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*J Immunol* 1998; 160:3790-3796; ;
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Antiviral Cytotoxic Activity Across a Species Barrier in Mixed Xenogeneic Chimeras: Functional Restriction to Host MHC

Yolonda L. Colson,* Ralph A. Tripp,† Peter C. Doherty,‡ Sherry M. Wren,§ Michael Neipp,§ Ashraf Y. Abou El-Ezz,§ and Suzanne T. Ildstad2§

Reconstitution of lethally irradiated mice with a mixture of mouse and rat bone marrow cells (mouse + rat→mouse) results in mixed xenogeneic chimerism and donor-specific tolerance. The current study demonstrates that mouse and rat T lymphocytes that have developed in xenogeneic chimeras are restricted to Ag presentation by mouse, but not rat, APC. Restriction to host Ags results in functional immunocompetence with generation of antiviral cytotoxic activity in vivo, within and across species barriers. These data demonstrate for the first time that the host thymus is sufficient to support development and positive selection of functional cross-species T lymphocytes. The superior immunocompetence, as compared with fully xenogeneic (rat→mouse) chimeras, may prove to be of significant benefit in the clinical application of xenotransplantation to solid organ transplantation and immune reconstitution for AIDS. The Journal of Immunology, 1998, 160: 3790–3796.

T lymphocytes recognize conventional Ags presented in the context of self MHC molecules (1–8). Studies from semiallogeneic radiation bone marrow chimeras demonstrated that negative selection, or clonal deletion, of T cells is mediated by the APC of the bone marrow donor (9, 10). In contrast, the positive selection of T lymphocytes necessary for the recognition of conventional Ags is determined by the MHC stromal phenotype of the host environment in which the T cells have developed (1, 4–7). Tolerance to MHC determinants, however, was not, in itself, sufficient to result in the recognition of those determinants for the purpose of Ag presentation. Singer et al. demonstrated that unfracticated spleen cells from fully allogeneic bone marrow chimeras (A→B), which were tolerant to both host and donor Ags, did not recognize an antigenic stimulus, such as trinitrophenol (TNP), when presented in the context of donor MHC molecules (strain A) (1). This immunoincompetence could be overcome with the direct addition of host-type APC (3, 4), or with the preparation of mixed allogeneic chimeras (B + A→B), in which the genotypically appropriate host APC (strain B) are present in the bone marrow in oculum at the time of transplantation (1). The failure of donor T cells to recognize Ag was therefore due to the absence of host-type APC to which the donor T cells were restricted, and not due to an inherent defect in T cell function. Therefore, T cells specifically recognize those self MHC determinants expressed on radiation-resistant host elements encountered by developing T cells in the thymus at a critical point in development. This phenomenon was also shown in vivo using a model of intranatal infection of mice with HK×31 influenza A virus. Intranatal infection results in severe pneumonia and a massive enlargement of mediastinal lymph nodes (MLN) that drain the respiratory tract (11, 12). However, this infection is nonlethal in immunocompetent recipients, as clearance of the virus occurs through the generation of viral-specific CTL activity and the recruitment of influenza A-specific CTL precursors (CTLp). Viral-specific CTLp are not detected (>106) within MLN and spleen before infection, but rapidly increase following infection in immunocompetent recipients (13). Mixed allogeneic and syngeneically reconstituted recipients survive due to generation of viral-specific CTLs, while fully allogeneic chimeras succumb to infection (14).

Until recently, the lack of a model for T cell development and tolerance induction in a xenogeneic stromal environment has not permitted similar analyses for xenotransplantation. Whether positive, as well as negative selection events associated with the development of a functional T cell repertoire can occur across a species barrier has remained an uncertainty. Clearly, the successful clinical application of xenogeneic bone marrow chimera requires the presence of both donor-specific tolerance and immunocompetence to respond to infectious agents.

