Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 342 (2006) 782–790
BBRC
www.elsevier.com/locate/ybbrc

Upregulation of ICOS on CD43+ CD4+ murine small intestinal intraepithelial lymphocytes during acute reovirus infection

Dina Montufar-Solis [a], Tomas Garza [a], Ba-Bie Teng [b], John R. Klein [a,*]

[a] Department of Diagnostic Sciences, Dental Branch, The University of Texas Health Science Center at Houston, Houston, TX 77030, USA
[b] Research Center for Human Genetics, Institute of Molecular Medicine, The University of Texas Health Science Center at Houston, Houston, TX 77030, USA

Received 3 February 2006
Available online 17 February 2006

Abstract

Murine intestinal intraepithelial lymphocytes (IELs) can be classified according to expression of a CD43 glycoform recognized by the S7 monoclonal antibody. In this study, we examined the response of S7+ and S7− IELs in mice during acute reovirus serotype 3 (Dearing strain) infection, which was confirmed by virus-specific real-time PCR. In vivo proliferation increased significantly for both S7− and S7+ IELs on day 4 post-infection as determined by BrdU incorporation; however, expression of the inducible costimulatory (ICOS) molecule, which peaked on day 7 post-infection, was upregulated on S7+ CD4+ T cells, most of which were CD4+8− IELs. In vitro ICOS stimulation by syngeneic peritoneal macrophages induced IFN-γ secretion from IELs from day 7 infected mice, and was suppressed by treatment with anti-ICOS mAb. Additionally, IFN-γ mRNA increased in CD4+ IELs on day 6 post-infection. These findings indicate that S7− and S7+ IELs are differentially mobilized during the immune response to reovirus infection; that the regulated expression of ICOS is associated with S7+ IELs; and that stimulation of IELs through ICOS enhances IFN-γ synthesis during infection.

Keywords: Cell activation; Cell surface molecule; Intestinal; T cell; Rodent; Viral

CD43 is expressed at high density on the surface of bone marrow cells, thymocytes, and peripheral T cells and some B cells [1–3]. Functionally, the CD43 molecule contributes to T cell recognition and activation [4–7] and has costimulatory activity with TCR/CD3 stimulation [8–12]. As part of our studies aimed at defining effector and regulatory populations of T cells in the gut epithelium, we recently determined that small intestinal intraepithelial lymphocytes (IELs) can be divided according to whether they express or lack a CD43 glycoform recognized by the S7 mAb to mouse CD43 [13]. That reagent demarcates two functional groups of IELs such that S7+ cells produce significantly more Th1 and Th2 cytokines, are more cytotoxic, and undergo more homeostatic proliferation than CD43 S7− IELs [13]. Additionally, CD43 S7+ IELs from IL-10−/− mice with intestinal inflammation spontaneously produce IFN-γ in the terminal ileum [13]. Consistent with those findings, gene array profiles of CD43 S7+ and CD43 S7− IELs revealed that CD43 S7+ IELs express genes of activated T cells (*cd6*, *cd44*, *cd97*) and genes of T cell-associated chemotactic and/or inflammatory mediators (*CCR2*, *CXCR3*, *MIP-1*β) [13]. In contrast, CD43 S7− IELs preferentially express NK activating/inhibitory receptor genes (*Ly49E-GE*, *Ly49G.2*, *CD94/NKG2*, *Ly49H*, *Ly49C*). Thus, CD43 S7+ IELs exhibit properties typically associated with adaptive immunity, whereas S7− IELs exhibit properties of innate immunity [13].

