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*Graft* 2003; 6; 21

DOI: 10.1177/1522162802239754

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# Overlap between Alloimmunity and Autoimmunity in the Rat and Human: Evidence for Important Contributions for Dendritic and Regulatory Cells

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Alloimmunity and autoimmunity share a number of important afferent, effector, and regulatory immunological pathways. It is likely that they also share overlapping specificities that at least partially explain the ability of allograft rejection to trigger autoimmune responses, the increased susceptibility of patients with autoimmune diseases to allograft rejection, and a strikingly similar histopathologic appearance of acute and chronic rejection to some organ-specific autoimmune diseases. Clinical and experimental data are presented and reviewed, showing some overlapping specificities of effector and regulatory mechanisms during alloimmunity and autoimmunity. The speculation is made that tissue damage during acute rejection uncovers self-reactive T cell clones via the process of epitope spreading, perhaps mediated by heat-shock-protein-peptide complex-priming of recipient dendritic cells. Conversely, donor dendritic cells are suggested as a candidate for stimulating regulatory T cells that help maintain allograft acceptance in the periphery.

**Keywords:** autoimmunity; alloimmunity; immune regulation; dendritic cells

## Introduction

Although genetically unrelated humans share more than 99% of their DNA sequences, when an attempt is made to transplant organs or cells, the immune system recognizes the tiny differences that are translated into structurally disparate proteins. These small differences trigger graft versus host disease (GVHD) after bone marrow transplantation and rejection after solid organ transplantation. Eventually, however, GVHD and acute and chronic rejection immune reactions diversify and evolve to include humoral and cellular reactivity against antigens shared between the donor and recipient.

Examples include the development of autoantibodies and cell-mediated immunity directed against various common cytoplasmic, nuclear, and matrix proteins.<sup>1-13</sup> This occurs because either the shared antigens or the immune system, or both, is altered by the transplant procedure and subsequent immunosuppression.

If one focuses on the similarities between individuals and antigenic targets in rejection and autoimmune reactions, rejection might be thought of as a type of autoimmune disease. Conversely, if focus is placed on the tiny differences, autoimmune disorders might be thought of as "rejection" of self. This

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DOI: 10.1177/1522162802239754

**Table 1 | THE EFFECT OF PRETREATMENT ON PROTECTION FROM THE CLINICAL MANIFESTATIONS, MORBIDITY, AND MORTALITY ASSOCIATED WITH HgCl<sub>2</sub>-INDUCED AUTOIMMUNITY**

GROUP	PRETREATMENT	n	CLINICAL MANIFESTATIONS AFTER HgCl <sub>2</sub> <sup>a</sup>				WEIGHT LOSS (%) <sup>b</sup>	DEATHS AT DAY 30	
			NONE	MINOR	MODERATE	SEVERE		n	%
I	Negative control	5	5	—	—	—	—	0	0
II	Positive control	15	—	2	6	7	22	7	47
III	FK	6	2	3	1	—	21	2	33
IV	BN bone marrow plus FK506	11	2	5	2	2	20	1 <sup>c</sup>	9
V	LEW bone marrow plus FK506	13	11 <sup>d</sup>	2	—	—	16 <sup>e</sup>	0 <sup>e</sup>	0

Although pretreatment with FK506 with or without syngeneic bone marrow (Groups III and IV) conferred some protection from HgCl<sub>2</sub> autoimmunity, animals treated with allogeneic bone marrow infusion plus transient FK506 at day -100 (Group V) had the most effective protection from the mortality and clinical features associated with the disease. All significant differences are indicated, and statistical analyses used are indicated in footnotes. Reproduced from Delaney et al.<sup>15</sup>

- a. Erythema affecting paws, chest, and perioral area.  
 b. Maximum mean weight change, as a percentage of initial body weight.  
 c.  $P < 0.01$  versus Group II, chi-square test.  
 d.  $P < 0.005$  versus Group II and IV, chi-square test.  
 e.  $P < 0.005$  versus Group II, III, and IV, Student's *t* test.

play of words highlights the obvious similarities between alloimmunity and autoimmunity: A specific organ is no longer "tolerated" by the immune system. In allografts, lack of central deletion and/or significant cross-reactivity between self-major histocompatibility complex (MHC)/nominal antigen complexes and allo-MHC/nominal antigen complexes is thought to underlie the brisk immune early response to allografts, at least the initial phases. Conversely, loss of peripheral regulatory control of naturally occurring, self-reactive clones is thought to greatly contribute to pathogenic autoimmunity. The work presented here suggests that peripheral mechanisms also significantly contribute to control of alloimmunity, especially in the regulation of long-term reactivity or nonreactivity to organ allografts<sup>14</sup> or chronic rejection.

