Pentostatin Plus Cyclophosphamide Safely and Effectively Prevents Immunotoxin Immunogenicity in Murine Hosts

Miriam E. Mossoba¹, Masanori Onda², Justin Taylor¹, Paul R. Massey¹, Shirin Treadwell¹, Elad Sharon², Raffit Hassan², Ira Pastan², and Daniel H. Fowler¹

Abstract

Purpose: The success of immunotoxin therapy of cancer is limited by host production of neutralizing antibodies, which are directed toward the Pseudomonas exotoxin A (PE) component. In this proof-of-principle study using a well-established murine model, we hypothesized that a newly developed immune depletion regimen consisting of pentostatin plus cyclophosphamide would abrogate anti-immunotoxin reactivity.

Experimental Design: BALB/c hosts were injected weekly with recombinant immunotoxin (RIT) SS1P, which is an antimesothelin Fv antibody fragment genetically fused to a 38 kDa portion of PE, and has been evaluated in clinical trials. Experimental cohorts received induction chemotherapy consisting of pentostatin (P) plus cyclophosphamide (C) prior to initial RIT exposure; some cohorts received further maintenance PC therapy of varying intensity just prior to each weekly RIT challenge. Cohorts were monitored for T, B, myeloid cell depletion, and for total anti-SS1P antibody (Ab) formation.

Results: Controls uniformly developed anti-SS1P Ab after the third RIT exposure. Induction PC therapy reduced the frequency of hosts with anti-SS1P Ab. Abrogation of antibody generation was improved by maintenance PC therapy: nearly 100% of recipients of intensive PC maintenance were free of anti-SS1P Ab after 9 weekly RIT doses. The most effective PC regimen yielded the greatest degree of host B-cell depletion, moderate T-cell depletion, and minimal myeloid cell depletion.

Conclusions: Induction and maintenance PC chemotherapy safely prevented anti-immunotoxin antibody formation with uniform efficacy. These data suggest that immunotoxin therapy might be used in combination with pentostatin plus cyclophosphamide chemotherapy to improve the targeted therapy of cancer. Clin Cancer Res; 17(11); 3697–705. ©2011 AACR.

Introduction

Immunotoxins (IT) composed of an antibody or antibody fragment linked to various bacterial and plant toxins are being actively developed for the treatment of cancer (1). Our laboratory has focused on the development of recombinant immunotoxins (RITs) in which the Fv portion of an antibody reacting with a tumor cell is directly fused to a 38 kDa portion of Pseudomonas exotoxin A (PE38; ref 1). We have observed a high complete and partial response rate with immunotoxin BL22 in patients with refractory hairy cell leukemia (2, 3). In these clinical trials, because the immune system of these patients is severely impaired, repeated treatment cycles of RIT can be given without neutralizing antibody (Ab) formation. We are also developing an immunotoxin (SS1P) targeting the mesothelin protein highly expressed in mesothelioma, and ovarian, pancreatic, and lung cancers (4–8). However, in patients with mesothelioma, where only a single treatment cycle can be given before antibodies develop, the responses have been much smaller and less frequent than with BL22 (9, 10). Specifically, in two recent clinical trials of SS1P therapy, neutralization of immunotoxin activity was observed in 88% (10) and 75% (9) of recipients. Importantly, such neutralizing Ab was typically present after the first cycle of therapy, thereby limiting the ability to provide a potentially effective number of RIT doses. Formation of neutralizing Ab was also observed in a diphtheria toxin-based clinical trial (11) and a Pseudomonas exotoxin (PE40)–based clinical trial (12). Host immunity to immunotoxins may limit the ability of RIT-based therapies to circulate and thus may represent a major therapeutic obstacle.

