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The Antigenicity of Serum Proteins and Their Role in Xenograft Rejection

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IT HAS been reported previously that following xenotransplantation, liver secretes proteins of donor origin into the serum of xenograft recipients, thus allowing them to retain their species-specificity.[1] The persistence and the function of hamster xenoproteins in long-surviving hamster → rat liver transplant recipients has already been well established.[2] In the present study, we have extended this concept further by evaluating the possible immunogenicity of donor (hamster) serum proteins. To this end, we have attempted to (1) characterize the hamster serum proteins that elicit an antigenic response in the rat and (2) identify donor proteins in the recipient serum that share common epitopes with those on the cell surface of the vascular endothelium, which are the primary targets of antibody-mediated rejection of vascularized xenografts.

MATERIALS AND METHODS

Animals and Operative Procedures

Syrian hamsters and LEW rats were used as donors and heterotopic heart transplants recipients, respectively. Based on the treatment regimen, the transplant recipients (rats) were grouped as follows: Group I (n = 6): sensitized with hamster serum (HS) alone; Group II (n = 10): sensitized with HS emulsified in Freund's incomplete adjuvant (FIA); Group III (n = 5): sensitized with rat serum + FIA; Group IV (n = 5): FIA alone. For sensitization, three injections of cell-free HS were administered to rats at 14-day intervals, and the challenge hearts were transplanted 5 days after the last immunization.

Immunofluorescence (IF)

Using indirect IF technique, the deposition of rat α-hamster IgG and IgM antibodies was determined by incubating (ex vivo) naive hamster hearts with hyperimmune serum (HIS) obtained from animals in Group II. Similar analyses were also performed in hamster hearts transplanted into rat recipients treated postrevascularization with 2 mL (IV) of HIS.

Immunoelectrophoresis (IEP)

Normal HS was electrophoresed against rat-α-hamster IgG antibodies, the bands of precipitation being indicative of the recognition by the latter of hamster proteins.

Western Blot (WB)

The HS used for this assay was depleted of IgG (by absorption with Protein G; Sigma, St. Louis, MO) and albumin (by absorption with Cibacron Blue 3 GA; Sigma). The treated HS was electrophoresed in SDS-PAGE, transferred onto a membrane, and then stained with Ponceau red or incubated with HIS, or with a similar amount

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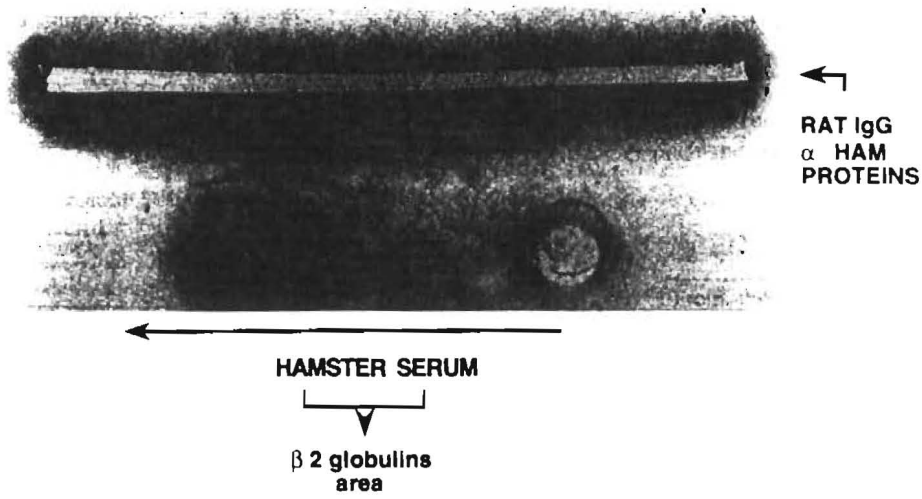

Fig 1. Immunoelectrophoresis of normal hamster serum with that obtained from a rat immunized with hamster sera. There are four distinct bands of precipitation, three of which are in the area of the β2-globulins.

of IgG eluted from rejected (hamster → rat) liver or heart xenografts.

RESULTS AND DISCUSSION

Accelerated (within 48 hours) rejection (AR) of the transplanted hamster heart was witnessed in 50% of Group I and 30% of Group II animals. On the contrary, hamster hearts transplanted into rats in Groups III and IV maintained a strong palpable beat until the day of rejection (day 3 or 4). Interestingly, hearts transplanted into Group I and Group II animals that had escaped AR showed a significantly weaker pulsation with fibrillation by the third postoperative day, suggesting that these organs were undergoing "slow motion" rejection. When tested in IEP assays, the serum obtained from normal hamsters showed four distinct arcs of precipitation, three of which were in the area of β2-globulin (Fig 1). This result indicates that within the serum of normal hamsters there are at least four proteins (and perhaps more), which by virtue of being immunogenic elicit an antibody response after infusion of this serum into naive rats. Western blot analysis using HIS confirmed the presence of four predominant bands with apparent molecular weights of 180, 100, 90, and 48 kDa and four additional bands of relatively lower intensity.

Of greater interest, however, was the recognition by IgG antibodies eluted from rejected hamster liver or heart xenografts of three of the four primary bands revealed by HIS. Immunohistochemistry revealed the deposition (primarily) of rat IgG in normal hamster hearts incubated ex vivo with HIS and also in the hearts transplanted into rats passively treated with HIS. Taken together, the results of this study indicate that even in closely related species such as hamster and rats, there are some serum proteins that may be immunogenic. However, our ability to prolong graft survival (with appropriate immunosuppression) in the hamster → rat strain combination suggests that, despite their obvious immunogenicity, the serum proteins do not play a major role in the initiation of xenograft rejection. Further characterization of these serum proteins is currently underway.

REFERENCES

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