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Immune Accessory Functions of Human Endothelial Cells Are Modulated by Overexpression of B7-H1 (PDL1)

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ABSTRACT: B7-H1 (PDL1) is a B7-related protein that inhibits T-cell responses. Human endothelial cells (EC), which can support polyclonal stimulation (by anti-CD3 or Phytohemagglutinin (PHA)) or direct alloantigen stimulation of T cells, basally express B7-H1 and increase expression in response to IFN-γ or coculture with allogeneic T cells. Previous studies have suggested that endogenous B7-H1 on EC reduces T-cell responses. We engineered overexpression of B7-H1 in EC (B7H1-EC) to evaluate whether this manipulation could reduce T-cell responses even further. Compared with green fluorescent protein-transduced EC (GFP-EC), B7H1-EC support less anti-CD3 or PHA-induced proliferation of CD4+ memory T cells; naive CD4+ T-cell or CD8+ T-cell responses were less inhibited. The effect of transduced B7H1-EC was more apparent when the EC were fixed prior to coculture, a manipulation that reduces the strength of costimulation and prevents upregulation of the endogenous B7-H1 molecule. T-cell activation markers, including CD25, CD62L, CD152 (CTLA-4), and CD154 (CD40L), were not altered by EC overexpression of B7-H1, whereas there was a reduction in CD69. B7-H1 reduced secretion of IL-2 and IL-10 by memory T cells. B7H1-EC were less able to stimulate allogeneic proliferation of CD4+ memory T cells than control EC. These data suggest that B7-H1 overexpression may be a useful approach for reducing allogeneic CD4+ memory T-cell responses to EC. *Human Immunology 67, 568–578 (2006).* © American Society for Histocompatibility and Immunogenetics, 2006. Published by Elsevier Inc.

KEYWORDS: Endothelial cells; T lymphocytes; cellular proliferation; transplantation

ABBREVIATIONS

EC	endothelial cell(s)
GFP-EC	EC transduced with a retroviral vector expressing enhanced green fluorescent protein
B7H1-EC	EC transduced with a retroviral vector expressing human B7-H1
PD1	B7-H1 receptor

INTRODUCTION

Cell-mediated immune responses are initiated by T lymphocytes, which respond to cognate peptides bound to MHC molecules on the surface of an antigen-presenting cell (APC). Specific recognition of the cognate peptide is mediated by the clonally expressed T-cell receptor (TCR), which upon binding the peptide-MHC complex, can deliver an activation signal (signal one) to the T cell via the TCR-associated CD3 complex [1]. Each individual TCR is selected in the thymus to recognize only a few structurally similar nonself peptides associated with a particular self-allelic form of an MHC molecule. However, many TCRs display cross-reactivity with a variety of peptides bound to nonself (allogeneic) MHC molecules, a phenomenon called direct allorecognition [2]. Signal one can also be provided experimentally by antibodies that bind to the TCR by CD3 antibodies or by certain lectins, such as PHA. Effective activation of T cells requires additional signals, provided by nonpolymorphic costimulators on the surface of the APC (signal two), *e.g.*, CD58 (LFA-3), which interacts with T cell CD2 [3] or CD80 (B7-1), and CD86 (B7-2), which interacts with T cell CD28 [4] as well as by cytokines (signal 3), *e.g.*, IL-2 or IL-15. In many instances, signal

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one and signal two activate T cells to produce sufficient IL-2 (signal three) for autocrine stimulation of growth in the absence of exogenous cytokine [5]. T-cell activation is generally self-limited, in part because activated T cells express receptors, such as CD152 (CTLA-4) [6] or programmed death 1 (PD1) [7, 8] that mediate inhibitory signals from the APC. CD152 binds B7-1 or B7-2, *i.e.*, the same ligands that signal positively through CD28, whereas PD1 is engaged by unique proteins, designated B7-H1 (PDL1) and B7-DC (PDL2 or B7-H2) [9–12].

