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The Role of Fas/FasL Apoptotic Pathway in the Development of Chronic Rejection

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FAS (CD95), a member of the tumor necrosis factor-receptor family is universally expressed on various tissues of the body.¹ On the contrary, its ligand (Fas L) has a more restricted pattern of expression being found predominantly on activated T cells and on discrete cells in the eye and in the testis.² It has been recently shown that the interaction between Fas and FasL plays an important role in inducing apoptotic cell death.³ Furthermore, interactions of these molecules have also been shown to mediate tissue destruction witnessed during acute cellular rejection (ACR) of organ allografts.⁴ This latter finding has prompted many to evolve novel therapeutic strategies aimed at interrupting Fas/FasL binding to mitigate or abrogate AC R. However, the role of Fas system in the pathogenesis of chronic rejection (CR) is as yet undetermined. To address this issue, FasL-deficient mice were used as donor and/or recipients of aortic allografts; reported herein is the outcome of this study.

MATERIALS AND METHODS

Animals

Inbred male C3H/HEJ (H-2^k), C3H/HEJ FasL^{sid} (FasL⁻), C57B1/6 (H-2^b: B6), and BALB/C (H-2^d) mice were purchased from Jackson Laboratory (Bar Harbor, Me) and housed in a pathogen-free facility for use at 10 to 12 weeks of age.

Experimental Design

The aorta (AO) was transplanted according to a method described previously;⁵ the recipients did not receive any treatment during the postoperative follow-up. Depending on the donor-recipient combination, the animals were divided into four groups (Table 1).

In Vitro Analysis

At approximately 40 days after transplantation, the recipient spleen, serum, and aortic grafts were harvested for in vitro analysis. Using a method described elsewhere,² antidonor cellular and humoral responses were determined by MLR and microcytotoxicity assays, respectively. Additionally, the morphology of the transplanted AO was also evaluated using routine hematoxylin and eosin staining and immunostaining for alpha-smooth muscle actin (α -sMA), elastic fibers and collagen (Verhoeff-van-Gieson's).

Table 1. Experimental Groups and the Observed Outcome in Recipient Aortic Grafts

Groups	Strain Combinations	n	Intimal Proliferation	Antidonor Reactivity	
				Cellular*	Humoral†
I	B6 → B6	5	-	-	-
	C3H → C3H	5	-	-	-
II	B6 → C3H	5	+++	+++	+++
	C3H → B6	5	+++	+++	+++
III	C3H FasL → B6	5	+++	+++	+++
IV	B6 → C3H FasL	5	-/+	+	+++

*Determined by MLR assay.

†Determined by microcytotoxicity.

All analyses were performed at 40 days post-transplantation.

RESULTS AND DISCUSSION

When transplanted across syngeneic barriers, the morphology of the AO harvested at 40 days after transplantation remained unchanged being very similar to that to native AO (Table 1). As anticipated, these syngeneic recipients did not exhibit any donor-specific cellular or humoral responses (Table 1). On the contrary, grafts obtained from wild-type allogeneic recipients (Table 1; group II), had profound intimal thickening which was largely due to proliferation and/or accumulation of α -sMA⁺ cells. Associated with this histological aberration was heightened antidonor proliferative response and the presence of increased titers of circulating donor-specific alloantibodies (Table 1). Analogous observations were also made in B6 recipients of C3H FasL⁻ allografts (Table 1; group III).

On the contrary, the majority of the B6 allografts harvested from C3H FasL⁻ recipients at 40 days after transplantation had remarkable preservation of morphology (Table 1; group IV). This was associated with markedly reduced donor-specific proliferation of enriched T cells, whereas the antidonor humoral responses remained unchanged (Table 1). Taken together, these observations

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suggest that the presence of functionally competent T cells which are capable of initiating a cellular immune response is required to induce changes in an allograft which eventually leads to the development of CR. Furthermore, the presence of high titers of alloantibodies in group IV recipients with remarkable preservation of allograft morphology is an intriguing observation. However, it could be argued that while cellular immune responses play an important role in initiating changes characteristic of CR, alloantibodies are primarily involved in the perpetuation of this lesion.

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