#### **Experimental Evidence for Common Regulatory and Effector Pathways in Alloimmunity and Autoimmune Reactions in Brown Norway (BN) Rats**

Treatment of naive BN rats with 5 injections of HgCl<sub>2</sub> consistently induces autoimmune disease,

manifest as lymphocytic infiltration and damage to liver, kidney, and skin and autoantibody production.<sup>15-19</sup> Peak IgG autoantibody production against laminin occurs between day 12 and day 14, and by day 20, the animals develop autoimmune lymphocytic dermatitis, interstitial nephritis, and hepatitis.<sup>18,20-22</sup> In our experience, these manifestations spontaneously resolve in 55% of the BN rats that survive the acute syndrome, and the survivors are resistant to another HgCl<sub>2</sub> challenge.<sup>15</sup>

In 1996, we showed that induction of donor-specific allograft tolerance using an allogeneic bone marrow infusion and transient immunosuppression simultaneously protects BN rats from the humoral and cellular-immune-mediated manifestations of HgCl<sub>2</sub> autoimmunity (see Table 1).<sup>15</sup> Just like the animals that became resistant to HgCl<sub>2</sub> injections because they recovered from the disease,<sup>18,20,22,23</sup> BN rats previously challenged with an allograft showed no mortality, and significantly less autoantibody production, autoimmune dermatitis, interstitial nephritis, and hepatitis when treated with HgCl<sub>2</sub> injections.<sup>15</sup> The protection from autoimmunity was closely associated with allogeneic mi-

**Table 2 | THE EFFECT OF HgCl<sub>2</sub>-INDUCED AUTOIMMUNITY ON SUBSEQUENT CHALLENGE CARDIAC ALLOGRAFT SURVIVAL**

GROUP	TREATMENT	DAY OF TRANSPLANT <sup>a</sup>	n	GRAFT SURVIVAL (DAYS)			
				M	SD	P vs. A <sup>b</sup>	INDIVIDUAL RESULTS
A	Nil		9	7	0.7		8, 7, 8, 7, 7, 6, 7, 6, 7
B	H <sub>2</sub> O	30	3	7.3	0.5	0.36	8, 7, 7
C	HgCl <sub>2</sub>	7	6	9.3	3.3	0.08	8, 4, 8, 10, 11, 15
D	HgCl <sub>2</sub>	14	8	2.1	3.2	< 0.005	0, 0, 0, 3, 3, 3, 4, 4,
E	HgCl <sub>2</sub>	30	5	10	0.6	< 0.001	10, 10, 10, 9, 11
F	HgCl <sub>2</sub>	> 60	3	9.7	0.9	< 0.001	9, 9, 11 <sup>c</sup>

Fully allogeneic LEW hearts were heterotopically transplanted in the abdomens of BN rats. Graft survival was evaluated by blinded abdominal palpation and confirmed by histological analysis. Transplants were carried out in naive BN rats (Group A) and BN treated with carrier only (Group B) as a control.

a. Days after first injection with HgCl<sub>2</sub>.

b. Student's *t* test.

c. = Day 90 animal.

crochimerism and an activated or upregulated baseline immune status that included increased class II expression on T cells, increased numbers of B cells, and increased baseline lymphocyte proliferation but reduced proliferation during autologous mixed lymphocyte response reactions.<sup>15</sup>

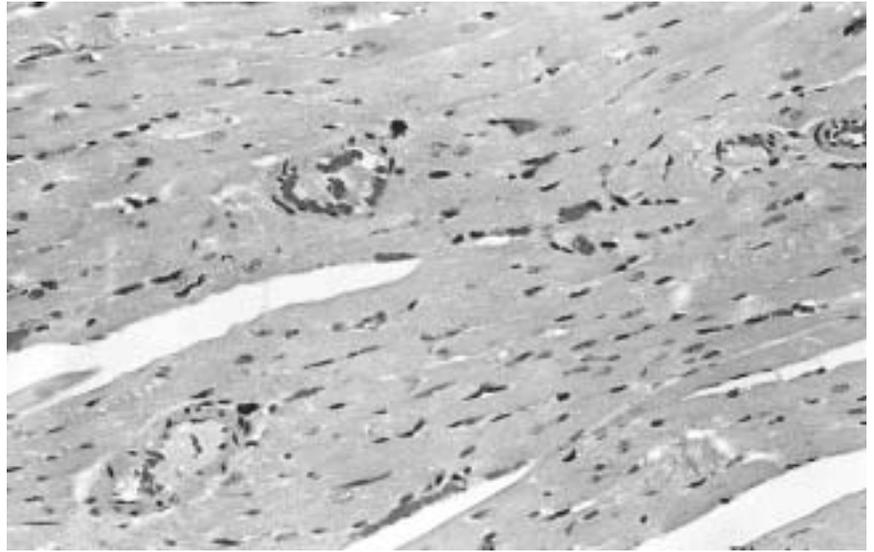
On the basis of this evidence, we suggested that alloimmune- and autoimmune-mediated injury share active regulatory circuits<sup>15,24,25</sup> that require ongoing immune stimulation for their maintenance.<sup>15,25</sup> Other evidence supporting this assertion in the HgCl<sub>2</sub> autoimmunity model includes amelioration of the disease by treatment with IL-10<sup>26</sup> and TGF- $\beta$ <sup>27</sup> during its peak and upregulation of IL-12<sup>28</sup> and increased levels of  $\gamma$ -IFN mRNA and circulating protein<sup>29</sup> during the regulatory phase. In addition, the regulatory phase can be partially blocked by administration of anti-IL-2 receptor antibodies.<sup>30</sup> One possible explanation for a common regulatory circuit between alloimmunity and autoimmunity is that the circuit is triggered by overlapping effector pathways.

