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Choice of resident costimulatory molecule can influence cell fate in human naïve CD4+ T cell differentiation

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ABSTRACT

With antigen stimulation, naïve CD4+ T cells differentiate to several effector or memory cell populations, and cytokines contribute to differentiation outcome. Several proteins on these cells receive costimulatory signals, but a systematic comparison of their differential effects on naïve T cell differentiation has not been conducted. Two costimulatory proteins, CD28 and ICAM-1, resident on human naïve CD4+ T cells were compared for participation in differentiation. Under controlled conditions, and with no added cytokines, costimulation through either CD3+CD28 or CD3+CAM-1 induced differentiation to T effector and T memory cells. In contrast, costimulation through CD3+ICAM-1 induced differentiation to Treg cells whereas costimulation through CD3+CD28 did not.

1. Introduction

Naïve CD4+ T cells are quiescent, non-activated cells recently emigrated from the thymus with the ability to travel between blood and the lymphatic system in search of cognate antigen. Recognition of antigen triggers differentiation to any of several types of effector and memory cells. It is accepted [1,2] that naïve T cells are successfully activated by a series of signals delivered by Ag appropriately presented to the TCR (signal 1) plus a costimulatory signal (signal 2). Signal 2 is received by engagement of any of several proteins resident on the T cell surface and the best studied of these is CD28. Equally accepted is the concept that specific cytokines delivered to the intercellular milieu can influence outcome of the differentiation event. As examples, the Th1 cytokine IFNγ influences differentiation to Th1 cells whereas Th2 cytokines such as IL-4 foster Th2 cell differentiation [3]. So evidence to date suggests that full activation of the naïve T cell by the two signals, augmented by specific cytokines, guides the choice of differentiation pathway.

It also has become clear that a multiplicity of costimulatory molecules participates differentially to regulate activation of T cells as they become effector cells and during cell survival and outgrowth. As was reviewed recently [4], costimulatory proteins from the CD28 family (e.g. CD28 and ICOS), and the TNFR family (e.g. CD40/CD40L, 4-1BB, CD27/CD70, GITR/GITR-L) participate in activation of T cells and are opposed by co-inhibitory proteins (e.g. PD-1, CTLA-4). The participation of counter receptors on opposing cells in delivering these signals implicates the local cellular microenvironment as participating in differential regulation and evokes the possibility of specialized stimulation [reviewed in 5,6]. The best studied example of this concept is the ability of engagement of the T cell surface protein CTLA-4 (CD152) to divert cell activation induced by costimulation through CD28 by competition for the same counter receptors (CD80 and CD86) [7] on antigen presenting cells. It is of interest to learn the degree to which additional proteins constitutively expressed on a naïve T cell contribute to the activation process and to learn which additional sets of stimuli might participate in determining the ultimate fate of the differentiating naïve T cell.

The surface phenotype of naïve T cells has been partly characterized in human and mouse with overlapping results. Human naïve T cells express TCR/CD3, CD4 or CD8, and the accessory molecules CD45RA, CD28, LFA-1, CCR7, CD62L, CD27, CD2, VLA-4, [reviewed, 8,9] as well as ICAM-1 (Intercellular Adhesion Molecule-1, CD54), a subject of the present work. Mouse naïve T cells express these same surface molecules; however, mouse naïve T cells express the CD45RB isoform and are generally characterized as $CD44^{dim}$. After activation, the profile of cell surface molecules expressed by the naïve T cell changes. Expression of, for example, ICOS, 4-1BB, OX40, CD40L, CTLA-4, and PD-1 is induced, some existing molecules such as CD28, LFA-1, and ICAM-1 are upregulated, some like CD27 can be downregulated, and CD45 isoform

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expression changes [reviewed, 8,10]. Interactions between CTLA-4 or PD-1 and their ligands are generally thought to attenuate T cell activation.

Our laboratory is investigating the hypothesis that costimulatory proteins expressed on resting naïve T cells, can differentially influence cell fate as these cells differentiate after encountering cognate antigen. We previously reported that stimulation of T cells through resident ICAM-1 can serve as a legitimate costimulatory event [11] and that costimulation of human naïve CD4+ T cells through ICAM-1 induces formation of effector and memory T cells with the same efficacy as costimulation through CD28 in an *in vitro* system [12]. Because use of antigen presenting cells to provide signals 1 and 2 engages several additional surface proteins, we isolate stimulation to only the specific T cell surface proteins of interest by stimulating with immobilized Ab. The present manuscript uses this *in vitro* system of human naïve CD4+ T cell differentiation to suggest that the choice of costimulatory molecule can influence cell fate and indicates a new role for ICAM-1 as a co-inducer of human regulatory T cell differentiation.

2. Materials and methods

2.1. Cell purification

Human naïve CD4+ T cells were isolated from peripheral blood of healthy donors using Ficoll-Paque PLUS (GE Healthcare, Piscataway, NJ) density gradient centrifugation followed by negative selection using Human Naïve CD4+ T Cell Enrichment Kits (StemCell Technologies, Vancouver, BC) as we have described previously [12]. Naïve cells for this study were defined as CD4+ CD45RA+ CD45RO(−)CD11a^{lo}CD27+ CCR7+ CD62L+ and routinely were >98% CD45RA+ as determined by flow cytometry (example in Fig. 1A). Cells were cultured in complete RPMI 1640 medium (Mediatech, Herndon, VA) containing 10% FBS (Atlanta Biologicals, Lawrenceville, GA), 2 mM L-glutamine, 50 units/mL penicillin, and 50 μg/mL streptomycin (Invitrogen, Carlsbad, CA).

