ELSEVIER

Tolerance Induction With Peripheral Blood Lymphocyte Depletion Using Monoclonal Antibodies: Tip of the Iceberg?

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IMMUNOMODULATION with short courses of monoclonal antibodies (MAb) to deplete CD4 and/or CD8+ peripheral blood lymphocytes results in donor-specific tolerance to heart, skin, and renal allografts in rodents.[1,2] This approach is less successful in transplantation of highly immunogenic organs such as the small bowel.[3] It is also not as effective in larger animals that possess a much larger and more complex immune system.[4] We hypothesized that transplant tolerance in situations where either the graft is of high immunogenicity or the host immune system is complex may require depletion of graft-reactive cells not only in peripheral blood but also in other lymphoid tissues (lymph nodes, thymus, spleen). The first report of successful tolerance to primate renal allografts using an anti-T-cell agent that effectively depletes lymphocytes not only in peripheral blood but also lymph nodes[5] lends support to this hypothesis. In order to arrive at dose schedules of anti-CD4 and anti-CD8 MAbs capable of achieving rat intestinal allograft tolerance, we commenced experiments to determine the efficacy of escalating doses of anti-CD4 and anti-CD8 MAbs in depleting lymphocytes in rat blood and other lymphoid tissues. We present here the results from our initial experiments.

AIMS

To determine the effect of administration of a single dose of 5 mg/kg each of anti-CD4 and anti-CD8 MAbs on lymphocyte subsets in (a) rat blood at 6, 12, 18, 24, and 48 hours and in (b) rat mesenteric lymph nodes, spleen, and thymus at 48 hours.

MATERIALS AND METHODS

Leukocyte preparations were prepared from freshly obtained Lewis rat blood, spleen, thymus, and mesenteric lymph nodes. These were then subjected to two-color staining to detect CD4+, CD8+, and CD3+ cells by flow cytometry. FITC-labeled mouse anti-rat CD3 (IgG3) was used to label CD3+ cells. Biotinylated mouse anti-rat CD4 IgG2a and mouse anti-rat CD8 IgG1 treated with streptavidin-phycoerythrin were used to label CD4+ and CD8+ cells, respectively. Cell sorter two-dimensional dot plots were analyzed to determine T-cell subset proportions in tissues from untreated rats (controls). In the experimental groups, rats were treated with 5 mg/kg each of anti-CD4 (MRC OX 35) and anti-CD8 (MRC OX8) MAbs. Peripheral blood leukocytes were prepared and analyzed at 0, 6, 12, 24, and 48 hours, and lymph node, thymus, and spleen preparations at 48 hours.

RESULTS

The proportions of CD4 and CD8+ lymphocytes in rat blood fell from 40% and 24%, respectively, prior to treatment to undetectable levels 6 hours after MAb administration. The levels remained undetectable till 48 hours. The respective proportion of CD4+ cells in mesenteric lymph nodes, thymus, and spleen were 45%, 32%, and 11% in controls and 35%, 30%, and 10% 48 hours after MAb treatment. The respective proportion of CD8+ cells in mesenteric lymph nodes, thymus, and spleen were 16%, 14%, and 5% in controls and 12%, 12%, and 6% 48 hours after MAb treatment.

DISCUSSION

In this study, a combination of anti-CD4 and anti-CD8 MAbs administered to rats at a single dose of 5 mg/kg each was effective in depleting peripheral blood CD4+ and CD8+ cells from 6 to at least 48 hours. This treatment, however, did not result in any appreciable lymphocyte depletion in the mesenteric lymph nodes, thymus, and spleen at 48 hours.

Higher doses of these MAbs may be successful in achieving a more complete lymphocyte depletion. These experiments are underway. The efficacy of high-dose regimens of MAbs in inducing tolerance to rat intestinal allografts will then be determined.

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