For example, fully allogeneic Lewis heart allografts are consistently rejected by normal BN rats within 8 days of transplantation (Table 2, Group A). However, LEW heart transplants carried out during the peak of serological HgCl<sub>2</sub>-induced disease (day 14, Group D) cause accelerated allograft failure, with findings consistent with antibody-mediated rejection with increased deposition of an-

tibody and evidence of antibody-mediated damage (Fig. 1). In contrast, LEW heart allografts placed in BN animals during the convalescent phase of HgCl<sub>2</sub>-autoimmunity (day 30, Group E) revealed a slight but significant prolongation of graft survival, an effect maintained for at least 90 days after commencing HgCl<sub>2</sub> (Group F). These findings suggest that many of the antigens targeted during rejection and autoimmunity are similar and likely to be autoantigens exposed because of tissue injury from preservation injury or some other perioperative insult or "danger" signal,<sup>31</sup> or an initial allogeneic reaction that exposes common autoantigens. However, the regulation exerted by alloimmunity over HgCl<sub>2</sub>-induced autoimmunity was much stronger than the other way around.

To determine whether the protection or accelerated rejection was antibody mediated, naive BN animals underwent allogeneic heart transplant and simultaneously received 1 ml of serum from animals with HgCl<sub>2</sub>-induced autoimmune disease. Serum from animals with peak serological disease (day 14 after starting HgCl<sub>2</sub>) reduced mean graft survival, although this did not reach statistical significance because of a large variability between animals. In contrast, transfer of serum from convalescent, day 30 animals had no effect on graft survival. These equivocal results suggested that antibody-mediated injury may contribute to the effector pathways but

A



B

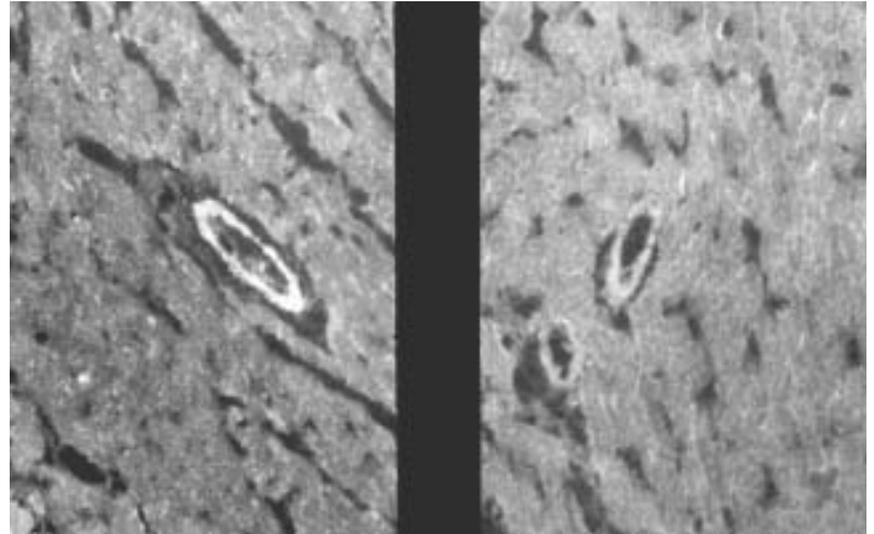


Figure 1. Light microscopic and immunohistochemical examination of grafts placed in animals at the acute serological phase (day 14) of HgCl<sub>2</sub> disease. (A) The grafts were rejected at an accelerated pace (Table 2, group D), and histological findings consistent with humoral rejection were seen, including platelet aggregation, intra-arterial thrombosis, and mast cell degranulation. (B) Immunofluorescence examination showed antibody deposition on the endothelial surface in rats treated with day 14 serum but not in those given naive BN serum.

that cellular mechanisms were likely responsible for the regulatory control.

