Heparinized venous blood was obtained from the eleven donor and twenty-four recipient animals. After ficoll separation, the peripheral sheep PBL (1 × 10⁷ cells/ml). Cell fragments were then pelleted and the

Antibodies:

nine months postoperatively. Thereafter, serum samples were taken at monthly intervals until sacrifice at –80ºC until used. Serum samples of recipient animals were taken at two-week intervals until the third postoperative month.

MATERIALS AND METHODS

Mature outbred black mutton sheep were used as both donor and recipient animals. Forty-eight grafts were procured from both tubias of 12 donor animals by cutting two grafts out of the mid-diaphyseal section of each bone. Grafts were stripped of soft tissues and the bone marrow was removed. The grafts were 30 mm long and used to replace a defect of similar size created in the mid-shaft tubia of the left hind leg. Fixation was achieved with an intramedullary nail. Two animals received a control allograft.

Plasma and Cells:

Heparinized venous blood was obtained from the eleven donor and twenty-four recipient animals. After ficoll separation, the peripheral blood lymphocytes (PBL) were adjusted to a concentration of 1 × 10⁷ cells/ml. The cell suspensions were centrifuged in one-milliliter portions at 10,000 × g for 1 min. The supernatants were discarded, and the pellets stored at –80ºC until used. Serum samples of recipient animals were taken preoperatively and at two-week intervals until the third postoperative month. Thereafter, serum samples were taken at monthly intervals until sacrifice at nine months postoperatively.

Antibodies: The mAbs PT85A (VMR, Inc. Pullman, WA 99163) and H58A (VMR, Inc. Pullman, WA 99163) were used to capture MHC class I gene products. These antibodies recognize sheep MHC class I antigens. After one-dimensional isoelectric focusing (1D-IEF) and Western Blot analysis, the sheep MHC class I gene products were detected by rabbit antisheep IgG (Dianova GmbH, Hamburg, Germany). Two human B-lymphoblastic cell lines (M1 and M2) with known isoelectric points (IP) of the HLA-A and -B antigens served as markers to estimate the relative running positions of sheep MHC class I antigens. To detect sheep alloantibodies against donor MHC class I gene products, alkaline phosphatase (AP) labeled rabbit anti-sheep IgG (Dianova GmbH, Hamburg, Germany) was used in the ELISA format.

MHC class I Typing:

The typing of sheep MHC class I antigens was performed by 1D-IEF originally described by Neeles et al. 1 using solubilized sheep PBL (1 × 10⁷ cells/ml). Cell fragments were then pelleted and the supernatant was transferred to a fresh tube and incubated with 5 µl of mAb PT85A (IgG2a), 5 µl of H58A (IgG2a), and 5 µl of 1 M PMSF. The immune complexes were precipitated by 100 µl of immunomagnetic beads coated with goat anti-mouse IgG. 1D-IEF and Western blot were carried out as described by Kubens et al. 3. The sheep MHC class I 1D-IEF variants were consecutively numbered, starting with the most basic gene products. The sheep MHC class I antigens from donor and recipient animals, samples were run side by side in parallel. Examples of two matched (columns A and B) and two unmatched (columns C and D) donor-recipient pair are shown in Figure 1.

Detection of Donor-Specific Alloantibodies: To capture sheep MHC class I antigens, donor cell lysates (100 µl) were added to microtiter plates and incubated. Unbound antigen was removed by washings. Next, 100 µl of the respective recipient serum were added, and plates were incubated. To detect donor-specific alloantibodies from the recipient serum samples bound to donor MHC class I gene products, 100 µl of alkaline phosphatase (AP) labeled rabbit anti-sheep IgG (1:1000 dilution in PBS) were added to the well and incubated. The substrate (1mM 4-Methylumbelliferylphosphate in 1 M diethanolamin and 0.5 M MgCl₂) was added. The reaction was stopped by 50 µl of 1 M NaOH after 30 min. The fluorescence intensities were measured immediately (Ex.355nm/Em 460 nm). Each serum sample was tested in duplicate. Serum samples of recipient animals taken before bone allograft transplantation and of the donor animals taken at the time of allograft procurement served as specific negative controls. Fluorescence intensities greater than two times that of the specific negative controls were considered indicative of a positive reaction for donor specific MHC class I alloantibodies.

RESULTS

Twenty-two different 1D-IEF variants were detected in the 35 animals analyzed. Only three donor-recipient pairs were perfectly matched for class I MHC antigens. Two class I mismatches were observed in four donor-recipient pairs and the remainder of the animals had one class I mismatch. Therefore, donor-specific MHC class I antibodies could have developed in 22 recipient animals. However, donor-specific alloantibodies were detected in only two recipient animals, both of which had one MHC class I mismatch. One of these two animals received a control allograft. The other animal received a laser-perforated and partially demineralized allograft. In these two recipient animals, antibodies were transient. They were detectable from the third to the fifth postoperative month. No other recipient animal showed evidence of antibodies against the donor as demonstrated by the negative ELISA reactions of post-transplantation sera.

CONCLUSIONS

Cortical bone allografts used in this study did not appear to be immunogenic. Seven of the eight control (untreated) grafts and fourteen of the sixteen treated cortical bone allografts did not stimulate an immune response. These results strongly suggest that humoral antibody stimulation by cortical bone allografts may be diminished or abolished by removal of the soft tissues and the bone marrow in the graft. Others have made analogous observations by demonstrating that the immunogenicity of cortical bone allografts can be reduced by irrigation with Triton-X' or by lipid extraction'.

REFERENCES


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