MHC Class II Presenting Cells Are Necessary for the Induction of Intrathymic Tolerance

John A. Goss, M.D., Yuji Nakafusa, M.D., and M. Wayne Flye, M.D., Ph.D.

From the Department of Surgery, Washington University School of Medicine, St. Louis, Missouri

Objective

This study determined the form of cellular donor MHC alloantigen necessary for the induction of intrathymic tolerance.

Background

The authors have achieved indefinite donor-specific tolerance, to a fully MHC-disparate rat heterotopic cardiac allograft, after the pretransplant intrathymic injection of unfractionated donor splenocytes and a single injection of rabbit anti-rat lymphocyte serum (ALS), without subsequent immunosuppression.

Methods

Male 4–12-week-old Buffalo (RT1^b) rats underwent an intrathymic injection of either fractionated Lewis (RT1^1) red blood cells (purified by Ficoll gradient) or T lymphocytes (purified by nylon wool column and plastic adherence), both of which express only MHC class I alloantigens, or B lymphocytes, macrophages, and dendritic cells (purified by plastic adherence) which express both MHC class I and class II alloantigens. At the completion of alloantigen injection the Buffalo recipient rats were given 1 ml of ALS intraperitoneally. Twenty-one days later a heterotopic Lewis heart was transplanted.

Results

The intrathymic injection of the fractions of Lewis MHC class I and class II expressing B lymphocytes, macrophages, and dendritic cells induced a donor-specific tolerance that resulted in indefinite Lewis cardiac allograft survival (MST > 125 days) in all recipients without further immunosuppression, whereas groups receiving MHC class I expressing red blood cell or T lymphocyte injections plus ALS rejected Lewis cardiac allografts with a MST of 7.3 and 16.5 days, respectively, thus indicating that the MHC class II expressing cell is necessary for the induction of intrathymic tolerance. Buffalo recipients with a long-term surviving Lewis cardiac allograft, after Lewis MHC class II expressing cells were still able to reject a third-party heterotopic ACI (RT1^a) cardiac allograft in normal time (MST = 7.0 days), but did not reject a second Lewis cardiac allograft (MST > 100 days). Additionally, the intrathymic injection of MHC class II expressing cells resulted in decreased Interleukin-2 (IL-2) production and an 80% decrease in *in vitro* donor-specific cell mediated cytotoxicity, whereas the cytolytic response to a third party was unaltered.

Conclusion

Donor MHC class II, and not class I, expressing cells are the cells in donor splenocytes, injected intrathymically, responsible for the development of donor-specific allograft tolerance.

Organ transplantation continues to be the only effective treatment for patients with end-stage isolated organ failure. Allograft rejection, initially recognized by Medawar[1] to be the principal barrier to success of this therapeutic approach, involves the recruitment and activation of CD4^{+} helper T lymphocytes, and the recognition and lysis of foreign cells by alloreactive CD8^{+} cytotoxic T lymphocytes (CTL).[2] Recent technical advances in solid organ transplantation and pharmacologic advances in nonspecific immunosuppression have significantly improved the results of kidney, liver, heart, lung, pancreas, and small bowel allograft transplantation over the past decade.[3-5] However, the long-term use of systemic immunosuppression often results in drug toxicity that continues to be a major cause of patient morbidity and mortality.[6,7] These complications could be avoided if a state of donor-specific unresponsiveness could be achieved without subsequent immunosuppression.

The normal maturation of T lymphocytes involves a series of well-characterized phenotypic changes as stem cells migrate from the outer cortex of the thymus to the medulla. The interaction of the α/β T-cell antigen receptor (TCR) with major histocompatibility complex (MHC)-encoded molecules on thymic stroma and resident bone marrow-derived cells play opposite roles within this developmental sequence. Maturing thymocytes that bear TCR with specificity for self-peptides and self-MHC molecules react with bone marrow-derived antigen-presenting cells and are deleted by a process called negative selection to result in self-tolerance.[8,9] However, the positive selection of reactive T lymphocytes by bone marrow-derived cells is dependent on recognition of self-MHC molecules[10,11] and self-peptides on thymic stromal cells.[12] What dictates the selection by these diametrically opposed events presently is unclear. It is also unknown how immature thymocytes recognize self-MHC molecules in the absence of foreign antigens, while mature T lymphocyte activation is dependent on the corecognition of both the MHC molecule and foreign peptide antigen.

Shimonkevitz and Bevan[13] previously demonstrated that the transfer of semiallogeneic CD4^{-} CD8^{-} thymocytes into the thymus of irradiated mice resulted in a transient state of chimerism in host spleen, thymus, and lymph nodes. Our laboratory has achieved indefinite survival of donor-specific, fully MHC-disparate cardiac allografts, after the pretransplant intrathymic injection of

unfractionated donor splenocytes and a simultaneous single intraperitoneal injection of rabbit anti-rat lymphocyte serum (ALS), while donor alloantigen injections at other sites did not prolong allograft survival.[14,15] Additionally, we have demonstrated an associated marked decrease in donor-specific cytotoxic T-lymphocyte precursor frequency (pCTL) without alteration in the peripheral CD8^{+} T lymphocyte phenotypic frequencies.[15] This study demonstrates that the form of donor MHC alloantigen placed in the thymus is critical for the induction of this donor-specific tolerance and allograft acceptance.