To examine the specificity of antibodies contained in day 14 autoantibody serum, naive normal LEW heart sections were incubated with serum from normal BN rats or animals treated for 14 days with

HgCl<sub>2</sub>. Freshly harvested LEW hearts and LEW allografts that had been transplanted and reperfused in naive BN recipients for 2 h (to allow reperfusion-induced injury to occur) were used as targets in this immunohistochemical assay. As evident in Figure 2, there was almost no deposition of immunoglob-

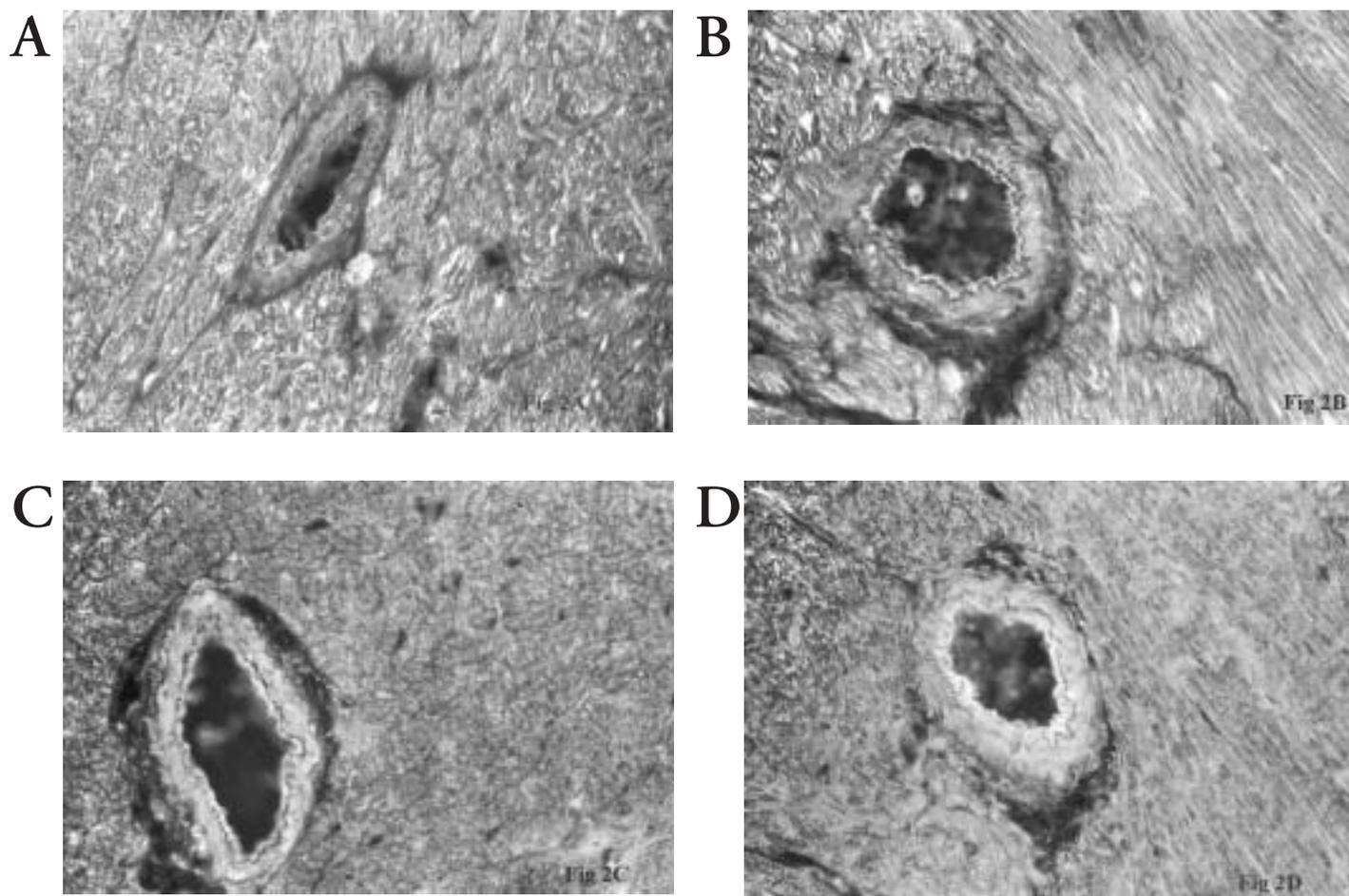


Figure 2. Sections of normal and reperfused donor cardiac allografts were incubated with normal and acute phase BN serum and examined blindly for specificity of IgG and IgM isotype autoantibodies present in sera of animals with HgCl<sub>2</sub> autoimmunity. The results for IgM deposition are shown, but similar results were obtained for IgG. (A) Normal LEW heart treated with naive BN serum showed no deposition of IgM antibodies. (B) However, a reperfused LEW heart showed mild deposition of naive BN serum on the arterial endothelial cells and very weakly on myocytes. (C) There were heavy deposits of antibody on the arterial endothelium and cardiac myocytes when a normal LEW heart was treated with day 14 autoimmune serum. (D) The heaviest antibody deposits were detected in reperfused LEW hearts treated with day 14 autoimmune serum. Furthermore, sections from allografts reperfused in the recipient for 2 h revealed a consistent, albeit modest, increase in antibody deposition from normal and acute phase sera when examined blindly.

