



Adenovirus-Mediated CD40Ig Expression Attenuates Chronic Vascular Rejection Lesions in an Aorta Allograft Model

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THE ACTIVATION of T cells is a key event leading to chronic vascular rejection. The costimulatory pathway involving CD40-CD40L interactions mediates both T-cell and antigen-presenting cell (APC) activation. CD40-expressing cells such as macrophages, vascular smooth muscle cells, endothelial cells, and B cells receive activation signals following CD40L binding.¹ Blockade of CD40-CD40L pathway using mAb prevents or delays acute allograft rejection processes. However, in murine studies, anti-CD40L mAb treatment did not abrogate the development of chronic rejection unless combined with other treatments.² CD40Ig is a receptor fusion protein including the murine extracellular portion of the CD40 receptor and the human Fc portion of IgG1 that blocks CD40-CD40L interactions. We hypothesized that local and sustained expression of CD40Ig through adenovirus-mediated gene delivery would prevent or attenuate the development of graft arteriosclerosis.

MATERIALS AND METHODS

Rat thoracic aortic grafts from Lew1W (RT1^u) were transplanted in Lew1A (RT1^a) recipients. The aortic grafts were transplanted at the abdominal aorta level using termino-lateral anastomosis at both the proximal and distal levels, with ligation of the native aorta. We analyzed four groups: nontreated allografts ($n = 10$), allografts treated with noncoding adenoviruses (Add1324, $n = 6$), allografts treated with recombinant adenoviruses coding for CD40Ig (AdCD40Ig, $n = 9$), and isografts ($n = 6$). Recombinant adenoviruses were constructed, propagated, and purified as previously described.³ Gene transfer into donor aortic grafts were done using *ex vivo* intra arterial administration of 10^{10} infectious particles of Add1324 or AdCD40Ig. After incubated at 37°C for 40 minutes the transplants were grafted into recipients. At 30 days following transplantation the aortic grafts were harvested for morphometric and immunohistochemical studies. Quantitative morphometric analysis of aortic grafts stained with hematoxylin/eosin (H&E) and von Weigert (elastin staining) were scored using a computer-assisted system (Scion image software). Immunohistological studies were performed on cryostat sections. Infiltrating leukocytes were analyzed using mouse mAb: a mixture of two anti-leukocyte CD45 mAbs, anti-monocytes/macrophage CD68, anti- $\alpha\beta$ TCR, anti-CD4, anti-CD8, and irrelevant mouse mAb (3G8). Thereafter slides were incubated with biotin-conjugated anti-mouse IgG (Jackson Laboratories), followed by HRP-conjugated streptavidin (60 minutes: Vector Laboratories) and VIP substrate. Graft adventitia-infiltrat-

ing leukocytes and their different subtypes were counted using a grid eyepiece as previously described.³

To evaluate systemic humoral and cellular responses of recipients, we evaluated responses to donor alloantigens and to cognate antigens after immunization with sheep red blood cells (SRBC) and keyhole limpet hemocyanin (KLH). SRBC (10^9 in 800 μ L of sterile PBS) were injected IV at the time of grafting. KLH (50 μ g) (Sigma, St. Louis, Mo) was emulsified in CFA and injected in the foot pad at day 22 after grafting. Antibody responses to SRBC and KLH were evaluated respectively at days, 17 and 30 after grafting. IgG and IgM antibody responses to SRBC was determined by cytofluorometry (FACScalibur; Becton Dickinson, San Jose, Calif), and anti-KLH response by ELISA as previously described.³ At day 30 detection of alloantibodies were accomplished using the following protocol: donor strain (Lew 1W) splenocytes that had been stimulated with Con A (7 μ g/mL) for 48 hours were harvested and incubated (30 minutes at 4°C) with heat-inactivated recipient serum serially diluted in PBS. Cells were then washed and incubated with either FITC-coupled anti-rat IgG (Jackson ImmunoResearch) or FITC-coupled anti-rat IgM (Jackson ImmunoResearch). Then cells were analyzed by cytofluorometry and reported as mean channel fluorescence. At day 30, the popliteal lymph nodes of KLH-treated animals were recovered and the lymphocytes cultured (3 days) in the presence of KLH (25 μ g/mL and decreasing doses) prior to pulsing with 1 μ Ci [³H]-Td. At day 30, one-way MLR responses were evaluated using host splenocytes cultured (10^5 cells/well) for 3 days with irradiated dendritic cells (5×10^4 cells/well) from either donor strain (Lew 1W) or third-party rats (Brown Norway, BN, haplotype RT1^b) before being pulsed with 1 μ Ci [³H]-Td.

RESULTS

At 30 days following transplantation, morphometric analysis of aortic grafts showed no significant increase in the intimal area of isografts (620 ± 125 pixels). The intimal area was decreased by 50% among AdCD40Ig-treated grafts (2979 ± 763 pixels) compared to nontreated ($6115 \pm$

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867 pixels) or Ad324-treated allografts (6042 ± 694 pixels) ($P = 0.005$). Adventitial leukocyte (OX1-OX30, CD45) infiltration was significantly decreased namely $39.9 \pm 6.5\%$ versus $17.3 \pm 4.3\%$ ($P = 0.03$) of cell population for control versus AdCD40Ig allografts. T cells were decreased among the adventitial infiltrating leukocytes namely, $6.9 \pm 2.0\%$ versus $1.9 \pm 1.0\%$ ($P = 0.02$) for control versus AdCD40Ig-treated allografts.

Systemic humoral IgG responses to alloantigens were significantly reduced in the AdCD40Ig-treated animals, whereas IgM levels were not affected. IgG levels observed at 30 days for control versus AdCD40Ig allografts were 361 ± 28 MCF versus 208 ± 46 MCF, respectively ($P = .03$). Antibody responses to SRBC and KLH were similar in both control and treated groups. Cell proliferative responses against alloantigens of either donor or third-party haplotypes were not affected in the AdCD40Ig group. Furthermore, the cellular proliferative response to KLH were also unaffected by the AdCD40Ig treatment.

DISCUSSION

Local delivery and expression of CD40Ig effectively attenuates graft arteriosclerosis development in rat aortic allografts. Ex vivo treatment of aortic grafts with AdCD40Ig

was associated with decreased infiltration by leukocytes and T cells. Graft-restricted expression of CD40Ig was associated with a slight but significant reduction in IgG alloantibodies at 30 days. However, the antibody responses to cognate antigens were not affected by local expression of CD40Ig. Furthermore, cellular proliferative responses in CD40Ig-expressing animals were not affected, indicating the functional capacity of the host immune system to respond against cognate antigens following gene transfer and local expression of immunomodulating molecules.

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