MATERIALS AND METHODS

Animals

Male Buffalo (RT1[b]), Lewis (RT1[l]), and ACI (RT1[a]) rats were used at 4–12 weeks of age (approximate weights 100–175 g), and were cared for according to specific NIH guidelines. Animals were provided a nutritionally balanced rodent diet (Purina Mills, St. Louis, MO) and water *ad libitum*.

Preparation and Purification of Donor Cellular Alloantigen

Erythrocytes

Lewis red blood cells were isolated as described by Wood et al.[16] Blood was collected into citrate-phosphate dextrose anticoagulant or heparin by aortic puncture and diluted 1:4 with isotonic saline before centrifuging at 300g for 10 minutes at 4 C. The supernatant and buffy coat were removed, the erythrocyte pellet was resuspended in 40 ml of saline, and the process was repeated. The erythrocytes were then resuspended in 20 ml of saline, overlaid on Ficoll-Hypaque (Litton Bionetics, Kensington, MD) and centrifuged at 1000g for 30 minutes at room temperature. After removing the lymphocytes, the erythrocytes were additionally purified by washing three times, and the Ficoll separation was repeated; 25×10^6 purified Lewis red blood cells (RBC) were then resuspended in a volume of 75 μl of saline and were used for intrathymic injection into a naive Buffalo recipient.

T Lymphocytes

A spleen cell suspension was prepared by passing the excised Lewis spleen through a 60 μm brass screen and the erythrocytes lysed by incubating for 3–5 minutes with Tris-ammonium chloride (0.83%) at 37 C. The resulting splenocyte population was washed three times with normal saline and resuspended in saline. The spleen cell population was then passed over a nylon wool column (0.6 g/10 cc), and the nonadherent population was

then incubated at 37 C for 1 hour in 100-mm plastic petri dishes (Costar, Cambridge, MA). Nonadherent cells were removed by three consecutive washes with warm saline and again passed over a nylon wool column to complete removal of adherent cells. The resultant T-lymphocyte population was then resuspended in saline at a concentration of 25×10^6 cells/75 μl for intrathymic injection.

B Lymphocytes, Macrophages, and Dendritic Cells

After the non-adherent Lewis spleen cells had been removed by three consecutive washes with warm saline, the adherent cell population was liberated by gentle pipetting and washing with 4 C saline. The harvested adherent spleen cell population was then counted and 25 $\times 10^6$ cells were placed in 75 μl saline for intrathymic injection.

Injection of Cellular Alloantigen into Recipient Thymus

Under Ketamine (0.1 ml IP/100 g) and Metafane inhalation anesthesia, the thymus of the male Buffalo rat was exposed through a partial upper median sternotomy. Direct visualization with the aid of a x12.5 operating microscope (model OPM212T, Jenoptik, Germany) allowed the injection of 25×10^6 Lewis cell fractions equally into both lobes of the thymus. The recipient rat then received 1 ml of rabbit anti-rat lymphocyte serum (ALS, Accurate Chemical Corp., Westbury, NY) intraperitoneally. Control Buffalo recipients either received no pretransplant treatment, the IP administration of 1 ml of ALS only without donor Lewis intrathymic alloantigen injection, or the intrathymic injection of 25×10^6 unfractionated Lewis spleen cells only without the IP injection of ALS.

Cardiac Transplantation

Heterotopic abdominal cardiac allografts were completed using the modified techniques of Ono and Lindsey.[17] The Lewis donor aorta and pulmonary artery were anastomosed end-to-side to the Buffalo recipient's abdominal aorta and inferior vena cava, respectively. Graft function was assessed by daily palpation and rejection was defined as cessation of cardiac contraction and was confirmed by histologic evaluation.

Experimental Design

Age-matched Buffalo recipients were randomly assigned to one of six experimental groups (Table 1). Group 1 received no pretransplant treatment before Lewis cardiac transplantation. Group 2 received rabbit anti-rat lymphocyte serum IP only, to deplete mature peripheral T lymphocytes, whereas group 3 underwent an intrathymic injection of 25×10^6 unfractionated Lewis spleen cells without the administration of IP ALS 21 days before heterotopic Lewis cardiac transplantation. It had been determined that by 21 days after receiving 1 ml of ALS the Buffalo rat had repopulated its peripheral T-lymphocyte population based on flow cytometry of CD4 and CD8 T-lymphocyte differentiation markers and a normal rejection time of cardiac (mean survival time, MST 7.0 ± 1.0 days) and skin (MST 9.3 ± 0.7 days) allografts. Group 4 Buffalo recipients received both 25×10^6 Lewis red blood cells intrathymically and ALS. In group 5, Buffalo recipients received 25 $\times 10^6$ fractionated Lewis T lymphocytes intrathymically with ALS, whereas group 6 underwent an intrathymic injection of 25×10^6 fractionated Lewis B lymphocytes, macrophages, and dendritic cells (adherent cells) plus ALS. All animals in group 1 through 6 received their respective pretransplant treatment 21 days before heterotopic Lewis cardiac allografting.