We have developed a model to achieve stable multilineage fully xenogeneic chimerism (rat→mouse) through reconstitution of mice conditioned with 950 centigray of total body irradiation with rat bone marrow (15). Recipient mice exhibit phenotypically normal rat T cell development, and are functionally tolerant to donor xenotransplants in vivo since donor-specific skin and islet xenografts were permanently accepted, while MHC-disparate third-party mouse and rat grafts are promptly rejected (15, 16). Mixed xenogeneic chimeras, in which T cell-depleted syngeneic mouse bone marrow is coadministered with either untreated or T cell-depleted rat bone marrow (mouse + rat→mouse), demonstrate similar functional donor-specific tolerance for skin, pancreatic islet, and cardiac xenografts in vivo (17–19). The rat- and mouse-derived T lymphocytes develop in a phenotypically normal fashion, and lymphocytes from the chimeras exhibit donor-specific functional tolerance in vitro by proliferative MLR assays (20).

We have now applied the model of mixed xenogeneic chimism (mouse + rat→mouse) to examine T cell repertoire selection

1 Abbreviations used in this paper: TNP, trinitrophenol; CML, cell-mediated lympholysis; CTLp, cytolytic T lymphocyte precursors; MLN, mediastinal lymph nodes.
events, namely: 1) the specificity of tolerance to xenointerigen for chimera-derived mouse and rat T cells; 2) whether rat-derived T cells that develop in a mouse thymus are restricted to Ag presentation by mouse or rat APC; 3) whether virus-specific cell-mediated lympholysis can be generated in a mixed xenogeneic microenvironment; and (4) whether virus-specific CTL recognition following intranasal infection with Hk31 influenza A virus can occur across a species barrier. We report for the first time that mouse- and rat-derived T lymphocytes that develop in mixed xenogeneic chimeras are each tolerant to both syngeneic mouse and donor rat Ags, yet competent to respond to MHC-disparate third-party rat and mouse Ags. In TNF-modified self studies, both mouse and rat T cells require Ag presentation by mouse, but not rat, APC. This Ag presentation by host elements is functional in vivo, as evidenced by the generation of viral-specific CTL activity. Furthermore, both mouse and rat cytotoxic T cells can recognize virally infected cells of the host. These findings suggest that the positive selection events, which result in a functional T cell repertoire, are operative in vivo, even across a species disparity.

Materials and Methods

Animals

Six- to eight-week-old male C57Bl/10SnJ (B10), B10.BR/SgSn (B10.BR), and BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Four- to eight-week-old fisher 344 (F344) (RT1.A1), and ACI (RT1.A2) male rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Animals were housed in a specific pathogen-free facility at Biomedical Science Tower at University of Pittsburgh (Pittsburgh, PA).

Mixed xenogeneic bone marrow reconstitution (mouse + rat→mouse)

Mixed xenogeneically reconstituted animals were prepared as previously described (15, 20). Briefly, inbred B10 male recipients were lethally irradiated with a single dose of 950 centigray from a Cesium source (Nordion, Ontario, Canada). Using sterile technique, a single cell suspension of bone marrow was prepared from the long bones of donor animals. B10 bone marrow cells were T cell depleted by treatment with a 1/60 dilution of rabbit anti-mouse brain serum plus guinea pig complement (Life Technologies, Rockville, MD), as previously described (20). Cells were washed twice and resuspended for i.v. injection. Recipient B10 mice were reconstituted within 4 to 6 h after irradiation via the lateral tail veins. Radiation controls were prepared to confirm adequacy of the radiation.

Characterization of chimeras by flow cytometry

Recipients were characterized for engraftment with syngeneic and/or xenogeneic donor lymphoid elements using flow cytometry to determine the percentage of PBL-bearing H-2b (B10) and RT1.A (F344) surface markers (16). Briefly, peripheral blood was layered over lymphocyte separation medium (Organon Teknika, Durham, NC) and centrifuged at 400 g for 30 min. The lymphocyte layer was aspirated and washed before being stained with mAbs against H-2b (Pharmingen, San Diego, CA) or RT1.A (gift from Dr. Heinz Kuntz, Pittsburgh, PA) Ags. FITC-conjugated sandwich was utilized for counterstaining when required.

