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Cytokine Profile in Graft-Versus-Host Disease After Small Bowel Transplantation

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GRAFT-versus-host disease (GVHD) remains a problematic immune reaction and has been a major concern after the transplantation of lymphoid-rich tissues, such as the small bowel. We recently developed a novel experimental animal model of GVHD.[1] This study describes the histopathologic changes and cytokine profiles of these animals before and after the onset of clinical signs of GVHD.

MATERIALS AND METHODS

Male ACI ($RT1^a$) and Lewis (LEW, $RT1^l$) rats were used as donors and recipients, respectively. Simultaneous orthotopic small bowel transplantation (portocaval drainage) with bone marrow (2.5×10^8 unfractionated cells) infusion was performed under immunosuppression of intramuscular FK 506 1.0 mg/kg/d for 2 weeks (day 0 to 13), followed by a weekly injection of the same dose thereafter. All recipients developed clinical signs of GVHD (skin rash, pile loss, and hyperkeratosis) between 98 and 107 days after transplantation. Recipients were sacrificed after clinical manifestation of GVHD (120 days after transplantation, group 1, $n = 3$) or prior to any overt sign of GVHD (65 days after transplantation, group 2, $n = 3$). Controls comprised FK 506 treated Lewis recipients of isografts, sacrificed either 120 days (group 3, $n = 3$) or 65 days (group 4, $n = 3$) after transplantation. At the time of sacrifice, the cervical lymph nodes (CLN), tongue (TG), graft (GSB), and recipient small bowel (RSB) were harvested for histopathologic examination and semiquantitative cytokine analysis by reverse transcription polymerase chain reaction (RT-PCR) using ^{32}P terminally labeled primer for interleukin (IL)-2, IL-4, IL-10, and interferon (IFN)-γ.[2] β-actin level in each sample was assessed for internal quality control and results were expressed as a ratio of cytokine/β-actin mRNA.

RESULTS

Isografted recipients did not show any histopathologic changes in any sample analyzed. The CLN and TG in group 1 animals showed typical changes of grade II GVHD according to the criteria detailed by Markus et al.[3] All animals from group 2 presented with grade I GVHD in the CLN and TG. GSB in these animals were essentially normal. No abnormality was observed in the RSB.

IL-2 and IL-4 mRNA levels were not increased in any samples. Before the onset of GVHD, levels of IFN-γ mRNA, compared with respective isograft controls, were upregulated in the TG ($0.66 \pm 0.30\%$ vs 0%, $P < .05$) and CLN ($0.30 \pm 0.10\%$ vs 0.01%, $P < .05$). mRNA for IL-10 increased in the CLN ($0.67 \pm 0.21\%$ vs 0.01%, $P < .005$). After the onset of GVHD, IFN-γ were upregulated in the TG ($0.70 \pm 0.29\%$ vs 0%, $P < .01$) and CLN ($0.38 \pm 0.18\%$ vs 0.01%, $P < .03$). IL-10 mRNA increased in the TG ($0.84 \pm 0.20\%$ vs 0.01%, $P < .002$) and CLN ($0.64 \pm 0.05\%$ vs 0.01%, $P < .0001$). GSB and RSB did not show any increased expression of these cytokines.

DISCUSSION

These results demonstrate the correlation between the upregulation of mRNA for IL-10 and IFN-γ, and lymphocyte infiltration in the tongue and cervical lymph nodes of animals with GVHD. Both cytokine and histopathologic changes preceded the clinical manifestation of GVHD. Interestingly, involvement of recipient small bowel was not clearly defined in this study either before or after the onset of GVHD.

In the TH1/TH2 paradigm, cytokines produced by TH1 cells have been associated with rejection and those of TH2 cells have been favored for tolerance induction. However, both sets of TH1 and TH2 cytokines have been shown to appear simultaneously at the site of immune reaction.[2] This study provides another example that upregulation of TH1 and TH2 cytokines was associated with the development and expression of GVHD after small bowel transplantation.

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