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Histone H1 Vaccine Therapy for Overcoming Acute Rejection in Experimental Organ Transplantation

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ABSTRACT

Objective. In a rat tolerogenic orthotopic liver transplantation (OLT) model, the recipient serum (post-OLT serum) shows strong immunosuppressive activity. In our previous reports, we suggested that autoreactive antibody (Ab) against histone H1 is a major immunosuppressive factor in this serum. The present study sought to determine whether up-regulation of anti-histone H1 Ab by histone H1 vaccination led to tolerance.

Materials and Methods. Using mixed lymphocyte reactions (MLR) and heterotopic heart transplantations (HHT), the alloreactive T-cell responses and allograft survivals of histone H1-immunized rats were compared with those of control rats. Cytokine and cellular profiles were determined by enzyme-linked immunosorbent assay (ELISA) and flow cytometry.

Results. The alloreactive T-cell response of histone H1-immunized rats was significantly lower than that of control rats, although there was no difference in nonspecific T-cell activation between the 2 groups. The allograft survival of histone H1-immunized rats was significantly prolonged after HHT. The major histocompatibility complex (MHC) class II and CD25 molecules of histone H1-immunized rats were significantly down-regulated compared with those of control rats. Moreover, the serum cytokine profile was modified by the immunization with histone H1.

Conclusions. These results suggest that histone H1 vaccination of transplant recipients leads to the production of immunosuppressive factors and the modification of cytokine/cellular profiles.

IN A RAT tolerogenic orthotopic liver transplantation (OLT) model (DA liver into PVG), recipient serum (post-OLT serum) shows immunosuppressive activity.[1,2] We previously reported that autoreactive antibodies (Abs) against histone H1 are a major immunosuppressive factor induced in early post-OLT serum.[3,4] These Abs inhibit the maturation of dendritic cells and activity of natural killer cells.[5] In the present study, we demonstrated that histone H1-immunized rats displayed up-regulation of antihistone H1 Ab and alloreactive T-cell unresponsiveness both in vitro and in vivo.

MATERIALS AND METHODS

PVG ($RT1^c$) and LEW ($RT1^l$) rats were immunized intraperitoneally every 2 weeks with histone H1 50 or 100 μg; Upstate, Charlottesville, Va, USA) in Freund's adjuvant (Wako, Osaka, Japan). Control rats were immunized with PBS or bovine serum albumin (BSA) on the same schedule as the histone H1 group. Sera collected every 2 weeks before immunization were assayed in duplicate for their Ab titer against histone H1 or the presence of cytokines by enzyme-linked immunosorbent assay (ELISA). To examine the effects of histone H1 vaccination on in vitro and in vivo alloreactivity, mixed lymphocyte reactions (MLR) and heterotopic heart transplantations (HHT) were performed, as previously described.[2,3] Splenocytes were incubated with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated Abs against major histocompatibility complex (MHC) class II or CD25 (1 μg; Immunotech, Marseille, France), and analyzed by an EPICS ALTRA Flow Cytometer (Beckman Coulter, Miami, Fla, USA).

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0041-1345/06/$–see front matter
doi:10.1016/j.transproceed.2006.10.078

RESULTS

To evaluate the effect of histone H1 vaccination in MLR conditions of DA ($RT1^a$) stimulator, of PVG responder, we first produced histone H1-immunized PVG rats. After up-regulation of the Ab titer against histone H1, the splenocytes of histone H1-immunized PVG rats were mixed with stimulator DA rat splenocytes. The allogeneic T-cell response of histone H1-immunized rats was significantly reduced by BrdU incorporation (A405 nm) to 0.444 ± 0.122 ($P < .05$) compared with that of control rats (1.020 ± 0.308). However, T-cell proliferation was restored to the control level when histone H1 (5 μg/mL) was added into the MLR culture (data not shown).

Subsequently, we produced histone H1-immunized LEW rats as recipients for HHT. DA heart allograft survival of histone H1-immunized LEW rats was significantly prolonged to a mean ± SD of 22.2 ± 12.0 days ($P = .005$), while all controls rejected their grafts within 14 days.

In order to explore the effects of histone H1 vaccination on cytokine and cellular profiles, we next examined serum cytokine levels and cell surface markers in histone H1-immunized or in control LEW rats. After vaccination the serum interferon-γ level of histone H1-immunized LEW rats was significantly higher (18.6–50.9 pg/mL) than that of control rats (<2 pg/mL), and interestingly, the serum interleukin-10 level was transiently up-regulated (max 210 ± 89.8 pg/mL) when the titer of anti-histone H1 Ab was increased in the serum. The levels of MHC class II antigen and CD25 marker on splenic cells in histone H1-immunized LEW rats were significantly lower (MFI = 34.9 ± 16.2 and 19.4 ± 12.0; $P < .05$) than control rats (63.0 ± 1.28 and 51.8 ± 5.1).

DISCUSSION

In summary, our present results indicated that up-regulation of antihistone H1 Ab by histone H1 vaccination led to down-regulation of alloreactive T-cell responses, resulting from the down-regulation of both MHC class II and CD25 molecules on splenic cells and modification of serum cytokine profiles after histone H1 vaccine therapy. These results suggested that histone H1 vaccination of transplant recipients, which leads to the production of an immunosuppressive factor (antihistone H1 Ab), modifies cytokine/cellular profiles. Further investigations are currently underway to elucidate the immunosuppressive mechanisms of antihistone H1 Abs in both cellular and humoral immunity.

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