Mixed lymphocyte reactions

MLR were performed as previously described (20). Briefly, splenocytes were ACK lysed (1.55 M ammonium chloride, 0.1 M potassium carbonate-MES, pH 7.4; prepared in our laboratory) and reconstituted in nonessential amino acids, 1 mM sodium pyruvate, 2 mM glutamine, 100 U/ml penicillin, 100 U/ml streptomycin (all from Life Technologies), 1 mM N-mono-methyl-arginine (NMA; Calbiochem, San Diego, CA), and 0.05 mM 2-ME (Sigma Chemical, St. Louis, MO). A quantity amounting to 5 x 10^6 responders was cultured 1:1 with irradiated stimulators for 4 days, pulsed with 1 mCi [3H]thymidine (DuPont NEN, Boston, MA), and harvested the next day with an automated harvester (PHD Cell Harvester Technology, Cambridge, MA).

Cell-mediated lympholysis (CML)

CML assays were performed using a modification of techniques previously described (21). Briefly, RPMI 1640 medium was supplemented as above, except that 10% FCS (both from Life Technologies) was used in place of normal mouse serum (CML-RPMI media). A quantity amounting to 5 x 10^6 responders was cocultured 1:1 with irradiated stimulators for 5 days. Target blasts were Con A (Miles-Yeda, Rehovot, Israel)-stimulated mouse splenocytes or rat lymph node lymphocytes stimulated with PHA for 2 days. In some experiments, EL4 (H-2b) and P815 (H-2d) tumor targets were used. After 5 days, effectors were harvested and resuspended at appropriate E:T ratios with 31Cr-labeled targets. After 4 to 5 h, supernatants were harvested and specific lysis was calculated. Spontaneous release was <25% of maximum release.

mAb depletion of mouse- or rat-derived cells

To examine whether donor-specific tolerance, as well as maintenance of anti-third-party responses, in mixed xenogeneic chimeras (B10 mouse + F344 rat→B10 mouse) was due to rat- or mouse-derived lymphoid cells, lymphocytes were isolated from the spleens of mixed chimeras. In vitro mAb-mediated depletion of mouse (H-2b) or rat cell populations was performed using anti-H-2b mAb, rat class I, or ACI anti-F344 alloantiserum (prepared in our laboratory) at a 1:20 dilution. Cells were washed and treated with rabbit complement twice before resuspension in CML-RPMI. Adequacy of depletions was determined by flow-cytometric analysis using non-cross-reactive biotinylated, protein-purified anti-H-2b and anti-F344 mAb and an FITC-avidin sandwich, as previously described (16). The limit of detection of a positive cell population was sensitive to 0.1 to 0.5% of gated lymphocytes (22).

Induction of Ag-specific CTL

Splenic lymphoid cells were harvested and pooled from four mixed xenogeneic chimeras. For all experiments (n = 6), the percentage of rat cells in mixed xenogeneic chimeras ranged from 50 to 60%. Ag-specific CTL activity was assessed against syngeneic cells modified by TNF, according to the technique developed by Shearer et al. (8, 23). Briefly, TNF-modified lymphocytes were prepared using irradiated lymphocytes from mouse spleen and rat lymph node leukocytes incubated in serum-free FBS containing 10 mM 2-ME (Sigma Chemical). These stimulators were washed with CML-RPMI with or without Con A-conditioned media (prepared in our laboratory). A quantity amounting to 5 x 10^6 TNF-modified stimulator cells was cocultured 1:1 with splenic lymphocytes from mixed xenogeneic chimeras. After 5 days, responders were harvested and assessed for CTL activity. Effectors and 35Cr-labeled TNF-modified blasts were cocultured for 4 to 5 h at appropriate E:T ratios, as described for CML.

