Suppressor T Cells in Mice Made Unresponsive to Skin Allografts

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We have induced suppressor T cells (Ts), capable of delaying allogeneic skin graft rejection, through the intravenous administration of allogeneic spleen cells under normal conditions. H-2 and non-H-2 incompatibility between recipient mice and donor skins induced strong graft rejection. However, when the Ts were transferred into recipient mice, the mean survival time was prolonged for every combination between recipient mice and donor skin. Studies using several strains of congenic mice revealed the antigen specificity of these Ts. Treatment with monoclonal anti-Lyt-2.2 or anti-Thy-1.2 antibody and complement abolished the suppression shown by the Ts of skin graft rejection. The suppression induced by these Ts, however, was resistant to treatment with monoclonal anti-Lyt-1.2, anti-L3T4, or anti-I-A antibody and complement. These results showed that the Ts were Lyt-1+2-, L3T4-, Ia-, T cells. J Invest Dermatol 91:333–335, 1988

In graft rejection, cytotoxic T cells (Tc) and T cells of delayed type hypersensitivity (TDT) are the principal effector cells [1–3]. However, the effective use of alloantigen-specific suppressor T cells (Ts) as a mechanism to prolong survival in allograft transplantation requires further study. We previously demonstrated that 4-hydroxy-3-nitrophenyl acetyl (NP)-specific Ts [4] and allogeneic skin antigen-specific Ts [5] can be induced in mice. Our results showed that afferent-phase Ts (Ts-aff) and efferent-phase Ts (Ts-eff) suppressed antigen-specific immune responses.

The purpose of the present study was to induce Ts by intravenous injection of allogeneic spleen cells and to analyze the characteristics of these Ts in skin graft transplantation.

MATERIALS AND METHODS

Mice The congenic strains used in this study and their H-2 haplotypes are shown in Table I. All the mice were maintained in our colony at the Department of Bacteriology, Yokohama City University School of Medicine, Yokohama, Japan. Only female mice (8–15 weeks old) were used in the present investigation.

Antibodies The ascites forms of hybridoma antibodies 25.9.17 (Ia.m25-specific, Aα-specific), GK1.5 (L3T4-specific) were obtained from the American type culture collection. Monoclonal Th-1.2-specific, Lyt-1.2-specific, and Lyt-2.2-specific antibodies were purchased from Cedarlane Laboratories, Ltd. (Hornby, Ontario, Canada).

Skin Grafting Two pieces of donor flank skin (0.9 cm in diameter) were grafted onto the flank of each recipient. A plaster bandage was used to cover the graft; it was removed on day 7 on average after transplantation. The fate of the grafts was followed by daily macroscopic inspection. Rejection was considered complete when the entire epithelial surface of the graft had undergone necrotic degeneration. Grafts rejected or absent at or before day 4 were regarded as technical failures and were excluded from the group. The results were expressed as the mean survival time ± SEM of grafts in groups of seven to nine mice.

Induction of Ts Mice were injected intravenously with 4×10⁷ allogeneic spleen cells in Hanks' balanced salt solution (HBSS) 7 d before transfer. On day 0, spleen cells were collected from the tolerant mice (donors of Ts) and single-cell suspensions were prepared in HBSS. These cells were used as the source of Ts.

Monoclonal Antibody Treatment Ts were treated with several monoclonal antibodies for 45 min at 37°C and then incubated with fresh rabbit complement for another 30 min at 37°C. The cells were then washed three times with HBSS and resuspended in HBSS at an appropriate concentration for cell transfers.

Adoptive Transfer A suspension of 4×10⁷ Ts was injected intravenously into each appropriate recipient.

Statistics Differences between the experimental and control groups were tested by Student's t test.

RESULTS Induction of Suppressor Cells It has already been reported that Ts can be induced by intravenous injection of antigens or antigen-

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Abbreviations:
ALS: anti-lymphocyte serum
DTH: delayed type hypersensitivity
GVH: graft versus host
HBSS: Hanks' balanced salt solution
MLR: mixed lymphocyte reaction
NP: 4-hydroxy-3-nitrophenyl acetyl
Tc: cytotoxic T cells
Ts: suppressor T cells
Ts-eff: efferent-phase suppressor T cells
Ts-aff: afferent-phase suppressor T cells

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Table I. Mouse Strains Used in This Study

