, reinject those into the recipient again and induce autoimmunity, or as Dr Batchelor would be advocating, producing enhancing antibodies. So in a cadaveric situation, to produce specific unresponsiveness towards a given donor after he has become a donor, as far as I can see is very difficult.

THORSBY Let me ask these three distinguished speakers, if we meet again in a couple of years from now, in the next EDTA Conference, and the same symposium is held, in which of these areas will the clinical breakthrough have occurred?

BRENT Is that all you want to know? I am convinced that in two year's time we shall know a great deal more about the direct effect of blood transfusion, the components in the blood which are primarily responsible for whatever effect is being obtained and the best ways of administering the blood. Many laboratories are working on this, including my own, and I am quite sure this is one aspect which will be very considerably clarified within the next two years. Of course, the other point which is being worked on in many transplantation centres, is the question of anti-D antibodies and the
role they may play and the effect matching for D antigens will have on the prognosis of kidneys.

WIGZELL Being a Swede, I have no hard views. However, I think that in a few years’ time we would know whether the principle I have been talking about today is feasible or not and in what areas it will function. I believe it will be established that the principal is valid but whether it is clinically applicable is not the same. The second thing that I think we will know at that time will be much more about the various sorts of molecules involved on these various kinds of sub-sets of T cells. For instance, it is already quite clear, I think, that the glycoprotein-binding patterns analysed for the killer T cells are distinct from the helper T cells. It might be that it is possible to create antisera, useful even in clinical situations, far more refined, for instance, than this kind of ‘nineteenth century’ approach using ALS, and actually directed against a minority of cells carrying a certain pharmacologically very active receptor. These other two things should be known in the period of about three years, let us say.

BATCHELOR Well, they have not left me very much to say. One of the things I would say, which actually does not bear specifically on this point, is the question that inducing some kind of non-reactivity by pretreatment raises special difficulties, as Erik Thorsby was saying. That is true if you are thinking in terms of one particular kidney, but it seems to me that although it is perhaps not all that neat, the cocktail approach is perfectly valid, and I can see no objection, for example, to pretreating a patient when you have them clinically in a desirable state as far as dialysis and so on is concerned. For a poor example, pretreating them - and I do not think that this is necessarily the only way of pretreating - using platelets from a number of different donors whom you know to carry the common types of HL-A incompatibilities. That would then absolve you from waiting for a specific kidney to turn up. About what will happen - I am sure things will be very messy because they never are tidy, and I guess that we will make advances in treatment without actually realising the reasons for them. I would say that there will be quite big changes in our transfusion practice. The crucial thing will be to introduce transfusion methods which avoid exciting the amplification stage of the MHC-activated immune response, and I can see that there will be a number of ways in which one could do this. One could either do it in the way that I am suggesting, or by D locus matching, but we have to depend on how polymorphic the D locus factors turn out to be. If they are extremely polymorphic, then we are back in the awkward situation that we are now presented with for the A B and C factors. So I think it will be a combination of events, and the crucial thing will be to eliminate this augmentation or amplification stage in immunisation.

THORSBY Thank you very much and thank you for attending. The answers to these questions will be given at the next EDTA Congress!