Viral infection

Mice were anesthetized with Avertin (2, 2, 2 tribromoethanol; Aldrich Chemical, Milwaukee, WI) by i.p. injection and infected intranasally with 30 μl PBS containing 240 hemagglutinating U of Hk31 influenza A virus (11, 13). This dose results in severe infection, but little mortality in immunocompetent mice. MLN and spleens were harvested and processed into a single cell suspension.

CTLp frequencies

The CTLp frequencies were determined as previously described (11, 13, 24). 35Cr-labeled normal and virus-infected MC57G (H-2b) fibroblasts were used as targets. Titrations of responder lymphocytes derived from MLN or spleens were added to 96-well round-bottom microtiter plates (Corning Glassworks, Corning, NY) with virus-infected syngeneic irradiated splenocytes in the presence of human rIL-2 (Boehringer Mannheim, Mannheim, Germany). All cultures were incubated for 7 days, before individual cultures were divided in half and incubated for an additional 6 h with 1 x 10^5 35Cr-labeled control or virus-infected target cells. Minimal estimates of CTLp frequency were obtained using the Poisson distribution, as the slope of the line relating the number of effector cells to the frequency of wells negative for cytotoxic activity. The slope was determined using χ^2 analyses.

Direct antiviral CTL activity

MC57G target cells (1 x 10^5) were mixed in round-bottom 96-well plates with effector cells from MLN or spleens at three different E:T ratios. Effector T cells of mouse and rat origin were isolated by flow-cytometric cell sorting using pan T cell mAbs directed against mouse CD3ε or rat OX52, respectively. Effectors and targets were mixed with a Dynatech Varsi-Shaker (Dynatech Laboratories, Chantilly, VA) for 30 s and then spun at...
1000 × g before incubating at 37°C for 5 h. Supernatant aliquots were collected and counted as described for CML.

**Results**

*Both mouse and rat lymphocytes from mixed xenogeneic chimeras exhibit MLR and CML reactivity*

As seen in a representative one-way MLR assay (n = 10), both B10 mouse and F344 rat lymphoid cells from mixed xenogeneic chimeras were specifically hyporeactive to syngeneic and xenogeneic donor strains of mouse and rat, while fully reactive (stimulation index 5.8) to MHC-disparate third-party mouse (BALB/c) and rat (ACI) Ags (Table I).

Similar to the results for MLR proliferation, donor-specific hyporeactivity was also observed in CML assays (Fig. 1). Splenocytes from the chimeras produced no detectable cytotoxic effectors for syngeneic (B10) or xenogeneic (F344) donor strain targets, yet produced effectors that were capable of lysing MHC-disparate third-party rat (ACI) and mouse (BALB/c) targets with an efficiency similar to that for normal B10 mouse and F344 rat responders prepared as controls. In addition, mixed chimeric lymphoid populations depleted of either rat or mouse cells remained specifically tolerant to both rat and mouse donor-type stimulator cells, yet reactive to third-party mouse and rat targets.

*Mouse- and rat-derived T cells are restricted to Ag presentation by mouse APC*

To determine the specificity of self-restriction, the ability of T cells to recognize TNP-modified targets in the context of either mouse or rat APC was assessed. In all experiments, an absolute restriction of recognition of TNP in association with the mouse host MHC was observed (Fig. 2). Upon stimulation with TNP-modified B10 stimulator cells, lymphocytes from normal B10

![FIGURE 1. Specific CTL lysis of $^{51}$Cr targets in one-way CML by rat or mouse splenocytes from mixed xenogeneic chimeras, B10 + F344→B10 spleen cells from three mixed xenogeneic chimeras were pooled and assayed for CML activity following no treatment (□), depletion of rat cells (chimera mouse cells only; ■), or depletion of mouse cells (chimera rat cells only; △) using Ab plus complement treatment. Efficiency of cellular depletions was confirmed by flow-cytometric analyses. The specificity of CML activity was determined using PHA-induced donor (F344) or third-party (ACI) rat lymph node targets or EL4 (H-2b; host) and P815 (H-2d; third-party mouse) tumor targets. Spontaneous release was <25%, unless otherwise indicated. This example is representative of three experiments."