Table 1. SURVIVAL OF LEWIS HETEROTOPIC HEART ALLOGRAFTS INTO BUFFALO RATS

Group	Pre-transplant Treatment*	No. of Rats	Days of Survival	MST	p Value vs. Control
1	No treatment	6	7, 7, 7, 8, 8, 8	7.5	—
2	ALS only	6	6, 6, 7, 7, 7, 8	6.8	NS
3	IT inj. only	6	5, 6, 6, 6, 7, 7	6.2	NS
4	RBC IT + ALS	6	7, 7, 7, 7, 8, 8	7.3	NS
5	T cell IT + ALS	6	7, 7, 8, 8, 9, >60	16.5	NS
6	(B cell, macrophage dendritic) adherent cells IT + ALS†	6	>100(×3), >150(×3)	>125.0	p < 0.001 vs. Group 1
	Unfrac. spl. cell + IT	25	6, 7, 14, >100(×6), >200(×16)	>153.1	p < 0.001 vs. Group 1

* Heterotopic Lewis cardiac grafts were placed 21 days after pre-treatment.
† Splenocytes adherent to plastic dishes were >90% B cells, macrophages, and dendritic cells.

Tumor Target Cells

The adenovirus transformed cell line A2/ASREB/IP/F4 [As-F4 from the Lewis rat strain (RT1[1])] originated in Dr. P. Gallimore's laboratory[18] and was provided by Dr. Donald Bellgrau (Denver, CO).[19] These tumor cells were maintained in RPMI-1640 supplemented with 1% L-glutamine (GIBCO Corp., Grand Island, NY), 100,000 U/l penicillin-streptomycin, 10 mmol/l HEPES (GIBCO Corp., Grand Island, NY), and 5% heat-inactivated fetal calf serum (FCS, low endotoxin, GIBCO, Grand Island, NY). Cell monolayers were subcultured twice weekly with trypsin-EDTA, by placing $2-4 \times 10^5$ cells in 10 ml of medium in a 25-cc tissue culture flask (Corning Glass Works, Corning, NY).

Generation of Blast Target Cells

ACI (RT1[a]) target cells were generated as described by Langrehr et al.[20] Briefly, target cells were prepared by culturing 7×10^6 lymph-node lymphocytes with Concanavalin A (2.5 µg/ml) in a final volume of 4 ml DMEM supplemented with 10% FCS for 48–72 hours. At the end of this culture period, target cells were harvested for use in cell-mediated cytotoxicity assays.

Generation of Specific Buffalo Anti-Lewis and Anti-ACI CTL

Lymph-node cells from nontreated control, RBC-treated, and T-lymphocyte–treated Buffalo rats 7 days after Lewis heart transplantation, at the cessation of cardiac contraction, were tested for the generation of primary Buffalo anti-Lewis and anti-ACI CTL, whereas lymph-node cells were harvested 30 days after Lewis cardiac transplantation from tolerant Buffalo rats which received B-lymphocyte, macrophage, and dendritic cell treatment; 20×10^6 responding lymph-node cells were cocultured in 10 ml of DMEM medium supplemented with 10% FCS, 1% L-glutamine, 10 mmol/l HEPES, and 100,000 U/l of penicillin-streptomycin in 25-cc flasks with 20×10^6 irradiated (2000 rads) Lewis or ACI lymph node cells at 37 C in 95% air/5% CO_2 for 7 days. At the completion of this incubation period, effector cells were harvested, washed, and resuspended in DMEM supplemented with 10% FCS.

⁵¹Cr Release Assay

Target cells (5×10^6) were labeled with 200 uCi ⁵¹Cr ($Na^{51}CrO_4$, 1 mCi/ml; Amersham Corp., Arlington Heights, IL) in 100 µl of DME supplemented with 10% FCS for 1 hour at 37 C in 95% air/5% CO_2, washed three times, counted, and resuspended at 200,000 cells/ml; 2000 cells in 100 µl DME with 10% FCS were added

to 96-well V-bottom microtiter plates (ICN, Costa Mesa, CA).

Effector cells were harvested, resuspended, diluted to the appropriate concentration, and dispensed into the V-bottom plates. The plates were spun at $50g$ for 2 minutes and incubated 4 hours at 37 C in 95% air/5% CO_2. At the completion of the incubation period, the plates were spun at $500g$ and 100 µl of the supernatant was harvested and counted on a LKB gamma counter (LKB, 1272 Clinigamma, Turku, Finland). The mean of the triplicate samples was calculated and percent ⁵¹Cr release was determined according to the following equation:

$$\text{percent } {}^{51}\text{Cr} = 100 \times \frac{[(\text{experimental } {}^{51}\text{Cr release} - \text{control } {}^{51}\text{Cr release})}{(\text{maximum } {}^{51}\text{Cr release} - \text{control } {}^{51}\text{Cr release})]}$$

where experimental ⁵¹Cr release represents counts from target cells mixed with effector cells, control ⁵¹Cr release represents counts from target cells incubated with medium alone (spontaneous release), and maximum ⁵¹Cr release represents counts from target cells exposed to 5% Triton-X 100.

