



Short Communication

Role of Adhesion Molecules in Chronic Allograft Rejection

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Abstract. Endothelial adhesion molecules play an important role in T cell recruitment to an allograft site. Therefore, it could be expected that their blocking may be beneficial for allograft survival. In this report, we show that T cells from patients with chronic rejection have an up-regulated ability to adhere to inflamed endothelium *in vitro*. Furthermore, this enhanced T cell: endothelial interaction could be blocked by anti-VCAM and anti-E-selectin monoclonal antibodies.

Key words: anti-VCAM-1; anti-ICAM-1; anti-E-selectin; endothelium; adhesion T cells; chronic rejection.

Introduction

Recent studies have emphasized the role of cell adhesion molecules (CAM) in tissue infiltration by leukocytes^{7, 8}. This phenomenon seems to be of particular importance in the immunopathology of allograft rejection, where graft-infiltrating T cells contribute decisively to transplant destruction. Therefore, factors interfering with T cell: CAM interactions should be beneficial in controlling allograft rejection^{1, 5, 6}.

In this communication, we have examined the influence of monoclonal antibodies targeting cell adhesion molecules on T cell: endothelial interactions in renal allograft recipients with stable graft function as well as chronic rejection.

Materials and Methods

Patients. T cells were isolated from 8 healthy blood donors, 13 recipients of a kidney allograft with an une-

ventful stable course (RAR-S) 56 ± 7 months post grafting (mean serum creatinine level 1.65 ± 0.11 mg/dl) and 6 with biopsy-proven chronic rejection (RAR-CH) 58 ± 13 months post grafting (mean serum creatine level 2.5 ± 0.4 mg/dl).

The patients were on a triple-drug therapy (prednisone 5–22.5 mg/day, azathioprine 75–175 mg/kg/day, and cyclosporine 2–3 mg/kg/day).

Mononuclear cells (MNC) and T cells isolation. Mononuclear cells were obtained from heparinized blood by Ficoll-Isopaque gradient centrifugation. A T cell-enriched fraction ($>90\%$ CD3⁺ cells, $\pm 5\%$ monocytes) was obtained by MNC passage through nylon-wool columns.

Adhesion of T cells to endothelium. We examined the adhesion of peripheral blood PMA (phorbol 12-myristate 13-acetate) -activated T cells to cultured resting and activated ($10 \mu\text{g/ml}$ LPS *E. coli*, serotype 055: B5, SIGMA) human dermal microvascular endothelial cells (HMEC-1) obtained from Emory Univer-

Abbreviation used: CAM – cell adhesion molecules, HMEC-1 – human dermal microvascular endothelial cells, monoclonal antibodies – anti-ICAM-1, anti-VCAM-1 and anti-E-selectin, RAR-S – recipients of kidney allograft with stable function, RAR-CH – recipients of kidney allograft with chronic rejection.

sity School of Medicine, Atlanta, Georgia. Monoclonal anti-ICAM-1, anti-VCAM-1 and E-selectin antibodies (Genzyme), in a concentration of 5 µg/ml, were used to block T cell: CAM interactions. Thereafter, the cultures were stained with 1% methyl blue and the number of T cells adhered assessed using inverted microscopy (Nikon)⁴.

Statistical analysis. The Mann-Whitney test and Statgraph software were used to determine statistical significance.

Results and Discussion

To determine the role of adhesion molecules in chronic graft rejection we used the monoclonal antibodies: anti-ICAM-1, anti-VCAM-1 and anti-E-selectin.

As shown in Fig. 1, 2 and 3, chronic rejection is associated with an increased binding of T cells to inflamed endothelium. Both anti-VCAM and anti-E-selectin antibodies diminished the enhanced T cell: endothelial reactivity (but not statistical significance). In contrast, anti-ICAM antibody caused only negligible effects.

In the normal kidney, monoclonal antibodies against VCAM-1 react with parietal epithelial cells of Bowman's capsule, with a cell in the proximal tubule, whereas vessels are devoid of VCAM-1. During acute renal graft rejection the expression of these adhesion molecules increased. E-selectin is not observed in normal renal tissue, but their expression increased during allograft rejection in capillaries and larger vessels. During this time, the measurement of circulating adhesion

molecules increased for ICAM-1 (3.5–5 fold) and (6 fold) for VCAM-1.

