Transplant Int (1992) 5 [Suppl 1]: S 583–S 586

TRANSPLANT
International

The "rejection reaction" is not confined solely to the allograft

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The rejection process refers primarily to the destruction of foreign tissues by host immune mechanisms. This process affects host lymphoid tissue profoundly and alters the migration patterns of lymphocytes in recipients of organ allografts [8]. It has been shown that specifically sensitized lymphocytes traffic both to and from the transplant [9, 10]. A considerable amount of knowledge has been gathered on the preferential migration pathways of lymphocytes through lymphoid and mucosa-associated lymphoid organs [1, 15]. The factors regulating lymphocyte migration through non-lymphoid tissue in normal conditions are not well known and even less well understood in the context of graft rejection.

In this article we described for the first time migration in a recipient non-lymphoid organ (heart) and it's potentially harmful effects in causing parenchymal damage during renal allograft rejection in the rat model. These lesions were detected during the process of developing a model of chronic renal allograft rejection. The pathogenesis of these cardiac lesions is not fully understood but possible mechanisms include upregulation of homing receptors/adhesion molecules, breakdown of peripheral tolerance and involvement of cross-reacting anti-endothelial antibodies.

Key words: Renal allograft rejection – Cardiac lesions – Pathogenesis

Materials and methods

Animals. Inbred male rats weighing 180 g to 300 g were used in all experiments, (Olac, Bicester, UK). PVG ($RT1^c$), AGUS ($RT1^l$), PVGRT1^u, PVGRT1^l, F344 ($RT1^{lvl}$) and (DA × PVG)F_1 were used as kidney donors; DA ($RT1^{avl}$), AO ($RT1^u$), LEWIS ($RT1^l$), PVG ($RT1^c$), PVGRT1^u, PVGRT1^l served as recipients.

Operative procedures. Orthotopic left kidney grafts were anastomosed end-to-end to the recipient's left renal vessels and an ureteroureteric anastomosis was performed using standard microvascular techniques. Seven days after transplantation the right kidney was removed and graft function was estimated by serial serum creatinine measurements. All animals that presented with evidence of localized or systemic infection at the post-mortems were excluded from this study.

Immunosuppression. Cyclosporin-A (CyA) (kind gift from Sandoz, Basel, Switzerland) dissolved in olive oil in an i.m. dose of 2.0, 3.5 and 5.0 mg/kg per day for 14 days post-transplant was administered to the recipient animals.

Histological examination. Full autopsies were performed on all animals that died during the post-operative period or that were culled due to development of graft insufficient. Graft insufficiency was defined as a level of serum creatinine double the normal control (40–50 umol/l). Samples of most tissues including lymphoid and various non-lymphoid organs were collected and routine haematoxilyn and special stains as required were performed.

Immunohistochemistry. Tissues obtained from autopsies were snap-frozen in liquid nitrogen. Five-micrometer-thick frozen sections were prepared and a two-step immunoperoxidase staining technique was used. The primary mouse anti-rat monoclonal antibodies were: OX-29 (leucocyte common antigen), OX-42 (macrophages and dendritic cells), OX-17 (anti-class II, Ia-E), OX-8 (T cytotoxic/suppressor), and W3/25 (T helper) from Serotec (Bicester, UK). The secondary antibody used was a sheep anti-mouse IgG horseradish conjugate (Amersham Int. Plc, UK).

Results

Histological and immunohistochemical characterization of the cardiac lesions. Postmortem findings showed that a great proportion of the rats that presented with rejection of the kidney graft at postmortem contained multiple foci of mononuclear cell infiltration in their own hearts. The cells were seen diffusely infiltrating the interstitium and also attacking and destroying isolated or bundles of myocardial fibres. In places of more advanced and extensive cell damage there were also foci of interstitial fibrosis with evidence of recent and old hemorrhage. In three cases acute fibrinoid necrosis of medium-sized arteries was present and two long-term survivor rats showed non-atherosclerotic intimal thickening of medium-sized ar-

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Table 1. Table showing the experimental groups and the incidence of the cardiac lesions in those animals that presented with graft rejection at postmortem

Experimental groups	graft rejection	cardiac lesion
MHC + Minor mismatch with immunosuppression ($n = 104$)		
Acute rejection	31	26
Chronic rejection	20	15
MHC + Minor mismatch no immunosuppression ($n = 10$)		
Acute rejection	10	9
Minor mismatch alone no immunosuppression ($n = 46$)		
Acute rejection	3	3
Chronic rejection	2	2

Table 2. Table showing the development of the cardiac lesions after priming the recipients with splenic allogeneic cells

	Day after priming	Cardiac lesions
MHC + Minor	8	2/2[a]
Mismatch	15	5/6[b]
($RT1^c < - > RT1^u$)	22	2/2[b]
Minor Mismatch	8	2/2[a]
($RT1^u < - > RT1^u$)	15	2/2[b]
	22	1/1[a]

[a] Mild lesions
[b] Moderate lesions

teries. All these abnormalities were very similar to those seen in rejecting cardiac allografts.