Cytokine Assay

The CTLL-2 lymphocyte line was obtained from American Type Culture Collection (ATCC, Rockville, MD) and maintained by biweekly subculture in 10% Con A-conditioned medium. For the bioassay, 2×10^3 CTLL-2 were cultured in serial dilutions of Buffalo anti-Lewis and Buffalo anti-ACI mixed lymphocyte culture (MLC) supernatant (harvested 48 hours after culture initiation). On day 1, 2 uCi ³H-TdR (³H-TdR, New England Nuclear, Boston, MA) was added per well, incubated for 6.5 hours, harvested on glass filter paper strips, and counted in a liquid scintillation counter (1214 Rackbeta, LKB, Yurku, Finland). Cytokine levels (U/ml) were calculated using rmIL-2 (Genzyme Corporation, Mountain View, CA) as the standard.

Tissue Collection and Histopathologic Studies

Lewis cardiac allografts were excised from the Buffalo recipient at the time of rejection, or long-term surviving allografts were removed for histologic examination 100 days after transplantation. Tissues were fixed in neutral-buffered formalin, embedded in paraffin, sectioned at 4 µm in thickness, and stained with hematoxylin and eosin (H&E).

Statistics

Allograft survival data were evaluated for statistical significance by the Kaplan-Meier analysis. Proliferation

assay data is presented as the mean plus or minus the standard deviation. For CTL data presented in this paper, the standard error of the mean percent lysis was < 5% of the value of the mean.

RESULTS

Effect of Lewis Cell Fractions on Lewis Cardiac Allograft Survival

To determine which donor MHC alloantigens must be present, during intrathymic T lymphocyte maturation, to induce donor-specific tolerance to vascularized allografts, Lewis heterotopic cardiac allografts were performed 21 days after the intrathymic injection of fractionated Lewis donor cells and a simultaneous IP injection of 1 ml of ALS. The mean survival time of Lewis cardiac allografts in Buffalo recipients receiving ALS treatment alone was 6.8 days (Group 2) (Table 1), which is not significantly different from Buffalo recipients receiving no pretransplant treatment (Group 1: MST = 7.5 days) or an intrathymic injection of unfractionated Lewis spleen cells without ALS (Group 3: MST = 6.2 days). Likewise, the pretransplant intrathymic injection of Lewis MHC class I expressing red blood cells or class I expressing T lymphocytes plus ALS did not significantly prolong Lewis cardiac allograft survival (Group 4: MST = 7.3 days and Group 5: MST = 16.5 days). The prolonged Lewis cardiac allograft survival for one recipient in group 5 is postulated to be secondary to contamination by class II expressing, B lymphocyte, macrophage, or dendritic cells. In contrast, intrathymic injection of enriched Lewis MHC class II-expressing cells plus ALS treatment resulted in significantly prolonged (Group 6: MST > 125 days, p < 0.001) Lewis cardiac allograft survival in all Buffalo recipients. This data is similar to that obtained with intrathymic injection of unfractionated splenocytes[15] (Table 1) and indicates that the ability to produce a state of donor-specific unresponsiveness requires MHC class II expressing cells to be placed within the thymus where maturing T-lymphocyte populations will be educated before replacing the peripheral T-lymphocyte repertoire previously depleted by the administration of ALS.

To document the donor specificity of this tolerance, Buffalo recipients in Group 6 tolerant to Lewis cardiac allografts underwent a second ACI or Lewis cardiac allograft. Buffalo rats with a long-surviving Lewis cardiac allograft rejected a third party ACI (RT1[a]) cardiac allograft in a normal manner (MST = 7.0 days), while a second Lewis cardiac allograft was accepted indefinitely (MST > 100.0 days). This provides evidence that the induced tolerance is specific for the strain of donor splenocytes given IT (Table 2).

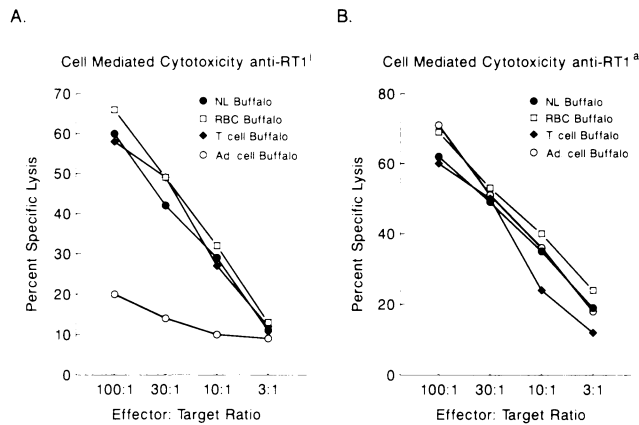| Table 2. SECOND ALLOGRAFT SURVIVAL IN LONG-TERM LEWIS HEART BEARING BUFFALO RATS	
Allograft	**Second Allograft Survival Time (days)**
Heart (ACI)	7, 7, 7
Heart (Lewis)	>100, >100, >100

