PART XI

Guest Lecture  INDUCTION OF SPECIFIC UNRESPONSIVENESS IN KIDNEY TRANSPLANT RECIPIENTS

Chairmen: M Broyer
INDUCTION OF SPECIFIC UNRESPONSIVENESS IN KIDNEY TRANSPLANT RECIPIENTS

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The recent results published by Opelz, Graver, and Terasaki [1] on high kidney graft survival rates in patients given multiple blood transfusions, if confirmed, give us genuine reason to hope that in transfusion we have a clinically acceptable regimen for inducing effective unresponsiveness with respect to kidney allografts. The salient facts of their study included the following:

a) the study was prospective;

b) with up to 20 transfusions 72% of patients did not form detectable lymphocytotoxic antibodies; 17% formed antibodies reacting with 50% or less of the test cell panel, and 11% with more than 50% of the cell panel;

c) there was a blood transfusion dose-related increase in graft survival from 23% (no transfusions) to 87% (> 10 transfusions) at one year.

The study shows that improvement in graft survival rates brought about by blood transfusion is not due to a selection effect. According to the selection hypothesis, highly responsive patients would develop HLA antibodies after receiving multiple blood transfusions, and in consequence would be less likely to receive a transplant because of a positive cross match test. Although 11% of the transfused population did develop antibodies reacting with more than 50% of the test cell panel, this could not be enough to account for the difference in actuarial graft survival rates between the non-transfused and transfused groups. Clearly, the improvement in graft survival rate must be due to a positive induction of unresponsiveness. This conclusion, together with the demonstration of the relationship to dose of transfusions points to the great similarity between the blood transfusion effect in man and in the experimental animal systems which have been extensively investigated over the past decade and longer. The use of these experimental systems for refining the questions which can only be finally answered in man, needs no further justification. There are many such questions, e.g. what are the component(s) in blood which cause unresponsiveness and can they be readily separated from those which induce sensitisation? What are the
most effective protocols and what is their specificity in terms of unresponsiveness to the major histocompatibility system antigens? Is there an identifiable group of subjects which is refractory to the induction of unresponsiveness?

Today I want to consider a problem which is central to the mechanisms of unresponsiveness. That problem is — why are major histocompatibility system (MHS) antigens such powerfully immunising antigens (‘strong’ antigens) capable of inducing rapid rejection of primary allografts or graft versus host disease in appropriate circumstances? What is it that distinguishes the major system antigens from those belonging to the minor systems? The reason for asking this question is that in practice major system incompatibility is the most important barrier to successful kidney allografting.

However MHS alloantigens are strong primary immunogens only in certain circumstances. They must be presented to the recipient on the surface of metabolically active tissue or cells and the incompatibility must include a Class 2 incompatibility (Class 1 antigens are HLA-A,B,C, and homologues in other species; Class 2 antigens are HLA-D/DR and homologues in other species). If MHS alloantigens are presented on dead cells, or cell membrane fragments [2] they have very weak or undetectable primary immunogenicity. It is worth mentioning that the position is entirely changed once the recipient has been sensitised, when dead cells, fragments etc can provoke secondary responses.

When investigating the mechanisms of immunological enhancement of the survival of rat kidney allografts, we found that the long surviving allografts did not behave as strong primary immunogens if they were re-transplanted, into immunologically naive recipients [3]. Studies with unlabelled and labelled antibodies had shown that the kidney allografts still carried their original donor-type alloantigen [4, 5], and recent investigations with monoclonal reagents showed that this included Class 2 MHS antigens [6]. The question therefore was why did a long surviving allograft have only a weak primary immunising effect whereas a normal kidney allograft which had just been harvested from its original donor animal provided a strong primary immunising stimulus which led to allograft destruction within 12 days? We attributed the difference to the former graft having no donor type passenger cells present in it, and it was proposed that the property of inducing strong primary alloimmunity was confined to the passenger cells.

Recent experiments, to be reported elsewhere in detail, have been designed to analyse what cell subpopulations of the passenger cells function as strong primary allo-immunogens. Our experimental system was identical to that previously described [3]. In brief, kidneys from (AS x AUG)F₁ donors were transplanted into AS recipients. Normally such grafts are acutely rejected within 12 days, but permanent graft survival can be ensured if a passive and/or active enhancement regimen is instituted. After four weeks, the kidney allografts were then re-transplanted into secondary AS recipients. As mentioned previously these are not acutely rejected and many survive indefinitely. At the time of re-transplantation we therefore injected into the secondary recipient various subpopulations of (AS x AUG)F₁ cells likely to be amongst the passenger cells and observed the effect on allograft survival.

