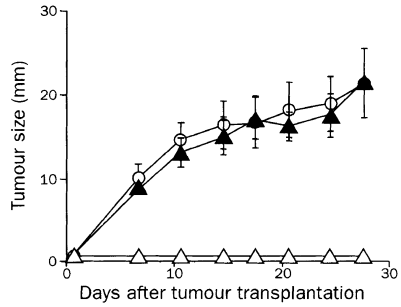
CD95 ligand in graft rejection

SIR — Bellgrau *et al.*[1] suggested that testicular allografts expressing functional CD95 ligand (CD95L) evade rejection by inducing apoptotic death of CD95-expressing recipient T cells activated in response to graft antigens. This implied that CD95L might be useful for creating immune-privileged tissue for a variety of transplant uses (see also ref. 2). We recently obtained a contradictory result indicating that CD95L expressed on the grafts induces a severe inflammatory rejection.

We transplanted a baby hamster kidney (BHK) fibroblast cell line, constitutively expressing transfected human CD95L complementary DNA, into nude mice lacking T lymphocytes. Xenogeneic BHK grew well in nude mice, but its CD95L transfectant was completely rejected (see figure). Administration of a neutralizing anti-CD95L monoclonal antibody[3] reversed the rejection, indicating that it was induced by CD95L. This rejection is apparently independent of cytotoxic T lymphocytes and natural antibodies, as the rejection was also observed in mice with severe combined immunodeficiency, lacking both T and B lymphocytes. Similar rejections were observed when murine CD95L-transfected BHK was transplanted into nude mice and when human or murine CD95L-transfected murine lymphoma cell lines[3] were transplanted into syngeneic or nude mice. *In vivo* depletion of natural killer cells, macrophages or granulocytes by administration of specific antibodies indicated that granulocytes are responsible for the rejection. Consistent with this was the massive infiltration of neutrophils observed in CD95L-expressing tumour grafts undergoing rejection.

Furthermore, CD95L-elicited peritoneal exudate neutrophils were strongly cytotoxic against the CD95L transfectants, and to a lesser extent against the nontransfectants *in vitro*. These results indicate that CD95L recruits neutrophils and activates their cytotoxic machinery, leading to acute graft rejection. Although the mechanism for the CD95L-mediated neutrophil recruitment and activation is unknown, it may involve the induction of interleukin-8 release from epithelial cells[4] and the destruction of neutrophils[5] by CD95L, resulting in local inflammation and non-specific graft damage. Nontransfectants were also rejected when transplanted together with the CD95L transfectants.

Bellgrau *et al.* used an allogeneic system, where graft rejection is mediated mainly by T cells, whereas we used a xenogeneic system, where rejection is mediated mainly by inflammatory neutrophils. However, this does not seem critical, as CD95L-expressing murine tumours were similarly rejected even in the syngeneic recipients. Bellgrau *et al.* used murine testis expressing murine



Rejection of CD95L transfectants. Parental BHK (○) or its CD95L transfectant (△, ▲) were transplanted subcutaneously into nude mice which had received an anti-CD95L monoclonal antibody (▲) or control IgG (△).

CD95L, but we used hamster fibroblasts expressing human CD95L. It has been shown that human, but not murine, CD95L can be released in a functional, soluble form[6], which may act chemotactically against neutrophils. This also does not seem critical, as murine CD95L transfectants were similarly rejected.

Bellgrau *et al.* transplanted grafts into the kidney capsule, whereas we performed subcutaneous grafts. It is well known that the site of transplantation greatly affects graft survival. The kidney capsule generally allows prolonged survival, as in the case of pancreatic islet grafts[7]; subcutaneous islet grafts are rapidly rejected even in syngeneic recipients[8]. The testicular allografts in the kidney capsule may not recruit neutrophils efficiently and may thus have evaded the neutrophil-mediated rejection. Alternatively, some factor other than CD95L, unique to testicular grafts, may be responsible for protection from neutrophils.

Neutrophil-mediated rejection induced by CD95L would severely limit its application for preventing graft rejection. Furthermore, CD95L is efficiently released from the transfectants[3], which could lead to liver damage, as demonstrated by administration of anti-CD95 monoclonal antibody in mice[9]. These serious problems must be solved before CD95L can be used to make immune-privileged grafts.

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BELLGRAU *ET AL.* REPLY — The results reported by Yagita and colleagues are unexpected in the light of recent findings published by our group and by Griffith and colleagues which indicate that CD95L expression by parenchymal cells in the testis and anterior chamber of the eye is required for the well-known immune-privileged status of these organs[1,10]. One prediction of our results is that CD95L could be exploited to facilitate successful transplantation of a variety of tissues[2]. This prediction has been borne out to a certain degree in that testis and the anterior chamber of the eye can protect allogeneic, as well as xenogeneic, tissues that do not express CD95L (for example, pancreatic islets) from graft rejection[11,12]. In direct contrast to our results, the data provided by Yagita *et al.* suggest that CD95L expressed by transplanted cells would provoke graft rejection rather than protect against it.

Freshly isolated neutrophils, as opposed to lymphocytes and monocytes, express high levels of CD95, a type-I membrane protein that transduces an apoptotic signal, and to undergo apoptotic cell death in response to treatment with anti-Fas antibodies[5,13]. A possible explanation of Yagita *et al.*'s results is that the CD95L-transfected tumour cells induced apoptosis of neutrophils, causing a nonspecific inflammatory response that destroyed CD95L-transfected as well as nontransfected tumour cells. Thus, one testable prediction is that *lpr* mice, which have neutrophils lacking functional CD95 expression, would be unable to reject CD95L-transfected tumours, in contrast to our preliminary results that testis tissue from BALB/c mice was rejected when transplanted under the kidney capsule of *lpr* mice[1].

Yagita *et al.* transplanted CD95L-expressing tumour cells subcutaneously, whereas we transplanted CD95L-expressing normal testis and Sertoli cells under the kidney capsule. The fate of CD95L-expressing testis and/or Sertoli cells transplanted subcutaneously is unknown, but preliminary results suggest that this site will not support engraftment of even syngeneic testis tissue (data not shown). Transplantation of various cells and tissues under the kidney capsule is thought to facilitate graft survival through more rapid vascularization than occurs subcutaneously. There is every reason to think that the kidney capsule in our system would contain as many neutrophils as the subcutaneous site chosen by Yagita *et al.*, especially considering the trauma associated with the transplantation procedure itself and the fact that Sertoli cells were transplanted in a fibrin clot.

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