Respiratory enteric orphan virus (reovirus) is a non-enveloped RNA virus. Both serotype 1 (Lang strain) and serotype 3 (Dearing strain) have been extensively used for murine studies of enteric virus infection given that both elicit strong anti-viral immune responses [14–16]. Oral infection of adult mice with reovirus serotype 1 or 3 generally

* Corresponding author. Fax: +1 713 500 4416.
E-mail address: john.r.klein@uth.tmc.edu (J.R. Klein).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.02.031

results in a self-limiting infection [17]; however, a role for the immune response in curtailing the infectious process is evident from studies in severe-combined immunodeficient (SCID) mice, which develop systemic reovirus infections leading to death of the host [17]. Anti-reovirus IEL cytotoxic T cell responses mediated principally by TCRαβ IELs develop in mice following oral infection with reovirus type 1 [18], and antigen-specific T cells and CTL have been shown to develop in response to reovirus types 1 and 3 infection [19–21]. Peyer's patch dendritic cells appear to play a role in antigen presentation to CD4+ T cells [22]. The participation of gut γδ T cells in immunity to reovirus remains unclear and natural killer cells, although present in significant numbers in the mouse intestinal mucosa, do not appear to contribute to the protective immune response to reovirus as witnessed by susceptibility of SCID mice [17], which have high levels of natural killer cells.

The goal of the present study was to determine the extent to which CD43 S7+ and S7− IELs are involved in the intestinal immune response to reovirus infection. In so doing, we have evaluated the in vivo proliferative changes of S7+ and S7− IELs during infection, and have examined the functional contribution of the ICOS costimulatory molecule in the local immune response to infection. As reported here, ICOS expression was upregulated on S7+ CD4+ IELs at day 7 post-infection. Moreover, stimulation of IELs through ICOS at day 7 post-infection enhanced IFN-γ synthesis. These findings indicate that S7 is an effective marker for differentiating murine IELs according to functional activity during a natural intestinal immune response in ways that have not been approached hitherto.

Materials and methods

Mice and reagents. Adult female C57BL/6 mice, 6–8 weeks of age, were purchased from Harlan Sprague–Dawley; Indianapolis, IN. Mice were housed under specific pathogen-free conditions. Animals were used according to protocols approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at Houston. Cell culture medium consisted of RPMI-1640 supplemented with FCS (10% v/v) (Invitrogen; Carlsbad, CA), 100 U/ml penicillin–streptomycin, 2 mM L-glutamine, and 5×10^{-5} M 2-ME (all reagents; Sigma–Aldrich, St. Louis, MO).

IEL isolation and sorting. Isolation of small intestine IELs was done in a manner similar to that described previously [13,23] with some modifications. Briefly, small intestine tissue pieces were stirred gently at 37 °C in 50 ml of Ca^{2+}/Mg^{2+} free PBS containing 2 mM DTT and 5 mM EDTA for 30 min. The tissue slurry was separated into two 25 ml volumes and each was passed through two 10 cc syringe barrels partially filled with wetted nylon wool. Each cell suspension was collected by centrifugation, mixed in 3 ml of 40% isotonic Percoll (Amersham Biosciences; Uppsala, Sweden), layered onto 70% isotonic Percoll, and centrifuged at 600*g* for 30 min. IELs were collected from the 40/70% interfaces, washed with cell culture medium, resuspended in 40% Percoll overlayed onto 70% Percoll, and centrifuged again at 600*g* for 30 min. IELs were collected from the 40/70% Percoll interface; this cell preparation was consistently 75–85% pure for IELs.

Enrichment of CD4+ IELs was done by autoMACS sorting (Miltenyi Biotec; Auburn, CA) by first conducting a negative sort to remove epithelial cells using the G8.8 mAb [24] as previously reported by our laboratory [13]. This yielded a cell preparation with 98–99% IEL purity. Positive autoMACS cell sorting was done by labeling cells with biotin-anti-CD4 mAb followed by streptavidin-beads (Miltenyi Biotec) to enrich for a population of CD4+ IELs, from which RNA was isolated for real-time PCR analysis of IFN-γ gene expression.

Virus preparation and animal infection. Reovirus type 3 (Dearing strain) was purchased from the American Type Culture Collection (Manassas, VA). Virus stocks were grown in L929 (American Type Culture Collection; cat. No. CCL-1) cell monolayers in RPMI-1640 supplemented as described above. Tissue supernatants were collected from monolayers showing 50–60% cytopathic effect; supernatants were clarified by centrifugation and virus titers were calculated to be $10^{7.5}$ pfu using a virus plaque assay with L929 cells. Mice were anesthetized by IsoFlo (Abbot Laboratories; Chicago, IL) inhalation and infected by oral gavage with $10^{7.5}$ pfu reovirus type 3 Dearing strain.