**Table I. Reactivity of mouse and rat splenocytes from mixed xenogeneic chimeras in one-way MLR (mouse + rat → mouse)**

<table>
<thead>
<tr>
<th>Responder</th>
<th>Anti-B10</th>
<th>Anti-F344</th>
<th>Anti-BALB/c</th>
<th>Anti-ACI</th>
<th>Anti-self</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal B10</td>
<td>1,060 ± 61</td>
<td>22,474 ± 101</td>
<td>24,099 ± 205</td>
<td>11,459 ± 753</td>
<td>1,060 ± 89</td>
</tr>
<tr>
<td>Normal F344</td>
<td>71,428 ± 1,500</td>
<td>2,254 ± 64</td>
<td>151,360 ± 1,507</td>
<td>99,113 ± 228</td>
<td>2,254 ± 202</td>
</tr>
</tbody>
</table>

Chimera

| Mouse and rat cells | 3,057 ± 412 | 4,514 ± 134 | 67,627 ± 585 | 11,050 ± 183 | 2,830 ± 68 |
| Rat cells only | 3,562 ± 124 | 3,748 ± 134 | 88,193 ± 1,200 | 17,568 ± 196 | 3,043 ± 83 |
| Mouse cells only | 5,854 ± 135 | 5,490 ± 92 | 98,723 ± 328 | 19,874 ± 220 | 3,296 ± 102 |

Stimulation index

| Normal B10 | 1.0 | 21.2 | 22.7 | 10.8 |
| Normal F344 | 31.7 | 1.0 | 67.2 | 44.0 |
| Chimera | Mouse and rat cells | 1.1 | 1.6 | 23.9 | 3.9 |
| Rat cells only | 1.2 | 1.2 | 29.0 | 5.8 |
| Mouse cells only | 1.8 | 1.7 | 30.0 | 6.0 |

*Representative MLR illustrating the proliferative response of splenocytes from B10 mice, F344 rats or mixed xenogeneic chimeras (mean ± SD of triplicate cultures). For each experiment (n = 10), spleens from three mixed chimeras were pooled and assessed for alloreactivity of mouse and rat cells in combination, mouse cells only (rat-depleted), or rat cells only (mouse-depleted). The proportion of rat cells in the pooled inoculum prior to depletion ranged from 40 to 60% in all experiments. Antibody plus complement treatment using anti-class I H-2b mAb (IgM, ATCC HB158) removed B10 mouse cells. Specific alloantisera (ACI anti-F344), prepared and characterized in our laboratory do not cross-react on H-2b, was utilized to remove F344 rat cells. Adequacy of depletion was confirmed using flow cytometric analysis.
mice and mixed xenogeneic chimeras generated specific CTL responses against B10-TNP targets. However, only minimal cytolytic activity was observed when responding splenocytes from the chimera were cocultured with TNP-modified F344 targets. As expected, mature lymphocytes from unmanipulated F344 rats were restricted to F344 MHC, and responded to TNP-modified F344 targets. TNP-stimulated responders from B10, F344, and mixed xenogeneic chimeras were also tested on irrelevant targets and unmodified syngeneic targets to examine specificity of killing. In all experiments, cytolyis of irrelevant targets was <11% at 100:1 E:T ratio, demonstrating that restriction was specific. In all mixed xenogeneic chimeras, cytolytic activity was substantially greater for TNP-modified B10 mouse than for TNP-modified F344 rat targets, demonstrating an absolute restriction of recognition of TNP in association with the host (mouse) MHC (Table II).