Immunocytochemistry showed that the infiltrating cells were leukocyte common antigen and class II positive. The OX-42 monoclonal antibody stained a fair number of macrophages within the mononuclear cell infiltrate. The majority of the cells stained positively with W3/25 (CD4) and to a lesser extent with the anti-CD8 monoclonal antibody.

Incidence of the cardiac lesions. The cases were divided into two main experimental groups: the MHC plus Minor mismatch and the MHC-matched/Minor mismatch groups (see Table 1). Most of the recipients with MHC plus Minor mismatched grafts received CyA as immunosuppression and a smaller number taken as control did not receive any immunosuppression. To date of the 104 transplants in the MHC plus Minor group that received immunosuppression, 51 had evidence of rejection at postmortem and of these 41 developed the cardiac lesions described above. All animals in the MHC plus Minor mismatch group that did not receive CyA died of rejection and 9 out of 10 showed the cardiac lesions. In the MHC-matched/Minor mismatch group the majority of the animals survived beyond 150 days and to date only 5 died secondary to rejection. All these five cases presented rather extensive and florid myocardial cell damage. The cardiac lesions have not been seen in the hearts of transplanted animals that did not show rejection of the allograft nor were they seen in normal non-transplanted controls.

When the rejection cases were divided into acute and chronic cases a high incidence of cardiac lesions was observed in both subgroups. These results point out the fact the this systemic organ damage can also occur in a chronic allograft rejection situation.

Is the cardiac lesion due to graft-versus host disease? Is it linked to the use of CyA? In order to address both questions in a single experiment kidneys obtained from 8 $(DA \times PVG)F_1$ were transplanted into 4 DA and 4 PVG parents without any post-transplant immunosuppression. In the F_1 to parent situation graft-versus-host cannot occur. All the DA and PVG recipients of $(DA \times PVG)F_1$ kidneys died of acute humoral and cellular rejection on day 8 after transplant and postmortem examinations showed the presence of cardiac lesions in all cases. In conclusion, neither graft-versus-host disease nor CyA are responsible for the development of the lesions present in the recipient's hearts.

The induction of the cardiac lesions in the recipient hearts is not specifically linked to renal allografts. Eight AO ($RT1^u$) male rats were primed intraperitoneally with 2×10^7 PVG ($RT1^c$) spleen cells (MHC plus Minor mismatch) and 5 PVG$RT1^u$ male rats were primed with 2×10^7 AO spleen cells (MHC-matched/Minor mismatched) on day 0. Controls were injected with saline. The animals were culled on days 8, 15 and 22 after sensitization and full postmortems performed. Histological examination of recipients' hearts showed that the lesions were already present on day 8 being most intense on days 14 and 22 after allosensitization in the MHC plus Minor mismatch situation. (Table 2) Repeat experiments with MHC-matched/Minor mismatched allosensitization produced similar results with the exception that on day 22 the intensity of the lesions was already subsiding. Overall, the severity of cardiac lesions induced by allosensitization with spleen cells was always less than the ones observed in the context of renal allograft rejection.

There is a temporal relationship between acute graft rejection and the development of the cardiac lesions. Ten DA ($RT1^{av1}$) male recipients received PVG ($RT1^c$) kidney allografts and were left with the right kidney in situ to maintain renal function. No immunosuppression was administered in the post-operative period. Animals were sacrificed on days 3, 7, 10, 16 and 23 after transplantation and full post-mortems performed. The intensity of the cardiac lesions were analyzed and scored (+ = minimal, + + = mild, + + + = moderate, + + + + = severe) by two different pathologists independently. Figure 1 demonstrates the direct relationship between the peak of unmodified graft rejection around days 7 and 10 after transplantation paralleled by the development of moderately severe parenchymal destruction in the recipients hearts. Saline-injected controls did not develop the cardiac lesions.

Involvement of other non-lymphoid organs. The recipients' own right kidneys that were either removed on

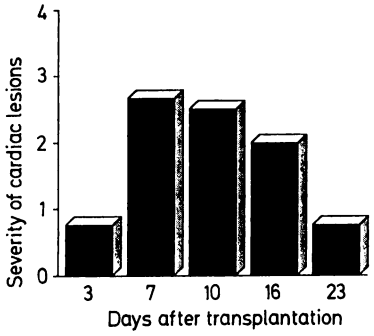

Fig. 1. Graph illustrating the temporal correlation between the intensity of the cardiac lesion and graft rejection

day 7 after transplantation or left in situ during the temporal relationship experiment were examined histologically. The findings included an increased mononuclear cell adhesion to vascular endothelium of veins, venules and peritubular capillaries. In places the cells were seen transmigrating the vessel wall and infiltrating the interstitium nearby. This latter finding, however, was infrequent and not accompanied by parenchymal damage. Preliminary observations in the recipients' lungs confirmed the presence of altered mononuclear cell trafficking, including increased leukocyte-endothelial cell adherence, transmigration and interstitial infiltration, very similar to the changes seen in rejecting lung allografts.