ulin from normal BN serum on normal LEW heart sections. Although deposition of antibodies from normal BN serum on reperfused sections was minimal, the deposits were significantly greater than that on normal sections. Moreover, acute phase day 14 serum showed heavy deposits of IgG and IgM isotype antibodies on normal and reperfused LEW heart sections. The antibodies were deposited most prominently on endothelial cell surfaces but were also present on the cardiac myocytes and vascular

smooth muscle cells. These findings suggested that perioperative trauma likely uncovers autoantigens in allografts that are targeted by autoantibodies generated during rejection and autoimmune reactions. Further studies were conducted to determine what these autoantigens might be.

We started by examining antigens known to be targeted by HgCl<sub>2</sub>-induced autoimmunity, as well as with heat shock proteins that are known to be associated with autoantigen presentation through se-

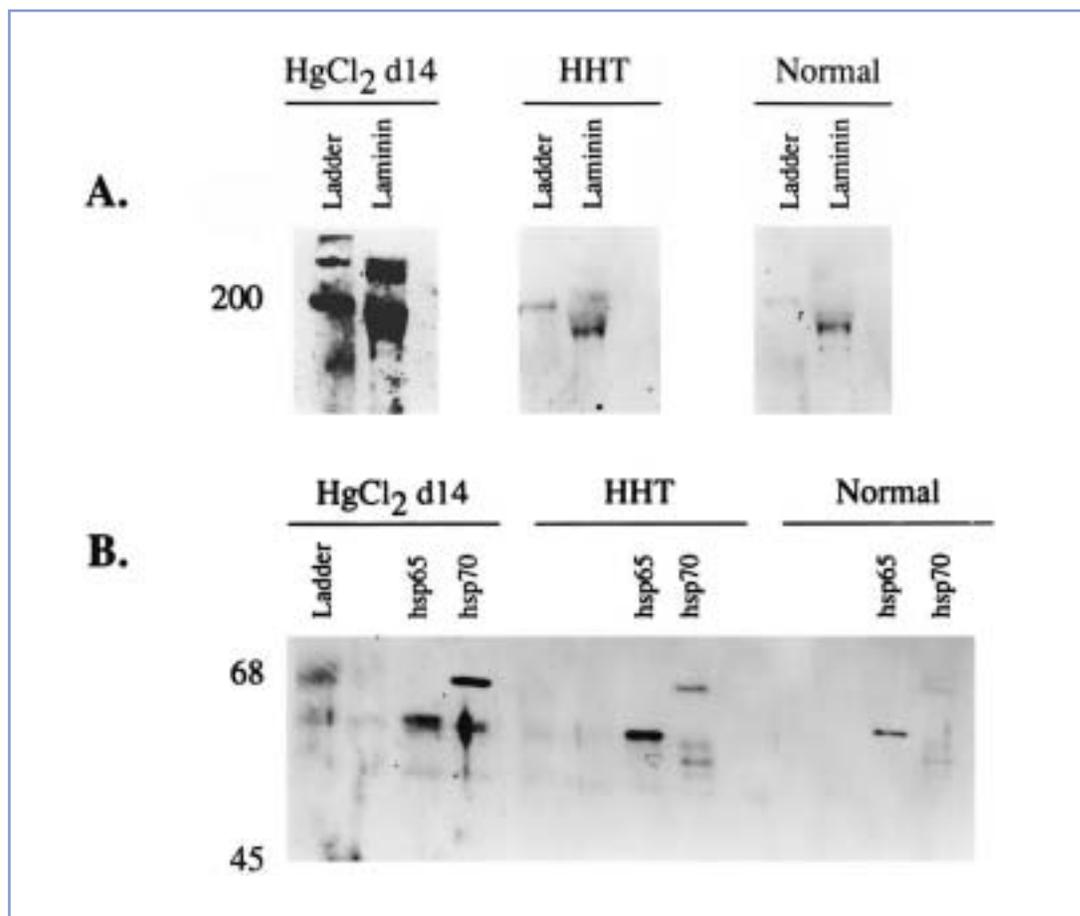


Figure 3. Immunoblots were performed to determine the specificity of antibodies contained in serum from normal BN rats, animals at day 14 of HgCl<sub>2</sub> disease, and naive BN rats that had just rejected cardiac allografts from an LEW donor. Laminin, hsp65, and hsp70 were run in gels, blotted onto nylon membranes, and incubated with the previously mentioned sera. Panel A shows the increased amount of antibodies recognizing hsp65 and hsp70 in animals after heart allograft rejection and particularly during HgCl<sub>2</sub> disease. Similarly, increased amounts of laminin-recognizing antibodies are present in sera from these animals (B).