Histopathologic Analysis of Lewis Cardiac Allografts

Lewis cardiac allografts removed from Buffalo recipients receiving no pretransplant treatment (Group 1), ALS treatment only (Group 2), intrathymic Lewis alloantigen injection only (Group 3), Lewis red blood cell intrathymic injection with ALS (Group 4) or Lewis T lymphocyte intrathymic injection with ALS (Group 5) at the time of cessation of cardiac contraction demonstrated a typical dense mononuclear cell infiltration and associated myofibril necrosis consistent with an unmodified rejection response. In contrast, the beating Lewis cardiac allografts removed 100 days after transplantation into a Buffalo recipient, who previously had undergone intrathymic injection of Lewis MHC class II expressing alloantigens with ALS, demonstrated healthy cardiac myocytes without mononuclear cell infiltration or evidence of tissue damage.

Effect of MHC Class I and Class II Expressing Lewis Alloantigens on Buffalo Anti-Lewis and Buffalo Anti-ACI Cell-mediated Cytotoxicity

Lymph-node cells harvested, from either Buffalo recipients with a long-term tolerated Lewis cardiac allograft after MHC class II intrathymic injection, from an acutely rejecting Lewis cardiac allograft after the intrathymic injection of MHC Lewis class I alloantigens, or from non-transplanted naive Buffalo rats, were analyzed for Lewis- and ACI-specific cell-mediated cytotoxicity. The intrathymic injection of Lewis MHC class II-expressing cells, with ALS, resulted in a marked decrease in the anti-RT1[1] (Lewis) cytolytic response in comparison to the naive control, or Lewis MHC class I intrathymically injected Buffalo recipients that demonstrated strong cytolytic activity against the RT1[1] targets (Fig. 1a). In contrast, the intrathymic injection of Lewis MHC class II-expressing cells did not alter the third party RT1[a] response when compared with naive controls or MHC class I-injected recipients (Fig. 1b). These data demonstrate that the intrathymic injection of Lewis MHC class II expressing cells specifically induce tolerance only to

A.

Cell Mediated Cytotoxicity anti-RT1^1

- NL Buffalo
- RBC Buffalo
- T cell Buffalo
- Ad cell Buffalo

Percent Specific Lysis

70 / 60 / 50 / 40 / 30 / 20 / 10 / 0

100:1   30:1   10:1   3:1
Effector: Target Ratio

B.

Cell Mediated Cytotoxicity anti-RT1^a

- NL Buffalo
- RBC Buffalo
- T cell Buffalo
- Ad cell Buffalo

Percent Specific Lysis

80 / 60 / 40 / 20 / 0

100:1   30:1   10:1   3:1
Effector: Target Ratio

Figure 1. Intrathymic injection of Lewis MHC class II expressing cells, with ALS, specifically inhibits the *in vitro* Buffalo anti-Lewis cytotoxic response. a: Primary Buffalo anti-Lewis specific CTL were assayed against As-F4 (RT1^1) targets after the intrathymic injection of MHC class II expressing B lymphocytes, macrophages, and dendritic cells (O), MHC class I-expressing red blood cells (□), or MHC class I-expressing T lymphocytes (△). Controls also included naive, non-thymic injected Lewis-specific Buffalo effector cells. b: ACI (RT1^a) specific CTL were analyzed after the intrathymic injection of the same cell populations as in (a). Additionally, ACI (RT1^a) specific effectors were generated from naive Buffalo responders. All effectors were tested against ACI Con A blasted lymphocytes.
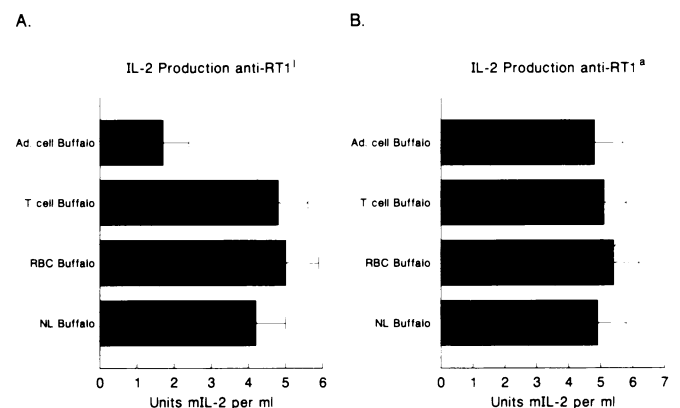
the donor strain injected intrathymically, and does not globally immunosuppress the host.