Several approaches have been investigated to reduce the immunogenicity of protein therapeutics. For example, protein B-cell epitopes can be masked by polyethylene glycol (PEG) or altered through mutagenesis (13–20).
Clinical trials have shown that Pseudomonas exotoxin (PE)-based immunotoxins mediate effective antitumor responses, thereby adding to the arsenal of targeted agents for personalized cancer therapy. However, host immune reactivity to the PE component generates neutralizing antibodies (Ab) that severely limit an ability to deliver repetitive immunotoxin doses required for optimal antitumor effects. For example, in clinical trials evaluating the antimesothelin PE immunotoxin SS1P, the majority of patients developed anti-SS1P Ab after only one round of therapy. In this proof-of-principle study, we have developed an immune intervention that overcomes this therapeutic obstacle. We show that a newly developed regimen of pentostatin (P) plus cyclophosphamide (C) safely abrogated murine host capacity to form anti-SS1P Ab with 100% efficacy. An ability to deliver repetitive doses of immunotoxin may enhance antitumor effects against a wide range of malignancies.

Materials and Methods

Mice

Female BALB/c mice (8–10 weeks old) were purchased from Frederick Cancer Research Facility. Mice were treated according to an approved animal protocol (MB-075) and maintained in a specific pathogen-free facility. Drinking water was supplemented with 100 mg/L of ciprofloxacin (Sigma).

Drug treatments and immunizations

Pentostatin (1 mg/kg/dose; Bedford Laboratories) and cyclophosphamide (50 mg/kg/dose; Bristol Myers Squibb) were injected intraperitoneally (i.p.) according to the schedules shown in Tables 1 and 2. As shown in Table 1,
a control cohort received immune suppression therapy with rapamycin (Sigma–Aldrich) by i.p. injection at a dose of 3 mg/kg/dose. Mice were injected weekly (i.p.) with SS1P recombinant immunotoxin (RIT; 5 or 10 μg/mouse); SS1P was manufactured in the NCI Laboratory of Molecular Biology according to previous methods (38). Control mice received saline injections in the absence of IT therapy. Between 200–250 μl of blood was drawn into sterile tubes containing 10 μl of heparin; samples were centrifuged for 10 minutes at 14 k rpm. About 100 μl of plasma supernatants were transferred to a new tube and kept at −80°C until analyzed.

**Determination of cell depletion**

Cohorts of mice were euthanized at week 3, 5, or 9 of immunotoxin therapy; spleens were harvested and single cells were quantified. Single cell suspensions were labeled with anti-CD4, -CD8, -B220, -CD11b, or -Gr1 conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), or allophycocyanin (APC; Becton Dickinson, BD) and

**Table 1. The PC regimen for abrogation of anti-SS1P antibody formation: experimental design**

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Week 1 (Induction cycle) Day of week</th>
<th>Weeks 2 and 3 (Maintenance cycles) Day of week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7</td>
<td>1 2 3 4 5 6 7</td>
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<tr>
<td>1</td>
<td>RIT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>RIT</td>
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<tr>
<td>(n = 10)</td>
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<td>2</td>
<td>RIT</td>
<td>RIT</td>
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<td>(n = 10)</td>
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<tr>
<td>3</td>
<td>P&lt;sup&gt;c&lt;/sup&gt;  P  P  P</td>
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<td>S&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>(n = 10)</td>
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**NOTE:** Anti-SS1P antibody was tested a week after last immunotoxin injection.

<sup>a</sup>Mice were immunized with SS1P immunotoxin ("RIT"; 10 μg).

<sup>b</sup>Rapamycin ("R") was administered at a dose of 3 mg/kg/dose (i.p.).

<sup>c</sup>Pentostatin ("P") and cyclophosphamide ("C") were administered (i.p.) at doses of 1 mg/kg/dose and 50 mg/kg/dose, respectively.

<sup>d</sup>Control mice received saline injections ("S"; i.p.).

**Table 2. Long-term administration of the PC regimen for abrogation of anti-SS1P antibody formation**

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Week 1 (Induction cycle) Day of week</th>
<th>Week 2 (Maintenance cycle) Day of week</th>
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<td></td>
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<td>2</td>
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<td>(n = 10)</td>
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<tr>
<td>3</td>
<td>P&lt;sup&gt;b&lt;/sup&gt;  C  C  C</td>
<td>P C  C  C  C</td>
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<td>4</td>
<td>RIT</td>
<td>RIT</td>
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<td>(n = 10)</td>
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**NOTE:** Cohorts 3 and 4 received weekly PC maintenance dosing, as indicated, through week 9 of the experiment. Anti-SS1P levels in the serum were tested at week 5 and week 9 of the experiment.

