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Characterization of Immunosuppressive Factors Expressed in Serum by Rat Tolerogenic Liver Transplantation

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ABSTRACT

Background. In a rat tolerogenic model of orthotopic liver transplantation (OLT), recipient serum after OLT (post-OLT serum) possesses strong immunosuppressive activity. This study aimed to identify immunosuppressive factors present in early post-OLT serum.

Methods. Immunosuppressive activity was evaluated in vitro by inhibition of the mixed-lymphocyte reaction (MLR). Autoantigens recognized by MLR-inhibitory IgG were identified by the internal protein sequencing.

Results. Recipient post-OLT serum inhibited MLR, and OLT-inducible IgG was the major immunosuppressive factor. IgG from post-OLT sera (2 to 3 weeks) specifically reacted to 31; 34; and 73-kd autoantigens on spleen cells. The internal sequences of the 31- and 34-kd antigens coincided completely with those of histone H1 molecules. Immunodepletion of anti-histone H1 antibodies (Abs) from early post-OLT serum abolished the MLR-inhibitory activity. Furthermore, rabbit polyclonal Ab-directed histone H1 not only significantly suppressed rat and human MLR but also prolonged survival of heart allografts. Flow-cytometric analysis revealed that some live PVG splenocytes were stained with antihistone H1 Abs, and that these positive cells increased on Con A stimulation. Western blot analysis indicated that several cross-reactive antigens against anti-histone H1 Abs were found in their membrane fraction.

Conclusions. In this study we provide evidence that autoreactive Abs, against histone H1 are a major OLT-induced graft survival factor, and may play at least a part in overcoming the acute rejection phase to establish solid allograft tolerance.

IN THE RAT tolerogenic OLT model (DA/PVG combinations), it has been known that post-OLT serum has an immunosuppressive activity,[1,2] raising the possibility that humoral immunosuppressive factors might mediate tolerogenicity. In this study, we provide evidence that autoreactive Abs against histone H1 are a major immunosuppressive factor induced in early post-OLT serum.

MATERIALS AND METHODS

In an OLT model, donor DA (*RT-1*^{a}) rat liver was implanted into PVG (*RT-1*^{c}) recipients by the cuff method.[3] Post-OLT serum was obtained from recipients at 7 to 83 days after OLT, and the immunosuppressive activity was evaluated by inhibition of the MLR.[4] To test the MLR-inhibitory activity of antihistone H1 antibody (Ab), rabbit antihistone H1 polyclonal Ab (Santa Cruz Biotechnology, Santa Cruz, Calif) was supplemented into the MLR culture. Normal rabbit IgG (Santa Cruz Biotechnology) was used as an isotype control. Rat heterotopic heart transplantation. (HHT) was performed as previously described.[2] Briefly, hearts

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0041-1345/05/$–see front matter
doi:10.1016/j.transproceed.2004.12.290

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from DA rats were heterotopically transplanted into the necks of LEW (*RT-1*^{l}) rats. Antihistone H1 antibody (180 μg) was injected intramuscularly into recipients four times at post-HHT days 0, 2, 4, and 6. A sham control group was treated with saline.

RESULTS

Post-OLT serum significantly inhibited allogeneic cell proliferation, and Western blot analysis demonstrated that IgG was significantly induced in the serum samples. IgG-depleted post-OLT sera failed to suppress MLR, whereas IgG, purified from post-OLT sera significantly inhibited MLR. In addition, IgG purified from post-OLT sera showed dose-dependent MLR-inhibitory kinetics quite similar to those observed in corresponding native sera. These results indicate that OLT-induced IgG antibodies are a major component of MLR-inhibitory factor in post-OLT serum. We hypothesized that the MLR-inhibitory activity of OLT-inducible IgG was executed through recognition of specific antigens expressed on stimulator (DA) or responder (PVG) cells. To test this possibility, we next attempted to detect these antigens. Western blot analysis indicated that early post-OLT serum specifically recognized 31-, 34-, and 73-kd antigens in the PVG spleen cells. To characterize the 31- and 34-kd autoantigens, these proteins were proteolytically or chemically digested, and N-terminal amino acid sequences were determined. A FASTA search revealed that an N-terminal sequence of a 31- or 34-kd fragment coincided completely with those of rat and human histone H1. The MLR-inhibitory activity of post-OLT serum was significantly abrogated by immunodepletion of antihistone H1 Ab, demonstrating that antihistone H1 Abs play a major role in the MLR-inhibitory activity of early post-OLT serum.

We next tested whether rabbit anti-histone H1 polyclonal antibody was able to inhibit MLR. Indeed, antihistone H1 antibodies significantly suppressed rat MLR in a dose-dependent manner. Furthermore, we also found that the antihistone H1 antibodies inhibited MLR culture using human peripheral blood mononuclear cells. We also examined the in vivo immunosuppressive effect of antihistone H1 antibody in a rat HHT model. The graft survival of recipient rats treated with antihistone H1 antibody was prolonged (mean graft survival = 21.5 $\pm$ 7.5 days [n = 6], $P < .005$), whereas all of the control counterparts rejected their heart allografts within 7 to 9 days (n = 10). The MLR-inhibitory action of antihistone H1 antibody may be exerted through the recognition of cell-surface cross-reactive antigens on splenocytes. Flow cytometric analysis revealed that some live PVG splenocytes were stained with antihistone H1 antibodies, and that these positive cells increased on Con A stimulation. Furthermore, Western blot analysis indicated that several cross-reactive antigens (31, 34, and 60 kd) were found in their membrane fraction.[4]

DISCUSSION

We have shown that antihistone H1 Abs, which are transiently induced following liver transplantation,[4] were able to suppress allogeneic immune response. Our recent results suggest that the expression of CD80/86 molecules on DCs and the cytotoxicity of NK cells were downregulated by antihistone H1 antibodies. These results suggest that in a rat tolerogenic OLT model the absence of rejection is due to induction of immature DCs and inhibition of NK cell activity. In addition, our preliminary data show that high-titer antihistone H1 autoantibodies may be associated with tolerance induction. Clinical evidence suggests that antinuclear autoantibodies are associated with the pathogenesis of autoimmune disorders, a finding that is in marked contrast with our data suggesting that these antibodies possess immunosuppressive activity. Thus, it would be of much interest to revisit the pathophysiologic role of antihistone autoantibody and focus on its possible immunosuppressive action.

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