Antibodies and flow cytometry. Antibodies used in this study were: FITC-anti-TCRβ (H57.597), PE-anti-TCRδ (GL3), biotin- and PE-anti-CD43 (S7), FITC-anti-BrdU, FITC-anti-CD8α (53-6.7), FITC-anti-CD8β (53-5.8), anti-CD16/32 (2.4G2), streptavidin-APC (all reagents, BD-PharMingen; San Diego, CA), FITC-anti-CD4 (BVD6-24G2), PE- and purified functional grade anti-ICOS (7E.17G9); biotin-anti-B7RP-1 (HK5.3), biotin-anti-CD4 (L3T4) (all reagents, e-Bioscience; San Diego, CA). Flow cytometric analysis was done on a FACSCalibur flow cytometer using CellQuest software (BD Bioscience; San Jose, CA).

In vivo BrdU labeling. Mice were injected i.p. twice at 24-h intervals with 1 mg of 5-bromo-2′deoxyuridine (BrdU) (Sigma–Aldrich) suspended in PBS. Forty-eight hours after the last injection, IELs were isolated and 1×10^6 IELs were reacted with anti-CD16/32 mAb for 15 min at 4 °C followed by PE-anti-CD43 S7 for 20 min at 4 °C. Cells were washed, and stained for BrdU incorporation using a BrdU staining kit (BD-PharMingen #559619). Cells were suspended in Cytofix/Cytoperm for 15 min at room temperature in the dark, washed, reacted with Cytoperm Plus for 10 on ice in the dark. Cells were washed with Perm/Wash and reacted with FITC-anti-BrdU for 20 min at room temperature in the dark. Cells were washed in Perm/Wash, resuspended in staining buffer, and analyzed for CD43 S7 expression and BrdU incorporation by flow cytometry.

IFN-γ assay and ICOS blockade experiments. IFN-γ synthesis was measured using IELs from day 7 C57BL/6 reovirus-infected mice. Cells were isolated from the small intestine and cultured with a transformed peritoneal macrophage line (PM) of C57BL/6 origin (ATCC number CRL-2458). PM cells were irradiated with 3000 rad, washed, and added to cultures at an IEL:PM cell ratio of 10:1 using 1.5×10^6 IELs in 2 ml of supplemented medium in a 24-well tissue culture plate. Interactions between IELs and PM cells mediated by ICOS were disrupted by the addition of 5 μg/ml anti-ICOS mAb to IELs for 10 min prior to culture with PM cells. Cell-free supernatants were harvested after 24 h and IFN-γ levels were measured by ELISA (e-Bioscience).

Q-RT-PCR. Intestinal tissues were collected from the mid-duodenum, mid-jejunum, and mid-ileum. Disruption and homogenization of tissues were performed with an Omni Tissue Master 240 (Omni International; Marietta, GA) for 30 s in RLT Buffer containing β-ME (Qiagen; Valencia, CA). Total RNA was isolated from 7 mm^3 (⩽30 mg) of intestinal tissue using an RNAeasy Protect Mini Kit-50; samples were treated with DNase using the RNase-Free DNase Set-50 (Qiagen) according to the manufacturer's protocol. RNA concentration was estimated spectrophotometrically at A_{260}. Forward and reverse primers (Integrated DNA Technologies; Coralville, IA) for reovirus type 3 were based on the design by Besselsen and co-worker [25], with a target amplicon size of 72 bp using a forward primer sequence (5′-TGATTTCCATTACTTCTGCTGCTT-3′) in nucleotide positions 1085–1108, and a reverse primer sequence (5′-TCCTGTTCACGATTCCATCAGAT-3′) in nucleotide positions 1156–1134 on the reovirus 3 (Dearing) sequence (GenBank Accession No. M27262). The validity of those to amplify a reovirus product was confirmed using 10-fold dilutions of RNA from reovirus-infected 3T3 cells, from which a standard curve was generated. IFN-γ primers (GenBank Accession No. NM_008337) were purchased from SuperArray Biosciences (Frederick,