questration or chaperoning of intracellular proteins and activation of dendritic cells.<sup>32</sup> Serum samples were collected from normal BN rats, BN rats 14 days after commencing HgCl<sub>2</sub> treatment, and naive BN rats that had completely rejected LEW cardiac allografts. Immunoblot analysis (Fig. 3) revealed that trace levels of antibodies to each antigen were present in normal sera (panels A and B). Animals that had rejected a cardiac allograft showed increased levels of antibodies to all 3 antigens tested, whereas animals at the acute phase of HgCl<sub>2</sub> disease had greatly elevated levels of all antibodies tested but especially those against laminin. These re-

sults suggest that damage that occurs either during rejection or autoimmunity exposed common antigens that are recognized by the immune system.

Experiments were also carried out to determine whether splenocytes from animals at different phases of HgCl<sub>2</sub>-induced autoimmune disease could affect the course of allograft rejection (Table 3). Approximately 1 splenic equivalent (1.25 X 10<sup>8</sup> cells) was intravenously injected into naive BN animals at the time of transplantation. A modest but statistically significant prolongation of allograft survival was seen with the transfer of day 30 (convalescent phase) splenocytes, which prolonged heterotopic

**Table 3 | DEMONSTRATION OF THE EFFECT OF ADOPTIVE CELL TRANSFER OF UNFRACTIONATED SPLENCYTES (FROM ANIMALS WITH HgCl<sub>2</sub> DISEASE) ON LEW TO NAIVE BN RAT CARDIAC ALLOGRAFT SURVIVAL**

GROUP	ADOPTIVE TRANSFER <sup>a</sup>	N	GRAFT SURVIVAL (DAYS)			INDIVIDUAL RESULTS
			<i>M</i>	<i>SD</i>	<i>P</i> vs. J <sup>b</sup>	
J	Normal BN	3	7.7	0.5		8, 7, 8
K	Day 14 HgCl <sub>2</sub> <sup>c</sup>	3	6.7	0.5	0.10	6, 7, 7
L	Day 30 HgCl <sub>2</sub> <sup>c</sup>	3	11.7	1.2	0.01	10, 12, 13
M	Day 90 HgCl <sub>2</sub> <sup>c</sup>	3	8	1.4	0.77	7, 7, 10

a. Adoptive transfer of  $1.25 \times 10^6$  splenocytes from animals treated with HgCl<sub>2</sub>.

b. Student's *t* test.

c. Splenocytes from animals 14, 30, or 90 days after HgCl<sub>2</sub>.

heart transplant survival to 11.3 days (Group L, Table 3). This effect was similar to that seen in vivo (Group E, Table 2). Heterotopic heart transplantation into animals up to 90 days after HgCl<sub>2</sub> injections showed a protective effect (Table 2, Group F); transfer of splenocytes from animals this far after autoimmunity did not have sufficient regulatory ability to reproduce the prolongation of graft survival (Group M). This may be because ongoing activation is needed and all of the mercury has been removed by this time. Studies to determine whether the ability to prolong cardiac allograft survival could be attributed to either the CD4 or CD8 subset of lymphocytes were inconclusive.

### Clinical Evidence of the Overlap between Regulatory and Effector Pathways in Alloimmunity on Autoimmunity

Solid organ transplantation and the associated immunosuppression generally exert a beneficial influence on autoimmunity. This becomes evident when replacing the organ targeted by an autoimmune reaction (e.g., the liver in autoimmune hepatitis or pancreas in diabetes mellitus). For example, recurrent autoimmune hepatitis and primary biliary cirrhosis recur in up to 50% of recipients by 5 years after liver transplantation, but reappearance of these diseases is not inevitable or universal, and when they do recur, they are usually less aggressive than before transplantation.<sup>33-42</sup> Lupus nephritis and other autoimmune renal diseases occur in a minority of kidney allograft recipients.<sup>43</sup> The same is true

of recurrent diabetes mellitus (preferential destruction of islets): It can occur after MHC-mismatched whole pancreas transplantation,<sup>44</sup> but it is distinctly uncommon. In contrast, recurrent autoimmunity and alloimmunity are thought to significantly contribute to the chronic destruction of islets allografts in mismatched isolated islet allografts.<sup>45-50</sup>