<table>
<thead>
<tr>
<th>Strain</th>
<th>H-2 Haplotypes</th>
<th>H-2 region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H-2 region</td>
<td>K</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>B6.C-H-2 bm.12</td>
<td>b*</td>
<td>b</td>
</tr>
<tr>
<td>C57BL/6.Igh*</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>C3H/He</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>BALB/cJ</td>
<td>g2</td>
<td>g2</td>
</tr>
<tr>
<td>B10.GD</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>B10.AM</td>
<td>h3</td>
<td>h3</td>
</tr>
<tr>
<td>B10.A(4R)</td>
<td>h4</td>
<td>h4</td>
</tr>
<tr>
<td>B10.BR</td>
<td>k</td>
<td>k</td>
</tr>
<tr>
<td>C3H/He</td>
<td>k</td>
<td>k</td>
</tr>
</tbody>
</table>


Coupled cells in several types of experimental systems [6,7]. In the present study, suppressor cells specific for alloantigens were induced by intravenous injection of normal allogeneic spleen cells into mice. Seven days after the intravenous administration of allogeneic cells, the suppressor spleen cells from the recipient mice were adoptively transferred. As shown in Table II, the suppressor spleen cells were adoptively transferred into syngeneic recipient mice. Within 1 h, donor skin was grafted onto the flank of the recipient mouse.

H-2 and non-H-2 incompatibility between recipient mice and donor skin induced strong graft rejection. However, long-lasting survival of C57BL/6. Igh* skin graft in a C57BL/6 recipient (Igh determinant-incompatible) was observed. The Igh gene encodes the immunoglobulin allotypic determinant. When alloantigen induced suppressor spleen cells were transferred into recipient mice, the mean graft survival time was delayed in every combination between recipient mice and donor skins.

Prolongation of Skin Graft in Alloantigen Suppressed, Ts Donor Mice To evaluate graft acceptance or rejection in alloantigen suppressed, Ts donor mice, the following experiment was conducted. As shown in Table III, C3H/He allogeneic skin graft survival time was prolonged in alloantigen suppressed, Ts donor C57BL/6 mice. These results indicate that the suppressor cell donors are tolerant of alloantigens and allow for prolonged skin allograft survival.

Monoclonal Antibody Treatment of Suppressor Cells We clarified the characteristics of the suppressor spleen cells obtained using several monoclonal antibodies and complement (Table IV). The treatment of suppressor cells with monoclonal anti-Lyt-1.2, anti-L3T4, or anti-A* antibody and complement increased the mean survival time. A reduction of mean survival time, however, was produced by treatment of these cells with monoclonal anti-Thy-1.2 or anti-Lyt-2.2 antibody and complement. Thus, we concluded that these suppressor cells were Lyt-1-2* , L3T4+, and Ia- Ts.

Antigen Specificity of Ts In the next experiment, antigen specificity of Ts was evaluated. Several different strains of mouse spleen cells were intravenously injected into BALB/cJ mice. After 7 d, spleen cells from alloantigen tolerant mice were transferred into naive syngeneic mice, and the recipient mice were grafted with C57BL/6 skin. As shown in Table V, C57BL/6 and C57BL/6.Igh* tolerizing spleen cells induced Ts in BALB/cJ mice that inhibited C57BL/6 skin graft rejection, but B6.C-H-2* C3H/He, B10.GD, and B10.BR spleen cells did not. These results indicated that these Ts were able to enhance the prolongation of skin graft rejection in an antigen-specific fashion.

Table II. Prolongation of the Survival of Transplanted Allogeneic Skin Grafts Using Suppressed Spleen Cells