Effect of Intrathymic MHC Class I- and Class II-Expressing Lewis Alloantigens on Donor-specific IL-2 Production

The lymph-node cells harvested from Buffalo recipients after the intrathymic injection of Lewis MHC class II- or class I-expressing cells, or no pretransplant treatment were analyzed for their production of IL-2 during stimulation with Lewis or ACI lymph-node cells. Buffalo lymph-node cells harvested from the recipients undergoing the pretransplant intrathymic injection of Lewis MHC class II-expressing allogeneic cells with ALS produced 50% less IL-2 upon stimulation with Lewis lymph nodes when compared with naive and Lewis MHC class I-injected Buffalo recipients (Fig. 2a). However, the intrathymic injection of Lewis MHC class II-expressing cells does not alter the recipients IL-2 production to a third party ACI lymph-node stimulation, when compared with naive and MHC class I-injected controls (Fig. 2b). These data further demonstrate that the tolerance associated with the intrathymic injection of donor MHC class II expressing cells results in a reduced production of the cytokine interleukin-2.

DISCUSSION

The understanding of the central role that the thymus plays in the development of the immune system and the control of MHC restriction has come largely from studies with bone marrow chimeras,[13] thymus-grafted mice,[21] and most recently from T-cell receptor constructed transgenic mice.[11] T-cell specificity is established in the thymus, where the differentiating thymocyte population is depleted of T lymphocytes bearing high affinity self-reactive TCR, while T lymphocytes that express T-cell receptors recognizing foreign antigen in association with self-MHC class I and class II gene products are allowed to differentiate into mature T lymphocytes. Endodermally derived thymic epithelial cells and accessory cells of hematopoietic origin, respectively, are implicated in performing the contrasting functions of positive and negative selection of differentiating thymocytes. Recent evidence indicates that self-tolerance occurs by clonal deletion (negative selection) of autoreactive T lymphocytes, which is thought to be mediated by the hematopoietically derived macrophage/dendritic cells of the thymic medulla[22] via a process of DNA and nuclear fragmentation followed by cell death termed apoptosis. Conversely, the positive selection of MHC-restricted T lymphocytes appears to be mediated by interaction of the T cell receptor and molecules such as CD4 and CD8 of the differentiating thymocytes,[23] with the MHC molecules of the thymic epithelial cells in the thymic cortex.[24]

A.

IL-2 Production anti-RT1^1

Ad cell Buffalo

T cell Buffalo

RBC Buffalo

NL Buffalo

0   1   2   3   4   5   6
Units mIL-2 per ml

B.

IL-2 Production anti-RT1^a

Ad cell Buffalo

T cell Buffalo

RBC Buffalo

NL Buffalo

0   1   2   3   4   5   6   7
Units mIL-2 per ml

Figure 2. Inhibition of Lewis-specific IL-2 production after the intrathymic injection of MHC class II-expressing Lewis allogeneic cells. a: Supernatants from Buffalo anti-Lewis mixed lymphocyte cultures were harvested after 48 hours and analyzed for IL-2. Buffalo recipients underwent the intrathymic injection of Lewis MHC class II adherent cells, Lewis red blood cells, Lewis T lymphocytes, or no pretransplant treatment. b: Buffalo anti-ACI 48-hour mixed lymphocyte culture supernatant was analyzed for IL-2 production from Buffalo recipients of intrathymic Lewis MHC class II adherent cells, red blood cells, T lymphocytes, or no pretransplant treatment.

Our findings demonstrate that the presence of MHC class II- expressing (B lymphocytes, macrophages, and dendritic cells) donor allogeneic cells within the thymic microenvironment during a period of thymocyte differentiation has a markedly different effect (i.e., allograft acceptance, decreased donor-specific cytolytic activity, and decreased IL-2 production) than do MHC class I-expressing donor red blood cells and T lymphocytes. The hematopoietically derived macrophages and dendritic cells needed for tolerance induction in this model are also necessary for the intrathymic deletion of auto-reactive cells and induction of self-tolerance. Injected MHC class II-expressing allogeneic cells probably interact with the differentiating host thymocytes, to either delete (clonal deletion) or functionally inactivate (clonal anergy) those maturing thymocytes possessing T-cell receptors with high affinity for the donor allogeneic MHC molecule. This hypothesis is supported by the inability to induce tolerance by the intrathymic injection of irradiated bone marrow cells while a naive bone marrow cell intrathymic injection resulted in indefinite cardiac allograft survival without subsequent immunosuppression as reported by Odorico et al.[25] Additionally, the intrathymic inoculation of semi-allogeneic stem cells into lethally irradiated mice reconstituted with donor bone marrow could induce tolerance in the recipient to the donor alloantigen.[24,26] Fuchs and Matzinger[27] postulated that the development of T-lymphocyte tolerance occurring within the thymic microenvironment is due to antigen exposure on the "professional antigen-presenting cells" (macrophage and dendritic cells), while exposure to antigen expressed on T and B lymphocytes is not capable of inducing intrathymic tolerance.