Less commonly, solid organ transplantation can worsen autoimmunity or trigger it in susceptible naive individuals. For example, liver transplantation and the associated immunosuppression can precipitate a syndrome that strongly resembles (or actually is, depending on one's perspective), autoimmune hepatitis. Children seem to be especially susceptible to this complication.<sup>36,37,51-53</sup> Although other organ allografts do not seem to provoke a similar attack of autoimmunity, there is substantial evidence showing that rejection and GVHD, especially chronic rejection and chronic GVHD, involve autoimmune effector pathways.<sup>1-5,13,54-58</sup> This is most likely attributable to the phenomenon of "epitope spreading."<sup>59,60</sup> Chronic injury continually uncovers new determinants from the damaged tissue that are processed and indirectly presented. This causes various donor MHC and tissue-specific or autoantigen reactive clones to appear in the recipient T cell repertoire. In fact, it is tempting to speculate that this phenomenon accounts for some of the striking similarities between the histopathologic appearance of autoimmune-mediated tissue injury and chronic rejection, such as the development of organized lymphoid nodules within the tissues.<sup>61,62</sup>

### Possible Mechanisms to Explain the Overlaps

Disparity in the incidence of recurrent autoimmune diabetes mellitus between whole pancreas and islets allografts has been attributed to passenger leukocytes emigrating from whole pancreas grafts.<sup>63,64</sup> The explanation is similar to the hypothesis used to explain drug-free allograft acceptance after short-term immunosuppression in experimental animals and humans.<sup>24,65-67</sup> We proposed a similar mechanism for the link between resistance to HgCl<sub>2</sub>-induced autoimmunity in allograft recipients and suggested that donor antigen-presenting or dendritic cells (DC) directly stimulated recipient regulatory T cells.<sup>15,24,25,67</sup> Subsequent studies have indeed shown active regulation of alloreactive cells in long-term allograft survivors<sup>68</sup> using the trans vivo delayed-type hypersensitivity model.<sup>69</sup>

When this hypothesis was proffered during the early 1990s, however, DC were thought to contribute only to direct alloantigen presentation. It was near heresy to suggest that they might contribute to tolerogenesis. In addition, suppressor or regulatory cell research had largely gone out of favor and was generally avoided in discussions of transplantation immunology.<sup>70,71</sup> Recent studies, however, have identified regulatory DC<sup>72</sup> and attest to the validity of CD4<sup>+</sup>/CD25<sup>+</sup> and other suppressor or regulatory cell phenotypes in both alloimmunity and autoimmunity (See *Immunological Reviews*, Vol. 182, 2001).

Regulatory T cells have been described in various experimental systems, and almost every lymphocyte subpopulation has been shown to contain regulatory cells or properties.<sup>14,70,73-78</sup> However, there is only one lymphocyte that so far has been shown to be apparently independent from the experimental system used. This is the naturally activated CD25<sup>+</sup> CD4<sup>+</sup> T cell subset that is present in the thymus and periphery of mice, rats, and humans.<sup>14,70,73-78</sup> These cells are generated in the thymus under the influence of thymic epithelium and require interaction with MHC class II molecules for their release.<sup>14,70,73-78</sup>

Studies show that thymic epithelial-selected regulatory cells can recruit nontolerant, tissue-reactive CD4<sup>+</sup> and CD8<sup>+</sup> T cells into similar regulatory functions on exposure to specific antigens.<sup>14,70,73-78</sup> Apparently, regulatory T cells develop in the thy-

mus independent of antigens expressed in the periphery, but the composition of the host determines whether these cells will survive in the periphery. For example, peripheral interactions of regulatory cells with antigen are mandatory for tissue-specific tolerance. Athyroid rats obtained by in utero treatment of pregnant animals with radioactive iodine contain thyroid-specific thymic regulatory CD4<sup>+</sup> T cells; however, the protective activity is absent from the periphery.<sup>79</sup> These findings suggest that regulatory cells must receive survival signals in the periphery by interacting with specific antigens.

Several investigators suggest that the best candidate for peripheral maintenance of regulatory cell survival is dendritic cells.<sup>14,70,73-78</sup> Zelenika et al.<sup>14</sup> proposed a local model of the immune regulation in transplantation tolerance. They suggested that indirect presentation of donor alloantigen by recipient antigen-presenting cells might stimulate the regulatory cells and recruit mast cells in a protective fashion. By increasing tryptophan utilization and serotonin production, the mast cells enhance local TGF- $\beta$  production, which in turn is immunosuppressive. The important point is that the regulating antigen must be present in the periphery and must have access to the lymphoid tissues presumably via the mobile antigen-presenting cells.