Discussion

The data presented in this paper suggested that the host response to allogeneic antigen was not confined to the allograft, and morphological abnormalities similar to rejection reaction could be seen in the host's own non-lymphoid organs. This response occurred in the context of both acute and chronic graft rejection and involved increased lymphocyte adherence and trafficking through a non-lymphoid organ, such as the recipient's own heart, with evidence of parenchymal damage. It was shown to be produced not only by the presence of a solid organ allograft like the kidney but also to a lesser extent by priming the recipient with allogeneic lymphocytes. The host response was seen in MHC plus non-MHC incompatibilities as well as in non-MHC disparities alone. Lastly, it was demonstrated that there was a temporal relationship between the peak of graft rejection and the development of the cardiac lesions.

The pathogenesis of these cardiac lesions is open to speculation. We showed that they were not due to graft-versus-host disease or Cyclosporin-A therapy. Other pathogenetic mechanisms that may be involved include upregulation of homing receptors/adhesion molecules secondary to the inflammatory response produced during graft rejection, breakdown of peripheral tolerance and the production of cross-reacting anti-endothelial antibodies.

Intense cellular reaction in host lymphoid tissues in response to untreated organ allografts has been previously reported [8]. However, our observation that recipient non-lymphoid organs, particularly the heart, could be involved during the process of graft rejection is unique. In lymphoid tissue, high endotheliac venules (HEV) cells lining the postcapillary venules control the nonrandom distribution of lymphocytes. The adhesion process between lymphocytes and HEV is mediated by lymphocyte homing receptors which show affinity for organ-specific endothelial ligands (vascular addressins) [3]. In non-lymphoid organs HEV are normally absent but ordinary endothelium can acquire HEV-like morphology and function when an inflammatory microenviroment is present [2]. Cytokines in chronic inflammatory sites can induce endothelial cells to develop HEV-like properties and promote lymphocyte-endothelial cell adhesion by enhancing the expression of organ-specific endothelial ligands. Lymphocytes bind to HEV in inflammed synovium via a recognition system that differs from homing receptors to lymphnode and mucosal HEV [1]. It is not known if the specificities observed are truly organ-specific or represent a general adhesive mechanism that is subject to regulation by inflammatory mediators. During acute renal allograft rejection the peritubular capillary endothelium obtains features similar to lymphnode HEV [10]. It has also been shown that the ligand responsible for the binding of lymphocytes to peritubular capillary is organ-specific and that the HEV in the kidney does not stain with a monoclonal antibody against rat lymphnode HEV. It seems that refined homing specificities allow the immune system to protect an organ from antigen-specific effector cells.

In addition to the tissue-specific homing receptor/ligand interaction it is now suggested that other families of adhesion molecules play an accessory role in lymphocyte-HEV adherence, in particular the integrins. They comprise the very late antigen (VLA) subfamily of integrins which function as receptors for extracellular matrix and the leukocyte integrins. There is some indication that extracellular matrix components have a co-mitogenic effect on T cells exposed to anti CD-3 antibody [13, 14]. Memory T cells express three to four times more VLA-4, VLA-5 and VLA-6 than do naive cells and bind more efficiently to fibronectin adn laminin [12]. Laminin has been shown to be a prominent heart cell surface protein [7]. It is possible that during the rejection reaction there is an enhanced expression of VLAs in circulating lymphocytes and this may promote transmigration of the lymphocyte with attachment to the surface laminin that normally surrounds both myocytes and capillaries in rat hearts [5]. It is possible that there may be cross-reactivity between glomerular basement membrane laminin and myocardial cell laminin.

The mechanisms of peripheral tolerance are not well understood. A general hypothesis is that whenever potentially self-reactive T cells in the periphery are isolated from their sources of "help" during antigen recognition these cells default to an anergic state [4]. If sufficient co-stimulation is provided, as in graft rejection, clonal anergy might be reversed and self-reactivity ensue. The pool of cytokines that are released during graft rejection could provide enough co-stimulatory factors to switch on anergic T cells in the periphery to respond to self tissues as if they were foreign. The production of cytokines upregulating the expression of MHC and adhesion molecules may lead to the generation of responses to other previously

"cryptic" determinant regions in non-lymphoid organs such as the heart during a systemic inflammatory event [14]. Increased levels of ligand could activate low-affinity T cells and memory T cells thus contributing to broadening the autoimmune response. The persistence of the antigenic source, as in the case of a chronically rejecting organ graft, may help to perpetuate this reactivity in vivo. Finally, it is possible that anti-endothelial antibodies produced to graft vascular endothelium may cross-react with recipient endothelial cell antigens to produce vasculitic lesions in addition to increased lymphocyte adherence and transmigration.

Our observations of lymphocyte infiltration and parenchymal damage in the non-lymphoid organs of an allograft recipient have not been reported previously. However it was not surprising that the rejection reaction, which is an intense inflammatory event, did indeed produce such systemic effects. We intend to conduct further experiments on the migratory behaviour of lymphoid cells in recipients bearing rejecting grafts in order to clarify the possible pathogenetic mechanisms involved in this systemic reaction.

Acknowledgements. We wish to thank Margaret McLeish for her expert technical help and Stephen P. Cobbold for immunological advice. We also thank C. A. P. E. S. of the Ministry of Education, Brasil and the East Anglian Regional Health Authority for the support of this work.

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