Another possible mechanism for this induction of tolerance via intrathymic injection of donor alloantigen involves the migration of the inoculum from the thymus into the peripheral circulation to function as a donor-specific transfusion. Intravenous injection of mice with viable MHC incompatible lymphoid cells can reduce a donor-specific proliferation,[28] donor-specific CTL generation,[29,30] and T-helper cell generation[31] in MLC and enhances survival of a donor specific allograft.[32,33] One possible explanation is that the injected donor lymphoid cells act as veto cells[34,35] to produce functional deletion rather than lymphocyte activation.[30,32] However, this mechanism would have to explain the following: injection of viable semi-allogeneic F1 (A × B) lymphoid cells into either parent A or parent B usually (but not always) induces immune response reduction and enhanced graft survival,[32] while injection of fully allogeneic B lymphoid cells into A seldom induces either immune response reduction or enhanced graft survival, although the veto hypothesis predicts that it should. Since the veto cell is

classically described as a CD8$^+$ T lymphocyte,[35] our achievement of donor-specific tolerance only with the injection of B lymphocytes, macrophages, and dendritic cells (not T lymphocytes) indicates that a veto cell mechanism is not occurring in this model.[35]

The donor-specific unresponsiveness in the donor specific transfusion model is dependent on the injected donor cells persisting in the recirculating lymphocyte pool of the host, since the failure of these cells to persist does not result in a reduction of donor directed immune responsiveness.[36] Our laboratory has shown that the injection of donor splenocytes in sites other than the thymus does not result in donor-specific tolerance and prolonged allograft survival.[15] Furthermore, to date there have been no reports documenting donor cells present within the recirculating lymphoid circulation in this intrathymic injection model of tolerance. Therefore, the veto mechanism of functionally deleting antigen-presenting cells in the recirculating lymphoid pool seems highly unlikely.

In summary, we have demonstrated that the induction of tolerance and prolonged allograft survival after the intrathymic injection of donor allogeneic cells is dependent on the presence of donor MHC class II-, and not class I, expressing cells. These findings further increase the understanding of donor MHC alloantigens necessary for intrathymic injection to successfully induce donor-specific intrathymic tolerance. Application of these principles could have implications for clinical transplantation.

References

1. Medawar PB. The behavior and fate of skin allografts and skin homografts in rabbits. J Anat 1944; 78:176–199.
2. Rosenberg AS, Mizuochi T, Sharrow SO, Singer A. Phenotype, specificity, and function of T cell subsets and T cell interactions involved in skin allograft rejection. J Exp Med 1987; 165:1296–1315.
3. Bach FH, Sachs DH. Transplantation immunology. N Engl J Med 1987; 318:489–492.
4. Foker JE, Simmons RL, Najarian JS. Highlights of recent progress in transplantation. In Najarian JS, Simmons RL, eds. Allograft Rejection. Philadelphia: Lea and Febinger, 1972, pp 63–145.
5. Todo S, Tzakis AG, Abu-Elmagd K, et al. Intestinal transplantation in composite visceral grafts or alone. Ann Surg 1992; 216:223–234.
6. Kahan BD. Cyclosporine. N Engl J Med 1989; 321:1725–1729.
7. Walker RG, d'Apice AJF. Azathioprine and steroids. In Morris PJ, ed. Kidney Transplantation: Principles and Practice. Philadelphia: W.B. Saunders, 1988, pp 319–341.
8. Kappler JW, Roehm N, Marrack P. T cell tolerance by clonal elimination in the thymus. Cell 1987; 49:273–280.
9. Sha C, Nelson CA, Newberry RD, et al. Positive and negative selection of an antigen receptor on T cells in transgenic mice. Nature 1988; 336:73–76.
10. Marrack P, Kushnir E, Born W, McDuffie M, Kappler J. The

development of helper T cell precursors in mouse thymus. J Immunol 1988; 140:2508-2514.

11. Teh HS, Kisielow P, Scott B, et al. Thymic major histocompatibility complex antigens and the α/β T-cell receptor determine the CD4/CD8 phenotype of T cells. Nature 1988; 335:229-233.

12. Nikolic-Zugic J, Bevan MJ. Role of self-peptides in positively selecting the T cell repertoire. Nature 1990; 344:65-67.

13. Shimonkevitz R, Bevan MJ. Split tolerance induced by the intrathymic adoptive transfer of thymic stem cells. J Exp Med 1988; 168:143-156.

14. Goss JA, Nakafusa Y, Flye MW. Intrathymic injection of donor alloantigens induces donor specific vascularized allograft tolerance without immunosuppression. Ann Surg 1992; 216:223-234.

15. Goss JA, Nakafusa Y, Yu S, Flye MW. Intrathymic injection of donor alloantigens induces specific tolerance to cardiac allografts. Transplantation 1993, in press

16. Wood KJ, Evins J, Morris PJ. Suppression of renal allograft rejection in the rat by class I antigens on purified erythrocytes. Transplantation 1985; 39:56-62.

17. Ono K, Lindsay N. Improved technique of heart transplantation in rats. J Thorac Cardiovasc Surg 1969; 57:225-229.

18. Gallimore PH. Viral DNA in transformed cells. II. A study of the sequences of adenovirus 2 DNA in nine lines of transformed rat cells using specific fragments of the viral genome. J Mol Biol 1974; 89:49-58.

19. Bellgrau D. Induction of cytotoxic T cell precursors in vivo: role of T helper cells. J Exp Med 1983; 157:1505-1512.

20. Langrehr JM, Dull KE, Ochoa JB, et al. Evidence that nitric oxide production by in vivo allosensitized cells inhibit the development of allospecific CTL. Transplantation 1992; 53:632-640.