Viral-specific CTL activity and CTLp are generated by mixed xenogeneic chimeras in vivo

Mixed xenogeneic chimeras generated measurable CTL responses to Hk×31 influenza A virus in both spleen and MLN compartments (Table III). The CTL response was comparable with that seen in syngeneically reconstituted animals at 10 and 14 days following viral challenge. In striking contrast, fully xenogeneic chimeras exhibited minimal CTL activity directed against influenza A. This was reflected in poor survival with 100% mortality due to infection by day 14. Evaluation of syngeneic and mixed xenogeneic chimeras for the presence of CTLp 2 wk following viral challenge demonstrated significant influenza A-specific CTLp frequencies in the spleen and MLN (Table IV).

Viral-specific CTL activity across a species barrier

To examine whether the viral-specific CTL activity of lymphocytes from mixed xenogeneic chimeras could occur across a species barrier, rat or mouse T cells were isolated from virally infected chimeras using electronic cell sorting, and the virus-specific activity was determined against 51Cr-labeled Hk×31 influenza A virus-infected fibroblast targets expressing the host (B10 mouse) MHC phenotype (Table V). Purified rat and mouse T cells from mixed xenogeneic chimeras each generated significant viral-specific CTL responses against infected host-type targets. These findings demonstrate that both rat and mouse lymphocytes are capable of generating viral-specific CTL activity, which is restricted to the host MHC molecules, even across species barriers.

Discussion

Cross-species transplantation has been suggested as a potential solution for the current shortage of solid organs for clinical transplantation or in clinical situations, which require that the transplanted tissue be resistant to infection with a human-specific pathogen (25). The most publicized applications of cross-species disease resistance are baboon hepatic xenotransplantation for hepatitis and the transplantation of baboon bone marrow in an attempt to achieve immune reconstitution in late stage AIDS (26–28). The major factor preventing successful clinical application of xenotransplantation is the current inability to control the vigorous rejection response, which ensues despite high dose conventional immunosuppression (25, 29). To overcome the limitations of xenograft rejection and the toxicities of immunosuppressive

Table II. Summary of cytotoxic reactivity for mixed xenogeneic chimeras: alloreactivity and restriction

<table>
<thead>
<tr>
<th>Effector</th>
<th>Target</th>
<th>B10</th>
<th>B10-TNP</th>
<th>F344</th>
<th>F344-TNP</th>
<th>BALB/c and ACI</th>
</tr>
</thead>
<tbody>
<tr>
<td>B10</td>
<td>Tolerant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F344</td>
<td>Alloreactive</td>
<td>Not restricted</td>
<td>Tolerant</td>
<td>Restricted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chimera</td>
<td>Tolerant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Alloreactivity and restriction specificity for CTL effectors derived from B10 mouse, F344 rat, or mixed xenogeneic chimera splenocytes were determined using host (B10), donor (F344), and third-party (BALB/c and ACI) target cell populations. Responses were characterized as either “Alloreactive” if cytolytic reactivity could be induced against a given target, or “Tolerant” if such a response was absent. Similarly, antigen presentation was “Restricted” to the MHC of the given TNP-modified target when cytolytic anti-TNP reactivity could be elicited. “Not restricted” characterized failure to induce an effective TNP-specific CTL response.
agents, we have developed a model to achieve stable donor-specific transplantation tolerance across closely related species barriers using bone marrow chimeraism (mouse + rat → mouse). Recipients exhibit excellent survival and stable multilineage mouse and rat chimeraism, and are functionally tolerant to donor skin, islet, and cardiac xenografts in vivo (15–19) and in MLR and CML assays in vitro (20). With the clinical application of mixed bone marrow chimeraism to induce tolerance to xenografts, or for the treatment of diseases such as AIDS, it is critical to establish that immunocompetence for primary immune responses, including antiviral responses, will be preserved.

MHC restriction of T cell reactivity in allogeneic models

MHC molecules play a key role in the functional specificity of cytolytic T lymphocytes (30). Doherty et al. demonstrated that CTL specificity for viral target Ags requires the appropriate MHC product and the inducing viral determinants to be present on the target cell (30). For conventional Ags recognized by T cells, the appropriate MHC products are self MHC determinants (8, 31), as dictated by the non-bone marrow-derived stromal host environment in which the T cells matured, rather than by the MHC molecules expressed on the T cells themselves (31–36).