Our study highlights the role of enhanced T cell: endothelial interactions in chronic rejection, pointing to the significance of VCAM-1 and E-selectin in T cell recruitment to the site of the allograft. It may be ex-

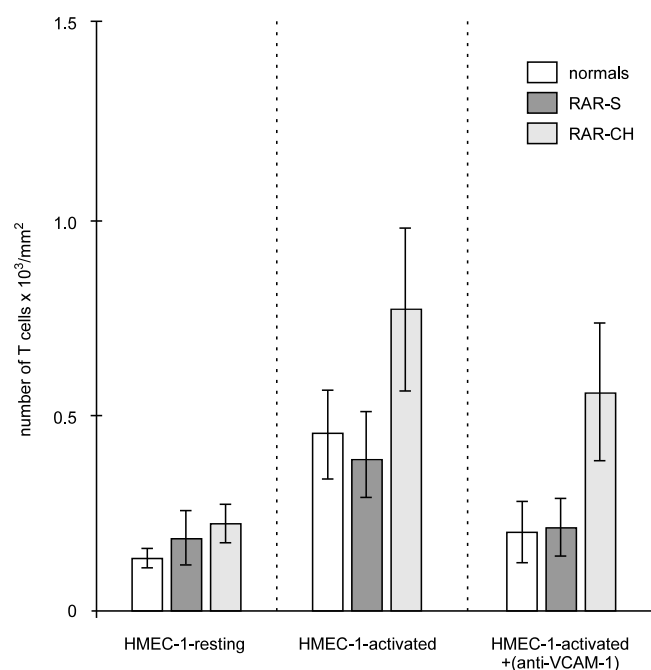


Fig. 2. The influence of anti-VCAM-1 antibody on adhesion of T cells to resting and activated HMEC-1 (data are presented as mean ± SEM)

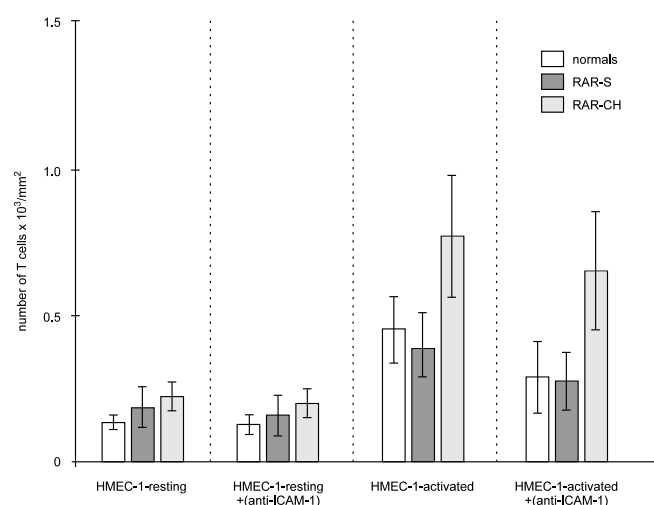


Fig. 1. The influence of anti-ICAM-1 antibody on adhesion of T cells to resting and activated HMEC-1 (data are presented as mean ± SEM)

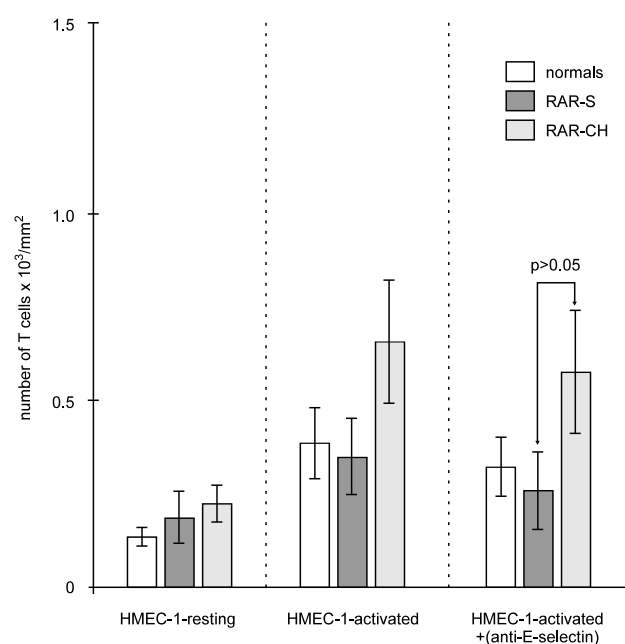


Fig. 3. The influence of anti-E-selectin antibody on adhesion of T cells to resting and activated HMEC-1 (data are presented as mean ± SEM)

pected that these results could lead to the introduction of a more efficacious means of the therapy of chronic rejection based on the clinical application of monoclonal antibodies against endothelial adhesion molecules in human transplantation^{2, 3, 6}.

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