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Combined Blockade of CD28/B7 and CD40/CD40L Costimulatory Pathways Prevents the Onset of Chronic Rejection

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SIGNALING through CD28/B7 and CD40/CD40L costimulatory pathways as a prerequisite for optimal T-cell activation has been well documented.[1,2] Furthermore, unlike that through CD40/gp39, blockade of signaling through the CD28/B7 pathway by perioperative use of CTLA4-Ig fusion protein has been shown to enhance allograft survival and mitigate the development of chronic rejection (CR).[3] In contrast, combined blockage of these two pathways has been shown to abrogate the development of posttransplant vasculopathy in a murine model of cardiac allotransplantation.[1] However, the utility of this latter model to study pathogenesis of CR is limited and, for its acceptance and mitigation of acute cellular rejection, the use of immunosuppressive drugs is required—agents themselves implicated in playing a role in the etiopathology of this lesion. It is for this purpose that we have developed an aortic allotransplantation (AOTx) model of CR in mice in which, for the acceptance of the graft, no immunosuppression is required and in which resultant changes established within 30 days posttransplantation are pathognomonic of CR.[4] Reported herein is the role of blockade of costimulation and the evolution of CR.

MATERIALS AND METHODS

AOTx was performed across the B10 ($H-2^b$)→C3H ($H-2^k$) strain combination by a method described previously.[4] Recipient treatment is detailed in Table 1. In addition to aortic transplantation (Tx) across untreated allogeneic (group B) and syngeneic (group A) recipients, those treated with irrelevant isotype-matched monoclonal antibodies (MAb; group C; human and hamster IgG; Jackson Immuno-Research Lab. West Grove, Pa) were also used as controls. Grafts were harvested at day 30 posttransplantation for histologic and immunohistochemical staining.

RESULTS AND DISCUSSION

Unlike group A, aortic allografts harvested from group B and C animals exhibited marked concentric intimal thickening with corresponding narrowing of the lumen (Table 1). This was accompanied by variable impairment of the elastic membranes and deposition within the intima of fibrous tissue and collagen. The intimal thickening observed in

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Table 1. Effect of Costimulatory Blockade on Development of Chronic Rejection*

Group	Treatment	Morphologic Changes (at Day 30 Posttransplantation)		
		Intimal Thickening	Presence of α-smA+ Cells	Disruption of Elastic Membrane
A	Untreated†	–	–	–
B	Untreated	+++	+++	+++
C	Irrelevant isotype-matched MAb‡	+++	+++	+++
D	CTLA4-Ig§	+++	+++	+++
E	CTLA4-Ig‖	+++	+++	+++
F	Anti-CD40L¶			
G	CTLA4-Ig§ + anti-CD40L¶	++	++	++
H	CTLA4-Ig‖ + anti-CD40L#	–	–	–

* All aortic allotransplants were between B10→C3H strain combination.
† Syngeneic (C3H→C3H) control.
‡ See Materials and Methods for details.
§ Dose of 200 μg IP on day 2 posttransplantation.
‖ Dose of 200 μg IP; 10 doses starting from day 2 and every 72 hours thereafter.
¶ Dose of 250 μg IM on Days 0, 2, and 4 posttransplantation.
# 250 μg IM; ten doses starting on day 2 and every 72 hours thereafter.

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animals in groups B and C were primarily due to accretion of alpha-smooth-muscle-actin (α-smA)-positive cells.

Use of CTLA4-Ig fusion protein alone either in a single (group D) or in 10 (group E) consecutive doses did not preclude the eventual development of CR (Table 1). Similarly, the use of anti-gp39 MAb alone (group F) was ineffective in averting the development of posttransplant vasculopathy. Conversely, treatment of the recipients with a short course of CTLA4-Ig fusion protein and anti-gp39 MAb (group G) resulted in marked diminution in morphologic changes pathognomonic of CR. Complete freedom from the development of posttransplant vasculopathy was, however, achieved in animals in whom treatment resulted in a more prolonged and stable blockade of the CD28/B7 and CD40/CD40L costimulatory pathways (group H; Table 1).

The observations reported suggest that, for the prevention of arteriopathy in clinical organ transplant recipients, prolonged perioperative disruption of costimulatory signaling may be required. This could be achieved by contemporaneous use of CTLA4-Ig fusion protein and anti-gp39 MAb, which, when used in appropriate doses, block signaling between CD28/B7 and CD40/CD40L pathways, respectively, and mitigate development of CR.

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