Shearer originally demonstrated that CTL could also be induced in vitro against syngeneic cells modified by TNP (23). These CTL only lysed TNP-coupled spleen targets in an MHC-restricted fashion, thereby revealing the identity of these self MHC determinants. Thymocytes from parent → F1 chimera (A → A × B) were unreactive in CML to both A and B MHC determinants and recognized TNP in association with both A and B parental haplotype. In contrast, in F1 → parent chimeras (A × B → A), thymic CTLp were restricted to recognition of TNP in association with only the MHC haplotype of strain A, even though they were tolerant to both A and B parental haplotypes (37). Transplantation of thymic grafts from genetically disparate donors into congenitally athymic nude mice demonstrated that for primary immune responses to sheep erythrocytes (SRBC) and TNP-modified target cells, T lymphocytes native to the host cooperated only with accessory cells matched to the allogeneic thymic donor (7). Similarly, both host and donor T cells from mixed allogeneic chimeras were restricted to host APC for primary immune responses (7, 38). These results further demonstrated that self-recognition by developing T cells was determined by the MHC phenotype of the intrathymic environment in which the lymphocytes differentiated. Of significant clinical importance is the fact that the presence of host APC in mixed allogeneic chimeras results in superior immunocompetence, compared with fully allogeneic chimeras, as assessed by SRBC plaque-forming assay, ability to eliminate virus, and overall survival (1, 7, 14, 38–40). Although the exact molecular or cellular events that lead to immunocompetence for primary immune responses have remained elusive, a number of factors, including normal T cell development and intrathymic selection events, are clearly critical to the process. Whether similar selection of the T cell repertoire would occur in a xenogeneic environment has not been demonstrated.

Using a model of mixed xenogeneic bone marrow chimeraism, we have now analyzed the reactivity of rat and mouse lymphocytes for the tolerance specificity and the restriction of Ag presentation across a species barrier. We present data to demonstrate that both mouse and rat lymphocytes that have developed in mixed xenogeneic chimeras are restricted to Ag presentation by mouse, but not rat, APC, as demonstrated in TNP-modified self assays. These data, therefore, establish that both positive and negative selection of the T cell repertoire can occur across a species barrier. Furthermore, the specificity of tolerance present in mixed xenogeneic chimeras is not due to two parallel responses of alloreactivity (mouse anti-mouse and rat anti-rat), but instead represents the competence of each donor phenotype to recognize MHC differences across a species disparity.

**Table IV. Influenza A-specific CTLp frequencies in lymphoid compartments of mixed xenogeneic chimeras**

<table>
<thead>
<tr>
<th>Reconstitution</th>
<th>Reciprocal of CTLp Frequency</th>
<th>Spleen</th>
<th>MLN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syngeneic</td>
<td>3,842</td>
<td>15,682</td>
<td></td>
</tr>
<tr>
<td>Mixed xenogeneic</td>
<td>9,933</td>
<td>8,847</td>
<td></td>
</tr>
</tbody>
</table>

*All mice were infected intranasally with 240 hemagglutinating U of Hk×31 influenza A virus. Normal and virally infected 3HCr-labeled MC57G fibroblasts were used as targets and limiting dilution analysis was performed to calculate CTLp frequency.*

**Positive selection of the T cell repertoire across a species barrier: host MHC restriction in mixed xenogeneic chimeras**

