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Experimental Animal Model of Graft-Versus-Host Disease (GVHD) After Small-Bowel Transplantation: Characteristics of the Model and Application to Developing Treatment Strategies

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ALTHOUGH graft-versus-host disease (GVHD) has been a major concern after small-bowel transplantation (SBTX), the lack of suitable experimental model has allowed only limited study of GVHD after solid-organ transplantation.[1] We recently developed a clinically relevant experimental animal model of GVHD of fully allogeneic SBTX. This study describes histopathological evolution of the disease, cytokine involvement, responsible donor cells, and preliminary results of treatment using recipient and third-party cell infusion.

MATERIALS AND METHODS

Lewis (LEW, RT1^l) rat recipients received orthotopic SBTX and simultaneous donor bone marrow cell infusion (BMC, 250 $\times$ 10^6) from ACI (RT1^a) rats. Tacrolimus (FK 506, 1 mg/kg per day) was administered on days 0 to 13, then continued as a weekly injection of same dose for 350 days. The number of donor cells in the recipient circulation was determined by flow cytometry using antibodies specific for donor and recipient MHC class I (MAbs MN4-91-6 and 163). Reverse-transcription polymerase chain reaction (RT-PCR) using ^{32}P-labeled primers was used to measure the levels of cytokine mRNA in the graft and recipient tissues.

RESULTS

Donor cells persisted in the recipients for more than 300 days after transplantation and accounted for 5% to 25% of lymphocytes in the circulation. The gradual increase of donor cells in these animals correlated with the appearance of clinical signs of GVHD (skin rash, hyperkeratosis, body weight, and hair loss) with a median onset of 104 days (range 98 to 114 days). These signs of GVHD waxed and waned for the rest of the experimental period, and eventually all animals died of GVHD or other transplantation-related complication, such as infection, with a median survival of 244 days. Cytokine analysis revealed the association of the histopathological changes of GVHD (grades I–III) and the upregulation of IFN-γ, TNF-α, IL-6, and IL-10 in the cervical lymph nodes and skin before and after animals showing clinical signs of GVHD. However, donor and recipient small bowel in this model did not show any increased expression of cytokine mRNA or histopathological changes.

Efficacy of immunomodulatory treatment of GVHD was examined using this GVHD model of SBTX and BMC. In vivo ACI (donor strain)-primed splenocytes were prepared from LEW (recipient strain) or PVG (third-party strain) rats that received simultaneous ACI heart transplantation and splenocyte infusion 10 days previously. Infusion of 2.5 to 3 $\times$ 10^8 in vivo primed splenocytes into GVHD animals 3 to 5 weeks after the onset of the disease was effective. The number of donor cells significantly decreased from pretreatment levels of 25.5 $\pm$ 6.1 and 25.1 $\pm$ 13.5 to 11.0 $\pm$ 6.2 and 12.5 $\pm$ 4.1 at 14 to 23 days after infusion with primed splenocytes from LEW (n = 2) and PVG (n = 4) animals, respectively.

Clinical signs of GVHD were also significantly improved (body weight gain and disappearance of skin rash) in these animals. In other groups of animals, fresh unprimed LEW or PVG splenocytes (3 $\times$ 10^8) were infused into GVHD animals at the same timing after the onset of GVHD. These recipients showed amelioration of clinical signs of GVHD; however, the number of donor cells remained at similar levels.

CONCLUSIONS

This study demonstrates a reproducible and clinically relevant animal model of GVHD and a new approach for GVHD treatment. The currently available experimental GVHD model is artificially based on the one-way paradigm of parent to F1 transplantation, in which the recipient immune system has been paralyzed. Immunomodulatory treatment with in vivo primed recipient or third-party strain splenocytes is based on the two-way paradigm of organ transplantation[2] and will provide a new strategy for the treatment of GVHD.

REFERENCES

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