In light of the above observations, it is likely that several mechanisms probably contribute to the cross-reaction and regulation between alloimmunity and autoimmunity. However, it is our opinion that the role of donor DC and regulatory or so-called internal image cells deserve particular attention.<sup>25</sup> In all of our experimental transplantation models, the best long-term, pathology-free allograft survival is associated with persistence of donor antigen-presenting cells or DC in the allograft and recipient lymphoid tissue.<sup>24,80,81</sup> The association is so strong, it led us to our proposal of the immunolymphatic theory of chronic rejection or tolerance induction.<sup>24</sup> The core premise of this hypothesis is that long-term, pathology-free survival requires an intact organ allograft-to-draining lymph node axis and organ-based donor and recipient DC traffic to the lymph node for (1) presentation of environmental pathogens and (2) maintenance of tolerance by direct allogeneic stimulation

of regulatory cells. In contrast, absence of the mobile donor and recipient DC traffic to the regional lymph nodes eventually leads to chronic rejection. The recently uncovered aspects of regulatory cell physiology, discussed above, are in accordance with this hypothesis (reviewed in *Immunological Reviews*, Vol. 182, 2001), but two aspects require further study. In allografts, it is uncertain whether the regulation is specific for MHC or tissue antigens, or both. Also, it is not known whether immature or regulatory donor dendritic cells can directly stimulate recipient regulatory T cells. Our observations would suggest that they can,<sup>24</sup> but this conjecture is purely speculative and must be rigorously tested experimentally.

A particularly interesting group of intracellular molecules, heat shock proteins (hsp), are likely to be an important link between alloimmunity and autoimmunity. These ubiquitous proteins<sup>32,82</sup> have been implicated in a variety of immune responses, including self-tolerance, the loss of self-tolerance, and protection against infection.<sup>83</sup> Hsp released by parenchymal cells injured during acute allograft rejection give rise to clones of hsp-reactive lymphocytes that can be isolated from rejecting human and animal allografts.<sup>9,84,85</sup> These hsp also carry with them fragments of other donor intracellular proteins, and the hsp-peptide complexes readily prime or activate allogeneic or synergistic DC,<sup>32</sup> which express specific hsp receptors. Thus, reactivity toward some of these non-MHC molecules may account for at least some of the large number of allograft-infiltrating cells that are known to be unreactive with donor-MHC.<sup>86</sup>

After the initial donor-specific attack on the allograft, we believe that a strong TH1-type and chemokine-rich response, typical of severe acute rejection, upregulates or exposes a large variety of non-donor-specific antigens such as the hsp family, laminin, and fibronectin on the allograft, which are shuttled to recipient DC via an hsp-peptide complex. These indirectly presented antigens subsequently become targets that are recognized by potentially autoreactive recipient lymphocytes and thus contribute to allograft rejection.

In the HgCl<sub>2</sub> experimental autoimmunity model, recovery from autoimmune disease automatically triggers a regulatory response toward a panel of au-

toantigens. When these antigens are exposed and antigen-reactive lymphocytes are activated during subsequent allograft rejection, the response is muted by the presence of the autoregulatory networks. Thus, a proportion of regulatory cells induced by HgCl<sub>2</sub> autoimmunity are likely directed toward hsp and extracellular matrix-reactive cells, and these regulatory cells partially protect the allograft from nonspecific components of rejection.

The above observations suggest that the early allospecific reaction favors a self-non-self discriminatory function of the immune system.<sup>87</sup> This phase is easily treated with increased immunosuppression. In contrast, late alloreactivity or chronic rejection favors a danger model<sup>31</sup> of immune responsiveness, which is more difficult to treat with increased immunosuppression, perhaps because of multiple specificities recognized. These conjectures are supported by the prevalent observation in all solid organ allografts that chronic rejection is almost invariably preceded by severe or persistent acute rejection episodes.<sup>88</sup> We would contend that the allograft damage from acute rejection increases exposure autoantigens and consequently facilitates epitope spreading, perhaps through hsp. If this were true, avoidance of acute rejection would seem to be the most effective therapy for chronic rejection.<sup>89,90</sup>

The approach of avoiding acute rejection with calcineurin inhibitors (or blocking signal 1 and 2 from DC) has been criticized, however, because potent immunosuppression is thought to inhibit activation-induced apoptosis and clonal deletion of donor reactive cells<sup>91,92</sup> which are thought to be necessary for tolerance induction. In fact, it has been suggested that the immunosuppression can actually cause chronic rejection. Thus, there are 2 conflicting approaches to immunosuppression after transplantation: (1) low immunosuppression and promotion of activation-induced apoptosis or (2) high-dose immunosuppression and avoidance of acute rejection and exposure of autoantigens and epitope spreading. The former approach is attractive because the adverse side effects of immunosuppression can be avoided. However, it remains to be determined whether this approach can be successfully implemented without increasing the incidence of chronic rejection.

## Acknowledgments

This work was supported by grants NIH RO1 DK49615-01A1 and RO1 AI38899-01A2.

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