Naive T cells are normally activated by foreign antigenic peptides in secondary lymphoid organs where antigens are presented by dendritic cells (DC) [13]. In contrast, effector memory cells may be activated directly in peripheral tissues, allowing them to conduct immune surveillance for the presence of nonself peptides resulting from infections. In humans, vascular endothelial cells (EC) display both class I and class II MHC molecules, potentially increasing the efficiency of immune surveillance. Specifically, we and others have proposed that human EC present peptides derived from microbial proteins produced locally in infected tissues to CD4+ (via class II MHC) and CD8+ (via class I MHC) effector memory cells as they pass through the microcirculation [14, 15]. Human EC can also provide signal two, largely through CD58 (LFA-3) [16, 17]. In addition, it has been reported that human EC can express B7-H1 (PDL1) which, in this context, serves to inhibit purified CD4+ or CD8+ T-cell synthesis of IL-2 and IFN-gamma [18, 19]. In the case of transplantation, direct allorecognition of nonself MHC molecules displayed on graft EC may initiate destructive immune response by memory T cells [20, 21].

We wondered if allogeneic transplantation could benefit further from modifying graft EC to constitutively express B7-H1 (PDL1), and if these engineered EC would effect markers of T-cell activation, *i.e.*, proliferation. To explore this hypothesis, we used retrovirus to modify cultured human umbilical vein EC, a cell type capable of providing costimulation to polyclonally activated T cells (*e.g.*, by antiCD3 mAb or PHA), and of stimulating an allogeneic cytokine and proliferative response from both CD4+ and CD8+ memory T cells [22–25]. Our results show that this strategy is partially effective and could be useful in conjunction with other therapeutic manipulations.

MATERIALS AND METHODS

Cell Isolation

Human umbilical vein EC were isolated from discarded tissue in accordance with a protocol approved by the Yale University Human Investigations Committee and serially cultured as described previously [26]. EC were cultured in M199 with 20% fetal bovine serum (FBS); 200 μM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (all from Invitrogen, Carlsbad, CA); 50 μg/ml EC growth factor (Collaborative Biomedical Products, Bedford, MA); and 100 μg/ml porcine heparin (Sigma, St. Louis, MO). All experiments were performed using EC at passage 3 or 4.

Peripheral blood mononuclear cells (PBMCs) were obtained from healthy volunteers by density-gradient centrifugation of leukapheresis products, as described previously [27]. PBMCs were depleted of monocytes by adherence to human plasma fibronectin-coated petri dishes for 45 minutes 37°C. CD4+ and CD8+ T cells were isolated from the decanted nonadherent PBMCs by positive selection using Dynabeads (Dynal, Lake Success, NY), which were routinely >98% positive. Naive (CD45RA+) or memory (CD45RO+) subsets of CD4+ T cells were isolated by further negative selection. CD4+ T cells were incubated with antiCD45RA mAb (B-C15, 1 μl/10^7 cells) or antiCD45RO mAb (UCHL-1, 5 μl/10^7 cells) (Biosource, Camarillo, CA), washed, incubated with magnetic beads coated with goat antimouse IgG antiserum (Dynal), and recovered by magnet. Cells were routinely >90% positive for CD4 and CD45RA, and >98% positive for CD4 and CD45RO. Isolated T cells were >98% viable as shown by trypan blue exclusion.

Real Time Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from EC cultures with an Absolutely RNA kit as per manufacturer's instructions (Stratagene, La Jolla, CA). EC were treated or not with 50 ng/ml IFN-γ (Biosource), 10 ng/ml IFN-α (R&D Systems, Minnesota, MN), 10 ng/ml Oncostatin M (R&D), or 10 ng/ml IL-4 (Sigma), with or without 10 ng/ml tumor necrosis factor (TNF) (R&D) for 24 hours at 37°C. Where indicated, EC were treated for 2 hours at 37°C with the transcriptional inhibitors, 5, 6, dichlorobenzimidazole riboside (DRB) (100 μM) or actinomycin D (10 μg/ml) (Sigma), prior to cytokine treatment.

RNA was reverse transcribed using TaqMan Gold RT-PCR kit and cDNA was subjected to qRT-PCR using SYBR Green PCR kit (both from Applied Biosystems, Foster City, CA), and primers developed (Primer3, [28]) for B7-H1 (**AF233516**) (B7H1 [107 bp]: 5′-dGCCGAAGTCATCTGGACAAG-3′ and 5′-dTCTCAGTGTGCTGGTCACAT-3′) on an iCycler (Bio-Rad, Hercules, CA, USA). Products were confirmed by melt curves and sequence. Standard curves utilized PCR templates isolated with QIAquick PCR Purification kit (Qiagen, Valencia, CA). Transcripts reported as copies per ng RNA as a percent of GAPDH ($\times 10^3$).