LETTER TO THE EDITOR

Pegylated TNF-α Receptor Does Not Prevent Acute Graft-versus-Host Disease in the Dog Leukocyte Antigen-Nonidentical Unrelated Canine Model

It has been suggested that cytokines in general and tumor necrosis factor α (TNF-α) in particular have important roles in the initiation of graft-versus-host disease (GVHD) [1,2]. Several recent publications have indicated that neutralization of TNF-α may be an effective treatment for acute or chronic GVHD [3-5]. Neutralization may be accomplished with a monoclonal anti-TNF-α (infliximab) or by the use of a soluble TNF receptor/immunoglobulin fusion protein (etanercept). An experimental polyethylene glycol (PEG)-treated form of the soluble TNF receptor/immunoglobulin (PEG sTNF-RI) has been recently constructed with the potential of decreasing immunogenicity and extending circulating half-life [6]. PEG sTNF-RI has been tested preclinically in the rat, cynomolgus monkey, chimpanzee, and baboon as a potential inhibitor against manifestations of rheumatoid arthritis in patients [7]. This new TNF-α inhibitor appeared to be an ideal candidate for evaluating the role of TNF-α in GVHD in the preclinical canine model.

We previously observed uniformly fatal acute GVHD in dogs when a dog leukocyte antigen (DLA) nonidentical recipient received 920-cGy total body irradiation (TBI) immediately before hematopoietic cell transplantation (HCT). On average, severe GVHD was observed within 14 days without treatment and delayed to day 20 when dogs were treated with the antimetabolite methotrexate (MTX) [8,9]. In this study, we investigated whether PEG sTNF-RI, administered in combination with MTX, could prevent or delay the initiation of GVHD in the DLA-nonidentical HCT model.

In preliminary in vitro studies, we first tested the cytotoxic effects of recombinant human TNF-α (rhTNF-α) or canine TNF-α, derived from lipopoly saccharide-treated canine peripheral blood monocytes, against the cell line WEHI using a standard tetrazolium dye (3-(4,5-dimethylthiazil-2-yl)-2,5-diphenyl tetrazolium bromide) reduction assay. Canine TNF-α and rhTNF-α were essentially equivalent in inducing cytotoxicity in a dose-dependent manner in the range of 1-8 ng/mL of rhTNF-α (Figure 1). Next, we assessed the inhibitory capacity of PEG sTNF-RI on cytotoxicity of recombinant canine TNF-α and rhTNF-α against WEHI cells. A concentration of 2.0 μg/mL of PEG sTNF-RI inhibited by 50% the cytotoxicity mediated by 2 ng/mL of canine or human TNF-α (Figure 1). Similar concentration ratios of infliximab or etanercept to TNF-α have been shown to inhibit TNF-mediated cytotoxicity against WEHI cells [10].

We then evaluated the effect of PEG sTNF-RI on 5 dogs given 920-cGy TBI followed by HCT from DLA-nonidentical donors. In the first group of 2 recipients, PEG sTNF-RI was administered twice weekly starting on the day after TBI. In the second group of 3 dogs, PEG sTNF-RI was administered every other day, starting the day before TBI. The dose of PEG sTNF-RI was 0.5 mg/kg, equivalent to the dose of etanercept recommended for children for the treatment of rheumatoid arthritis and within the range of doses of PEG sTNF-RI found to be effective in decreasing induced arthritis in Lewis rats [11]. MTX was administered at 0.4 mg/kg intravenously on days 1, 3, 6, and 11 and once weekly thereafter, as previously described [8,9]. HCT consisted of a mean of 4.6 × 10^8 marrow cells/kg (range, 1.8-7.8 × 10^8 marrow cells/kg) infused intravenously immediately after TBI (day 0) and a mean of 2 × 10^8 peripheral blood buffy coat cells/kg (range, 1.25-2.7 × 10^8 peripheral blood buffy coat cells/kg) infused on day 1. Toxicities were mainly gastrointestinal and hematologic owing to the effects of the 920-cGy TBI and MTX and comparable to those observed in historical controls.

The results are presented in Table 1. All dogs engrafted with complete donor chimerism. Four of 5 dogs developed acute GVHD and >30% weight loss, resulting in the need to euthanize between 13 and 18 days, independent of the PEG sTNF-RI treatment regimen. GVHD was confirmed by histopathology on necropsied tissue biopsies. The median number of days to survival was 17.5 compared with 20 days for 35 historical treated dogs in 1 study [8] and 19 days for 29 dogs in another study [9]. Statistical analysis of the data using a 2-sided Mann-Whitney U test compar-
son of nonparametric samples failed to note a significant improvement in survival in the dogs treated with PEG sTNF-RI plus MTX over dogs treated with MTX alone ($P < .05$). The fifth dog was euthanized on day 13, with histopathology confirming herpes virus infection.