<sup>a</sup>Mice were immunized with SS1P immunotoxin ("RIT"; 5 μg; i.p. injection).

<sup>b</sup>Pentostatin ("P") and cyclophosphamide ("C") were administered (i.p.) at doses of 1 mg/kg/dose and 50 mg/kg/dose, respectively.
analyzed on a FACS Calibur flow cytometer (BD). At least 10,000 live events were acquired; 7-AAD was used to exclude dead cells.

Immune complex captured—ELISA

An ICC–ELISA format was used to measure the native form of PE38-specific antibodies in plasma. Because the antigen–antibody reaction was carried out in solution, the antigen was kept in its native form (19). Briefly, microtiter plates (Maxisorp, Nalge Nunc) were coated with 100 ng/50 μl/well of mesothelin-rFc in PBS overnight at 4°C (39). In a separate tube, plasma collected from experimental recipients was diluted in blocking buffer (25% DMEM, 5% FBS, 25 mM HEPES, 0.5% BSA, and 0.1% azide in PBS); plasma was serially diluted, starting from 200-fold. After washing plates with PBS containing 0.05%Tween-20, the SS1P-serum mixtures were transferred to individual wells (50 μl/well). The amount of immune complex captured by the mesothelin-rFc protein was detected by rabbit antimouse IgG–HRP (Jackson–Immuno Research Laboratories) and tetramethylbenzidine (TMB substrate kit; Pierce). Each plate contained serial dilutions of anti-PE38 mAb IP30 as a concentration control standard (39). The antidrug antibody concentrations were expressed as mAb IP30 concentrations. A standard curve of the IP30 reactivity was created with a four-parameter logistic curve model by SoftMaxPro 4.0 (Molecular Devices).

Statistics

Student’s 2-tailed t-tests, 2-way ANOVAs, or log–rank tests were used to analyze data. For comparisons between the experimental groups in the immunogenicity study, the Mann–Whitney nonparametric test was used. Values of \( P < 0.05 \) were considered statistically significant.

Results

PC therapy (6-day regimen) depletes host lymphocytes and prevents anti-SS1P antibody formation

In the first experiment, cohorts received three weekly doses of RIT without an immune intervention or in combination with either pentostatin plus cyclophosphamide or rapamycin (experimental schema, Table 1); rapamycin was initially evaluated to prepare for the possibility that both immune depletion and immune suppression might be required to prevent anti-immunotoxin Ab responses. In the setting of fully MHC-mismatched murine HSC transplantation, we found that an 8-day regimen of pentostatin plus cyclophosphamide was required to prevent allograft rejection (24); we reasoned that host reactivity to the RIT would be less robust relative to alloreactivity, and therefore we initially evaluated a truncated 6-day PC induction treatment regimen. In addition, because of our goal of administering multiple weekly doses of RIT and in light of the robust capacity of murine hosts to regenerate immunity (40), we built in a weekly maintenance therapy with PC just prior to each RIT dosing. In previous studies (24), we found that the depth of immune depletion with pentostatin therapy related to the intensity of alkylator coadministration. As such, to assess the degree to which maintenance therapy contributed to abrogation of immunogenicity, we evaluated maintenance therapy that consisted of a single dose of cyclophosphamide or an intensified, double dose of cyclophosphamide. Comparison of drug dosing based on body surface area can determine whether doses in experimental animal studies approximate the human equivalence dose (41). We used pentostatin at 1 mg/kg, which translates to approximately 3 mg/m², which is within the recommended range of 2 to 4 mg/m² used in the clinic (31, 37). Similarly, we used cyclophosphamide at 50 mg/kg, which translates to approximately 240 mg/m², which is within the dose range used in the clinic (30, 37).