Passenger cells in a kidney allograft can be divided into intravascular and extra-
vascular compartments. Therefore the amount of blood remaining in an allograft prepared for transplantation was calculated using $^{51}$Cr labelled red cells as a marker. The data showed that approximately 0.1ml of blood remained in an average sized kidney of 1gm. This amount of blood was then tested for its immunogenic property by injection into naive AS recipients which also received long surviving, immunologically enhanced (AS x AUG)F$_1$ kidneys. It was found that 0.1ml of (AS x AUG)F$_1$ blood had a negligible effect on the survival of the re-transplanted kidneys. However a larger dose of blood — 0.5ml — had a significant effect. We concluded that blood does contain a cell population(s) in small numbers with the property of inducing a strong primary alloimmune response but whether or not that response is induced depends upon the amount of blood administered.

The effects of (AS x AUG)F$_1$ T and B lymphocyte enriched suspensions were then tested. In summary, these populations failed to induce acute destruction of the re-transplanted kidney allografts even when up to 5x10$^6$ cells were injected.

Because dendritic cells have been implicated in the process of antigen presentation to helper T cells, and are found in kidney tissue, these were the next sub-population tested. The results showed that cell suspensions enriched for dendritic cells were remarkably effective in provoking acute graft rejection. The minimum number required in our donor/recipient strain combination was approximately 1-5x10$^4$ cells.

Suspensions of plastic adherent cells derived from the peritoneal cavity or the spleen were also tested in the same experimental model. The majority of the cells in these populations were macrophages, but other cells, some of which may be precursors of dendritic cells, were also present. Adherent cell suspensions were clearly less active than dendritic cells in provoking acute graft rejection. For example, only two out of seven rats given 10$^6$ adherent cells died with acute graft destruction during the first two weeks after receiving re-transplanted kidney allografts. However more work is needed to identify the sub-populations in the adherent cell suspensions which are responsible for inducing acute graft rejection.

The important conclusion from these studies is that the major primary immunogenic stimulus of a kidney allograft is provided by the dendritic cells within the graft. Other cell types, although they may carry MHS alloantigens do not appear to provoke the same strong primary response.

The explanation of the unique position occupied by dendritic cells lies in their function. Studies by Steinmann and colleagues [7] and Mason et al [8] have demonstrated that dendritic cells are extremely potent stimulators of primary MLC reactions and the lineage may be unique in this regard. A primary MLC in essence measures cellular proliferation by the responders’ T helper cells [9]. The implication therefore is that dendritic or related cells are the only ones that can directly activate T helper cells. Since primary alloimmune responses are T dependent, it follows that dendritic cells perform the crucial activation of the recipients’ T helper cell population.

At this point the assumption is made that dendritic cells are identical to the accessory cell which has been demonstrated to be necessary for presenting conventional antigens to helper T cells [10]. If this assumption is accepted, primary
alloimmunisation proceeds by two routes:

1) Allogeneic dendritic cells situated in the incompatible tissues can bypass further antigen processing and presentation; they are able to activate the recipients' T helper cells directly.

2) All other MHS alloantigen of the graft is processed and presented by the recipients' own accessory cells.

According to our hypothesis the crucial distinction between MHC incompatible grafts and all other types of antigen is that by virtue of their content of viable dendritic cells, primary alloimmunisation can proceed by route 1 as well as route 2. Minor system incompatibilities activate T helper cells only by route 2.

For reasons which will not be discussed here, route 1 is likely to be an optimally efficient way of activating the recipient's T helper cells, and contrasts in this respect with route 2. Hence weak responses are to be expected if activation is confined to route 2. Furthermore, if an allograft is only a weak primary immunogen, any concomitant immunosuppressive property, e.g. by activating suppressor circuits, or eliminating antigen reactive cells by opsonisation [11], would have a profound effect upon the net response of the recipient.

Some of the clinical implications of thinking of immunisation and induction of unresponsiveness in these terms are reasonably clear. Probably the most important one is that the effect of removal or inactivation of dendritic cells and their precursors from transfused blood deserves investigation. It will also be necessary to exercise our imaginations in order to devise safe methods for eliminating these cells from allogeneic grafts.

References

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