2.2. Antibodies and reagents

Hybridoma producing anti-ICAM-1 (R6.5D6) was obtained from ATCC (Manassas, VA) and in early experiments antibodies were purified from serum-free hybridoma cultures; later Ab were obtained fromBioXCell (West Lebanon, NH). Anti-CD3ε (OKT3) was either purified from serum-free hybridoma culture (ATCC) or was purchased from eBioscience (San Diego, CA). The anti-CD3 antibodies from both sources were used with similar results. Anti-CD28 (ANC28.1) from Ancell (Bayport, MN) or anti-CD28 (CD28.2) from BD Biosciences (San Diego, CA) were used with similar results. Anti-human Foxp3-FITC and anti-human Foxp3-PE (clone PCH101) were from eBioscience and used with the accompanying Fixation/Permeabilization reagents. In some cases, anti-Foxp3-PE (clone 3G3) was from MiltenyiBiotec (Auburn, CA). Anti-CD25-FITC, anti-CD25-TriColor, anti-CD11a-FITC, anti-CD27-PE, and anti-CD45RA-TriColor were from Caltag Laboratories (Burlingame, CA). Anti-CD152-PE (CTLA-4) and anti-CD127-PE were from BD Biosciences. Isotype control antibodies for anti-Foxp3 were Rat IgG2a-FITC (eBioscience) and Rat IgG2a-PE (Caltag) or mouse IgG1-PE (Caltag), and isotype control antibody for anti-CD45RA-Tri was Mouse IgG2b-TriColor (Caltag). Ab used for cytokine blocking were anti-IL-10 (eBioscience), anti-IL-2 and anti-TGF-β1 (R&D Systems Minneapolis, MN). Controls in these experiments were rat IgG1 and mouse IgG1 (eBioscience). CFSE (5-(and-6)-carboxyfluoresceindiacetate, succinimidyl ester) was from Molecular Probes (Carlsbad, CA) and used at 2.5 μM. Flow cytometry was performed using a FACScan (BD, San Jose, CA) or an Accuri C6 (AccuriCytometers, Ann Arbor, MI). Data analysis was performed using CellQuest software (BD), CFlow (Accuri) and FlowJo software (Tree Star, Inc., Ashland, OR).

2.3. Naïve CD4+ T cell stimulation

Stimulation of human naïve CD4+ T cells was performed using plate-bound antibodies. As we have described previously [12], all stimulating antibodies were first titrated to determine the lowest concentration that provides maximum stimulation. Antibodies in PBS were adhered to tissue-culture treated 96-well plates (Midwest Scientific, St. Louis, MO) by incubation at 37 °C for 2 h. Wells were washed 3 times with PBS to remove unbound antibody. Antibodies were used at the minimum concentrations that resulted in maximum T cell proliferation (unpublished observations): anti-CD3 (1 μg/mL), anti-ICAM-1 (10 μg/mL), and anti-CD28 (2–5 μg/mL). Cells were stimulated at 1.5×10^6 cells/mL in 200 μL of complete RPMI 1640 with no exogenous cytokines added.

2.4. Cytokine ELISA

Cell culture supernates were collected from stimulated cultures and used after clarification by centrifugation. IL-10 production was measured using Human IL-10 ELISA Ready-Set-Go kits (eBioscience, San Diego, CA) or Human IL-10 Quantikine kits (R&D Systems, Minneapolis, MN). Levels of secreted TGF-β1 were determined using Human TGF-β-1 Quantikine kits (R&D Systems). Plates were analyzed using an Automated Microplate Reader (BioTek, Winooski, VT) and DeltaSoft software (BioMetallics Inc., Princeton, NJ).

2.5. Suppression assay

Naïve CD4+ T cells were stimulated for 10 days using anti-CD3 plus anti-ICAM-1. On Day 10, the stimulated cells were spun over Ficoll-Paque (GE Healthcare, Piscataway, NJ) to remove dead cells. The CD4+ CD25+ Treg cells were separated from the CD4+ CD25(−) cells using CD4+ CD25+ Regulatory T Cell Isolation Kits (MiltenyiBiotec, Auburn, CA). Also on Day 10, fresh peripheral blood was again obtained from the same donor and second bleed total T cells were isolated using Ficoll-Paque density centrifugation and a Human T Cell Enrichment Kit (StemCell Technologies, Vancouver, BC). The cultured CD4+ CD25+ Treg cells, the cultured CD4+ CD25(−) cells, and an aliquot of second bleed total T cells to be used as a control were each stained with PKH26 dye (Sigma, St. Louis, MO) at 2.5 μM concentration. An aliquot of second bleed total T cells to be used as responders was labeled with CFSE (Molecular Probes, Carlsbad, CA) at 2.5 μM concentration. Subsequently, the cells were cultured at Treg (or Control) Cell: Responder Cell ratios of 1:1, 1:2, and 1:4. Co-cultured cells were stimulated for 5 days using anti-CD3 plus anti-CD28 antibodies as described above (3×10^5 cells/well). Proliferation of the CFSE-labeled responder cell population was assessed using flow cytometry by gating out the PKH26-labeled Treg or control populations and analyzing proliferation of the CFSE-labeled responder population.

2.6. Human subjects

Peripheral blood cells were obtained after informed consent of healthy volunteers. Procedures were approved by the University of Kansas Institutional Review Board.