One week after the third immunization, recipients of the PC regimen had an approximate two-log reduction in host CD4⁺ and CD8⁺ T-cells and B220⁺ B-cells (Fig. 1A). As anticipated, immune suppressive therapy using rapamycin yielded only modest levels of T and B lymphocyte depletion. The immune interventions appeared to be toxic in this setting, as 3/10 recipients of rapamycin and 4/10 recipients of PC died during the 28-day experimental interval; by comparison, 1/10 recipients of RIT alone died. The control cohort that received RIT without any immune intervention developed an anti-SS1P immune response, as evidenced by positive titers in the ICC–ELISA (Fig. 1B; PE-specific Ab developed an anti-SS1P immune response, as evidenced by positive titers in the ICC–ELISA (Fig. 1B; PE-specific Ab concentration, 4.0 ± 1.7 μg/ml). Immune suppressive therapy greatly reduced anti-SS1P antibody production: the concentration of PE-specific antibodies in rapamycin recipients was 0.007 ± 0.02 μg/ml. By comparison, recipients of the PC regimen did not develop antibodies (PE-specific antibody was not detected in any sample; 0/9). The PC regimen was more effective for the prevention of antibody formation than rapamycin therapy (\( P < 0.05 \)). Because immune depletion therapy with PC was effective in preventing Ab formation, immune suppressive therapy with rapamycin was not further evaluated.

Because the 6-day PC regimen was effective but associated with lethality, we carried out an experiment to compare the safety of the 6-day PC regimen and a truncated 4-day PC chemotherapy regimen. We also considered the possibility that the PC regimen and immunotoxin may each contribute to host toxicity; as such, we also reduced the RIT dose from 10 μg to 5 μg. In this experiment, 100% of recipients in each cohort survived the 28-day experimental interval. Moreover, no significant differences in the percentage of baseline weight were found among the cohorts (data not shown). Based on these safety data, further experiments were conducted with the truncated 4-day PC regimen and the 5 μg dose of RIT.

PC induction and maintenance therapy safely permits immunotoxin dosing without antibody formation through 5 weekly injections

Using the 4-day PC regimen, experiments were carried out to: (1) quantify the balance between PC regimen-associated lymphoid cell depletion versus myeloid cell...
depletion; (2) determine the specific role of maintenance PC therapy, including an assessment of dose intensity; and (3) test whether such PC regimens could permit weekly RIT administration for 5 weeks or 9 weeks without antibody formation. The experimental schema for these experiments is detailed in Table 2.

After 5 weekly doses of RIT, recipients of the induction PC regimen without maintenance PC had had absolute numbers of CD4+ T-cells, CD8+ T-cells, and B220+ B-cells relative to recipients of RIT alone (Fig. 2A). To test whether weekly maintenance therapy with pentostatin and cyclophosphamide would preserve the cell depletion effects achieved by the induction PC regimen, we evaluated two regimens consisting of a single dose of pentostatin combined with either one or two doses of cyclophosphamide (Table 2). Recipients of PC induction combined with standard dose PC maintenance therapy (Table 2, cohort 3) or higher dose PC maintenance therapy (Table 2, cohort 4) had further reductions in both T- and B-cell populations; although these cohorts did not differ in terms of depth of T-cell depletion, the higher dose PC maintenance therapy cohort had more severe depletion of B-cells. Importantly, the level of myeloid cell depletion, as measured by the absolute number of CD11b+ and Gr-1+ cells, was only moderately reduced in the PC cohorts and was reduced to a similar level independent of whether maintenance PC therapy was delivered (Fig. 2A). All mice in each cohort were healthy at this post-5 week time point; survival was 100% (10/10 survival in each cohort).