<table>
<thead>
<tr>
<th>Recipient mouse</th>
<th>Donor skin</th>
<th>Difference</th>
<th>Suppressor cells</th>
<th>Mean survival time (days ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>bm12</td>
<td>A</td>
<td>C57BL/6 α bm12</td>
<td>14.7* ± 1.6</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>bm12</td>
<td>A</td>
<td>B10.AM α B10.BR</td>
<td>8.6 ± 1.0</td>
</tr>
<tr>
<td>B10.AM</td>
<td>B10.BR</td>
<td>D</td>
<td>13.8* ± 1.4</td>
<td>7.9 ± 1.1</td>
</tr>
<tr>
<td>B10.A(4R)</td>
<td>C57BL/6</td>
<td>K.A</td>
<td>9.8* ± 0.8</td>
<td>6.2 ± 1.2</td>
</tr>
<tr>
<td>B10.A(4R)</td>
<td>C57BL/6</td>
<td>K.A</td>
<td>14.8* ± 1.5</td>
<td>8.1 ± 0.7</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>C57BL/6</td>
<td>H-2</td>
<td>16.2* ± 1.1</td>
<td>&gt; 60</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>Ig*</td>
<td>Ig*</td>
<td>9.0 ± 1.7</td>
<td>&gt; 60</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>Ig* &gt; 60</td>
<td>Ig*</td>
<td>9.7* ± 0.6</td>
<td>&gt; 60</td>
</tr>
<tr>
<td>C3H/He</td>
<td>B10.BR</td>
<td>non-H-2</td>
<td>7.3 ± 0.9</td>
<td>&gt; 60</td>
</tr>
<tr>
<td>C3H/He</td>
<td>B10.BR</td>
<td>non-H-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6</td>
<td>BALB/cJ</td>
<td>All</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6</td>
<td>BALB/cJ</td>
<td>All</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6</td>
<td>C57BL/6</td>
<td>—</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Various mouse spleen cells were injected intravenously into naive mice. After 7 d, spleen cells were collected from mice. These spleen cells were transferred into recipient mice, which subsequently received grafted allogeneic donor skin.

b P < 0.01.

Table III. Prolongation of Skin Graft Survival in Tolerized Ts Donor Mice

<table>
<thead>
<tr>
<th>Recipient mouse</th>
<th>Donor skin</th>
<th>Suppressor cells</th>
<th>Mean survival time (days ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>C3H/He</td>
<td>C57BL/6 α C3H/He</td>
<td>12.0* ± 1.5</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>C3H/He</td>
<td>C57BL/6 α C3H/He</td>
<td>12.9* ± 0.8</td>
</tr>
<tr>
<td>(C3H/He suppressed, spleen cell donor)</td>
<td>C3H/He</td>
<td>—</td>
<td>11.6* ± 1.6</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>C3H/He</td>
<td>—</td>
<td>7.4 ± 0.9</td>
</tr>
</tbody>
</table>

* See footnotes to Table II.

b P < 0.01.
**DISCUSSION**

In the present study, we induced Ts, capable of delaying allogeneic skin graft rejection, through the intravenous administration of allogeneic spleen cells. Antigen specificity is important in the immune response. Our induced Ts had antigen specificity. This result is consistent with those obtained for distinct Ts-eff with the use of various antigens [5–7].

Following the demonstration by Gershon et al [8] that lymphocytes can have a suppressive effect on the immune response, the role of cellular interactions in the regulation of the response has become one of the major fields of immunologic study. Although suppressor cells have now been induced in many experimental situations, their role in allotransplantation is not reported. Several investigators have examined methods which prolong the process of skin graft rejection. Immunologic tolerance can be induced in immature or adult animals. Induction in immunologically immature animals requires that the animals be exposed to the tolerogen during the tolerance-responsiveness period.

Tolerance in adult animals can be induced in a variety of ways. One is to inject extracts containing histocompatibility antigens of the donor strain repeatedly into adult animals. For this purpose, liver extracts are particularly effective [9]. For the induction of tolerance to weak histocompatibility antigens, such extracts alone often suffice for stronger differences to be produced, however, the treatment has to be combined with one that damages the immune system of the recipient, such as the injection of anti-lymphocyte serum (ALS) [10], injection of hydrocortisone [11], or irradiation [12]. Often ALS treatment alone prolongs the survival of allogeneic grafts subsequently transferred to treated individuals. This prolongation is probably the result of not only T-cell depletion but also activation of suppressor cells [13]. Other ways of achieving unresponsiveness in adult animals is daily injection of lentil lectin into mice [14], or injection into adult rats of antibodies presumably specific for the idiotypes of the T-cell receptors recognizing RT1 alloantigens [15].

However, the characteristics of Ts in allotransplantation have not yet been analyzed. In the present study, our Ts delayed the time of allotransplantation graft rejection for which incompatibility between recipient and donor mice was of H-2d and/or non-H-2 type. It should be emphasized, however, that the survival of skin grafts was prolonged by only a few days. Other methods, utilizing antibodies, ALS, hydrocortisone, or irradiation may therefore be combined with Ts to delay skin graft survival when Ts are not affected by these methods.

**REFERENCES**