Serum levels of PEG sTNF-RI were determined by enzyme-linked immunosorbent assay on blood samples 24 hours after treatment and daily thereafter (Figure 2). The results indicated that mean serum levels of PEG sTNF-RI, although variable for the dogs treated twice weekly and moderately less so for the dogs treated every other day, were comparable between the 2 groups. These levels of PEG sTNF-RI were similar to those reported for the same dose (0.5 mg/kg) of PEG sTNF-RI administered on a weekly basis in chimpanzees [7].

Our results indicate that PEG sTNF-RI in combination with MTX failed to induce graft-versus-host disease (GVHD) in DLA-nonidentical HCT recipients. We believe that the reasons for the lack of a significant improvement in survival with PEG sTNF-RI plus MTX over MTX alone may include a higher incidence of herpes virus infection and, therefore, a need for shorter treatment duration. This hypothesis is supported by the observation that a higher proportion of dogs treated with PEG sTNF-RI plus MTX had evidence of herpes virus infection.

**Table 1.** Comparison of Survival in Dogs Given 920-cGy TBI, Hematopoietic Grafts from DLA-Nonidentical Unrelated Donors, and Postgrafting MTX plus PEG sTNF-RI*

<table>
<thead>
<tr>
<th>Dog</th>
<th>Cause of Death</th>
<th>Grade of GVHD†</th>
<th>Skin</th>
<th>Liver</th>
<th>GI Tract</th>
<th>%Chimerism</th>
<th>GVHD Start (days)</th>
<th>Survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G381</td>
<td>GVHD</td>
<td>4</td>
<td>3,4</td>
<td>3,4</td>
<td></td>
<td>100</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>G488</td>
<td>GVHD</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td></td>
<td>100</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>G549</td>
<td>GVHD</td>
<td>3,4</td>
<td>3,4</td>
<td>3,4</td>
<td></td>
<td>100</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>G574</td>
<td>GVHD</td>
<td>2,3</td>
<td>3</td>
<td>2,3</td>
<td></td>
<td>100</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>G581</td>
<td>CHV</td>
<td>NE‡</td>
<td>NE</td>
<td>NE</td>
<td>&lt; 100</td>
<td>Median</td>
<td>0</td>
<td>13</td>
</tr>
</tbody>
</table>

TBI indicates total body irradiation; DLA, dog leukocyte antigen; MTX, methotrexate; polyethylene glycol-treated form of soluble tumor necrosis factor receptor/immunoglobulin; GVHD, graft-versus-host disease; GI, gastrointestinal; CHV, canine herpes virus; NE, no evidence by pathology.

*All dogs received 920-cGy TBI on day 0. MTX was given intravenously at a dose of 0.4 mg/kg on days 1, 3, 6, and 11. PEG sTNF-RI was given subcutaneously at a dose of 0.5 mg/kg twice weekly on days 1, 4, 8, 11, 14, 17, etc (dogs G381 and G488) or every other day starting day −1 (dogs G549, G574, and G581). Percent chimerism was determined by documentation of donor microsatellite marker polymorphisms in nucleated peripheral blood cells by polymerase chain reaction [14].

†Grade of GVHD, with 4 being the most severe, was confirmed by histopathology of autopsy tissue.
‡Dog was euthanized due to a severe case of CHV.
§Median survival in days.
the model is valid for evaluating GVHD, because it has been used previously to identify clinically relevant drug combinations, such as MTX/cyclosporine [9], MTX/tacrolimus [12], and mycophenolate mofetil/cyclosporine [13]. A possible explanation for lack of efficacy is that the role played by TNF-α in this model of GVHD is relatively minor compared with donor anti-host major histocompatibility antigen immune recognition. In addition, earlier treatment of the recipients with PEG sTNF-RI before irradiation may be effective in limiting a “cytokine storm” after 920-cGy TBI.

ACKNOWLEDGMENTS

This work was supported by grants CA78902 and CA15704 awarded by the National Institutes of Health, Bethesda, MD. We acknowledge the generous gift of PEG sTNF-RI from Amgen Inc, Thousand Oaks, Calif. There was no conflict of interest regarding this work.

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