After 5 weekly doses of RIT, recipients of PC induction therapy alone (without maintenance PC therapy) had a similar magnitude of anti-SS1P formation relative to recipients of RIT alone (Fig. 2B). The anti-SS1P concentration was 62 ± 21 μg/ml in recipients of induction PC therapy and 61 ± 10 μg/ml in recipients of RIT alone. Addition of standard dose PC maintenance therapy to PC induction therapy was effective in reducing antibody formation (anti-SS1P concentration of 3.7 ± 2.9 μg/ml); the beneficial effect of standard dose maintenance therapy was statistically significant (PC induction vs. induction plus standard dose maintenance, P = 0.002). Remarkably, the higher dose PC maintenance therapy was even more effective at preventing antibody formation (0/10 cases of anti-SS1P positivity).

**PC induction and maintenance therapy safely permits immunotoxin dosing without antibody formation through 9 weekly injections**

To further test the stringency of this approach, additional cohorts were continued through 9 weekly administrations of SS1P recombinant immunotoxin. Similar to the results at the 5-week time point, standard dose PC maintenance therapy and higher dose PC maintenance therapy resulted in greater reductions in both T-cell and B-cell populations relative to recipients of induction PC therapy alone (Fig. 3A). Although these maintenance PC cohorts did not differ in terms of depth of T-cell depletion, the higher dose PC maintenance therapy cohort had a greater reduction in B-cell numbers. The direct correlation between
maintenance PC dose intensity and depth of B-cell depletion was therefore identified after both 5 weeks and 9 weeks of RIT therapy. Importantly, the level of myeloid cell depletion, as measured by the absolute number of CD11b+ cells, was only moderately reduced in the PC cohorts and was reduced to a similar level independent of whether maintenance PC therapy was delivered (Fig. 3A); the level of myeloid cell depletion, as measured by the absolute number of Gr-1+ cells, was more severe with maintenance PC therapy, but did not vary significantly between the standard dose and higher dose maintenance cohorts (Fig. 3A).

After 9 weekly doses of RIT, recipients of RIT alone or RIT combined with standard dose maintenance PC therapy developed anti-SS1P antibodies (Fig. 3B; anti-SS1P concentrations of 1290 ± 379 μg/ml and 1687 ± 463 μg/ml, respectively). Addition of standard dose PC maintenance therapy to PC induction therapy was not effective in reducing anti-SS1P antibody formation in a statistically significant manner (anti-SS1P concentration, 569 ± 261 μg/ml; P = 0.075 relative to RIT therapy alone). In marked contrast, recipients of the higher dose maintenance therapy were nearly universally protected against the formation of anti-SS1P antibodies (anti-SS1P concentration, 2.7 ± 2.7 μg/ml; P = 0.0001 relative to RIT therapy alone).

Conclusions

Host immunity to immunotoxin represents an obstacle to the further success of this type of targeted anticancer therapy. This limitation is particularly formidable in relatively chemotherapy naive and immune replete patients such as individuals with mesothelioma (9, 10), but is also observed in heavily-treated, immune-depleted patients with hairy cell leukemia (42) and other B-cell malignancies (2). Host production of neutralizing antibodies occurs against both Pseudomonas exotoxin-based (2, 3, 42, 43)
and diptheria toxin-based agents (11), thereby indicating the broad nature to the therapeutic obstacle. In this manuscript, for the first time, we have utilized an in vivo model to show that stringent host immune depletion averts immunotoxin immunogenicity during repetitive, prolonged immunotoxin dosing. These results establish a proof-of-principle for safe combination therapy using immunotoxins with immune-depleting pentostatin and cyclophosphamide chemotherapy.

The current results extend our recent identification of the PC regimen as a safe and potent immune depletion strategy for the abrogation of murine host rejection of fully MHC-mismatched allogeneic HSC grafts (24). In our previous murine experiments, we found that pentostatin operated synergistically with cyclophosphamide to ablate immune B- and T-cells with relative sparing of myeloid cells; most importantly, we showed that the PC regimen induced more severe host immune functional deficits than fludarabine-based regimens and was more effective in the prevention of allograft rejection. In an ongoing clinical trial (NCT00923845), we are evaluating a pentostatin plus cyclophosphamide regimen to facilitate allogeneic HSC engraftment; in this study, 11/11 patients who received a PC regimen had marked B- and T-cell depletion without significant neutropenia, and each patient achieved allograftengment (D. Fowler, unpublished data). These observations suggest that it may be possible to develop a safe and effective PC chemotherapy regimen for the clinical setting of immunotoxin therapy.