21. Fink PJ, Bevan MJ. H-2 antigens of the thymus determine lymphocyte specificity. J Exp Med 1978; 148:766-775.

22. Schwartz RH. Acquisition of immunologic tolerance. Cell 1989; 57:1073-1081.

23. MacDonald HR, Nengartner H, Pedrazzini T. Intrathymic deletion of self reactive cells prevented by neonatal anti-CD4 antibody treatment. Nature 1988; 335:174-176.

24. Lo D, Sprent J. Identity of cells that imprint H-2 restricted T cell specificity in the thymus. Nature 1986; 319:672-675.

25. Odorico JS, Barker CF, Posselt AM, Naji A. Induction of donor-specific tolerance to rat cardiac allografts by intrathymic inoculation of bone marrow. Surgery 1992; 112:370-377.

26. Saluan J, Bandeira A, Khazaal I. Thymic epithelium tolerizes for histocompatibility antigens. Science 1990; 247:1471-1474.

27. Fuchs EJ, Matzinger P. B cells turn off virgin but not memory T cells. Science 1992; 258:1156-1159.

28. Wilson DB, Nowell PC. Quantitative studies on the mixed lymphocyte interactions in rats: tempo and specificity of the proliferative response and the number of reactive cells from immunized donors. J Exp Med 1971; 133:442-452.

29. Miller RG, Phillips RA. Reduction of the in vitro cytotoxic lymphocyte response produced by in vivo exposure to semiallogeneic cells: recruitment or active suppression? J Immunol 1976; 117:1913-1918.

30. Rammensee HG, Fink PJ, Bevan MJ. Functional clonal deletion of class 1 specific cytotoxic T lymphocytes by veto cells that express antigen. J Immunol 1984; 133:2390-2396.

31. Kiziroglu F, Miller RG. In vivo functional clonal deletion of recipient CD4+ T helper precursor cells that can recognize class II MHC on injected donor lymphoid cells. J Immunol 1991; 146:1104-1111.

32. Heeg K, Wagner H. Induction of peripheral tolerance to class I major histocompatibility complex (MHC) alloantigens in adult mice: transfused class I MHC-incompatible splenocytes veto

clonal responses of antigen reactive Lyt-2+ T cells. J Exp Med 1990; 172:719-730.

33. Kast WM, vanTwuyver E, Mooijaart RJD, et al. Mechanism of skin allograft enhancement across an H-2 class I mutant difference: evidence for involvement of veto cells. Eur J Immunol 1988; 18:2105-2112.

34. Miller RG. An immunological suppressor cell inactivating cytotoxic T lymphocyte precursor cell recognizing it. Nature 1980; 287:544-547.

35. Fink PJ, Shimenkovitz RP, Bevan MJ. Veto cells. Annu Rev Immunol 1988; 6:115-146.

36. Sheng-Tanner X, Miller RG. Correlation between lymphocyte-induced donor-specific tolerance and donor cell recirculation. J Exp Med 1992; 176:407-413.

Discussion

DR. ARNOLD G. DIETHELM (Birmingham, Alabama): Wayne, I enjoyed your paper a great deal and it's an interesting and yet very complex set of experiments that you have presented to us. Let me limit my comments to maybe some of the mechanistic aspects. Do you think the cell needs to leave the thymus in order to achieve this level of tolerance? In other words, you're injecting a cell into the thymus, does that cell undergo a change and then leave the thymus or does something happen in the thymus that other cells that leave the thymus are now tolerogenic. And where do these cells go if they do leave the thymus? It would seem to me that if there's clonal deletion, it couldn't all happen in that very short period of time. Possibly I'm wrong. And what is the timing of the ALS and the tolerogenic effect of thymus? You mentioned, I believe, 21 days. Is that an important time event or can it be sooner or later? Obviously the time between the injection of the ALS and the tolerogenic result is critical when one considers any aspect of clinical transplantation. I very much enjoyed your paper. I think you're on to a very complex subject. It's going to be interesting to see how all of this plays out and whether or not the cells in the thymus have to leave the thymus or if something else happens to make the animal tolerogenic.

DR. JAY C. FISH (Galveston, Texas): The Australian, Kevin Lafferty, demonstrated 15 years ago that in a murine model if you take the thyroid and parathyroid out and culture it for 28 days in high oxygen that all the passenger leukocytes die off and you're left with a pure culture of follicular cells that bear only Class I antigens. Those cells can be transplanted in the mouse successfully without immunosuppression. Unfortunately neither he nor anyone else has been able to duplicate this finding in higher order species. In addition it has been difficult with other tissues. These studies demonstrate the importance of Class II antigens in donor tissue to stimulate the allergenic response. What we've seen today is the importance of Class II antigen cells in the donor tissue to produce tolerance. A certain inner logic of that might be predicted. What would be interesting to know is if it works as well with other tissues such as skin and how it works in higher order animals. I, too, am interested in knowing the fate or the final resting place of the cells in the inoculum to the thymus.