Positive selection chooses T cells that recognize host class I or II MHC molecules and simultaneously regulates development of the phenotypically mature CD8+ or CD4+ T cells, respectively (41).
Analysis of positive selection in a model in which rat T lymphocytes have developed in a xenogeneic mouse environment has not been possible in a non tolerant model. In the present model, in which both mouse and rat T lymphocytes are produced, develop, and differentiate in a lethally irradiated mouse environment (15, 20), both mouse and rat T cells are cotolerant, yet remain functional to recognize MHC-disparate transplantation xenoeigens. It is of interest that the ability to recognize Ag is restricted to presentation by mouse, not rat, APC for all T cells within these chimeras. The presence of reproducible cytolysis of TNP-modified targets from B10 mouse, but not F344 rat, is, we believe, the first demonstration that recognition of conventional transplantation Ags in a closely matched xenogeneic environment is restricted to the MHC haplotype of the thymic host. The response to TNP-modified B10 stimulator cells was virtually identical over all E:T ratios for splenocytes derived from normal B10 mice or mixed xenogeneic chimeras. As 50 to 60% of the chimera splenocytes were of the rat phenotype, failure of these cells to respond to TNP-modified B10 stimulators would have resulted in a lower level of cytotoxicity for a given E:T ratio as compared with the normal B10 effectors. Furthermore, if rat cells from the chimeras were restricted to F344 MHC Ags, a response to TNP-modified F344 stimulator cells by half of the chimeric splenocyte population should have been detectable at 100:1 E:T ratio, especially given the fact that the response of normal F344 effector cells was detectable at a 12.5:1 ratio. This restriction to mouse APC further supports the concept that rat T cells present in the xenogeneic chimeras have developed in the mouse stromal environment, and are not a result of expansion of a very small number of mature rat T cells transferred with the bone marrow inoculum at the time of reconstitution. Therefore, the positive selection events, which are known to shape the T cell repertoire in allogeneic chimeras, are also operative across a species disparity.

Evidence of functional viral recognition across a species barrier in mixed xenogeneic chimeras

A requirement for host APC has been clearly demonstrated for helper-dependent T cell responses in vitro, the generation of plaque-forming Ab responses in vivo, and the antiviral CTL response for vaccine and lymphocytic choriomeningitis virus (LCMV) in allogeneic chimeras (14, 38–40). We have now demonstrated that mixed xenogeneic chimeras are competent to generate viral-specific CTL activity in vitro. More importantly, both mouse and rat CTL are capable of functioning in vivo to generate specific responses to infectious agents, which infect host-type cells, even across species barriers. Although syngeneic reconstitution appeared to favor recruitment of CTLp to the spleen, these values suggest that in response to an infection with influenza A virus, a significant recruitment of viral-specific CTLp to both spleen and MLN can occur in vivo in a mixed xenogeneic environment. This is further reflected in the superior survival of mixed xenogeneic chimeras following a viral challenge compared with the universally fatal outcome seen with fully xenogeneic reconstitution. The recent baboon-to-human bone marrow transplant performed for the treatment of AIDS has brought the immunocompetence of mixed xenogeneic chimeras to the forefront (28). These results suggest that establishing a mixed chimeric state between cells of the AIDS-resistant baboon and the human should result in functional baboon lymphocytes capable of generating a CTL response against infectious agents, if sufficient quantities of human APC are available for Ag presentation.

In summary, the present data suggest that both mouse and rat T lymphocytes that have developed in mixed xenogeneic chimeras are functionally tolerant to both mouse and rat donor Ags, yet are capable of discriminating MHC xenoeigens disparities. Moreover, although cotolerant, both the mouse and rat T cells are restricted to respond to Ag presented by B10 mouse, but not rat, APC, suggesting that positive selection of developing rat T lymphocytes is mediated by the genotypic mouse thymic stroma. The apparent requirement for host APC in the generation of responses to infectious agents, across both allogeneic and xenogeneic barriers, suggests a major clinical advantage for mixed bone marrow chimerism even for xenograft recipients. These observations are important in view of the potential emergence of xenotransplantation for solid organ transplantation, and/or immune reconstitution, in the clinical setting.

Acknowledgments

We thank Michelle Waters, Debbie Shivers, and Gray Lorig for manuscript preparation; Drs. Susan McCarthy, Haval Shirwan, Beate Exner, and Richard Hodas for helpful suggestions; and Marissa Massochetti and the staff of the Central Animal Facility for excellent animal care.

References