In the current study, we have established that the PC regimen also has great utility for the abrogation of host neutralizing antibody responses against immunotoxin. It is important to note that the therapeutic effect in the current study was achieved with a 4-day immune depletion regimen, which is truncated relative to the 8-day regimen we established as being required for the abrogation of HSC rejection. It is likely that the ability of a truncated PC regimen to prevent antibody production is due to the more severe immune depletion achieved with the PC regimen in comparison to the fludarabine-based regimens.

Figure 3. PC chemotherapy prevents anti-SS1P antibody production after 9 weekly SS1P exposures: role of intensive maintenance PC chemotherapy. Mice were given nine weekly injections of SS1P recombinant immunotoxin (RIT) either without any immune intervention (“RIT” cohort) or with immune suppression consisting of either induction PC chemotherapy alone (PC) or in combination with maintenance PC chemotherapy of standard-intensity (“PC” cohort) or higher-intensity (“PC” cohort) according to the regimens detailed in Table 2; additional control cohorts received either saline injections (“S” cohort) or were left untreated (“NT” cohort) and did not receive RIT. Each cohort had n = 10 recipients. One week after the ninth weekly RIT injection, spleens were harvested and the absolute numbers of CD4+ and CD8+ T-cells, B220+ B-cells, CD11b+ myeloid cells, and A, Gr-1+ granulocytes were quantified by flow cytometry. Plasma was collected at this time point and analyzed for the presence of anti-SS1P antibodies using the ICC–ELISA. *P < 0.05, **P < 0.001.
regimen to prevent anti-RIT responses relates to reduced immunogenicity of the PE toxin relative to alloantigen. These observations have potential translational significance and might inform the design of next-generation immunotoxin clinical trials: that is, the host immune depletion target might be less stringent for the immunotoxin setting relative to the allogeneic HSC transplant setting.

Our experiments also point to the importance of maintenance PC chemotherapy to prevent immunogenicity during prolonged immunotoxin dosing. Indeed, we observed that maintenance PC regimens of higher intensity, which can be readily achieved by increasing the cyclophosphamide dose, were required for complete protection against anti-SS1P formation during nine weekly challenges with recombinant immunotoxin. It should be noted that our experiments were carried out in a tumor-free model. Further evaluation of combination RIT plus PC chemotherapy may be warranted in experiments that incorporate tumor cells, such as xenograft models incorporating the mesothelin-expressing human tumor cell line A431/H19 (43, 44). It is also interesting to note that thymic function, which is the primary driver of immune reconstitution capacity, is robust in mice of the age used in our experiments (40) and relatively constrained in adult humans (45). As such, it is possible that maintenance immune depletion may be less important in the clinic relative to the experimental model that we used.

It is also important to note that recipients of the more intensive maintenance PC chemotherapy had similar levels of CD4+ and CD8+ T-cell depletion relative to the other treatment cohorts, but had more exhaustive and persistent depletion of B-cells. It has long been established that T-cell treatment cohorts, but had more exhaustive and persistent depletion of B-cells. It has long been established that T-cell immunotherapy in mice of the age used in our experiments is robust in mice of the age used in our experiments (40) and relatively constrained in adult humans (45). As such, it is possible that maintenance immune depletion may be less important in the clinic relative to the experimental model that we used.

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**Disclosure of Potential Conflicts of Interest**

I. Pastan has ownership interest as an inventor on a NIH patent for SSIP.

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**References**


25. Vallera DA, Taylor PA, Sprent J, Blazar BR. The role of host T cell subsets in bone marrow rejection directed to isolated major histocompatibility complex class I versus class II differences of bm1 and bm12 mutant mice. Transplantation 1994;57:249–56.


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