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Proinflammatory Vδ2+ T Cells Populate the Human Intestinal Mucosa and Enhance IFN-γ Production by Colonic αβ T Cells

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In nonhuman primates, Vγ9Vδ2* (Vδ2) T cells proliferate and accumulate in mucosal tissues following microbial activation. Human Vδ2 T cells produce proinflammatory cytokines in response to bacterial species that colonize the gut, but the role played by Vδ2 T cells in intestinal immunity is unknown. We hypothesized that circulating Vδ2 cells can populate the human intestine and contribute to mucosal inflammation. Cell suspensions prepared from peripheral blood and intestinal biopsies were stimulated with microbial phosphoantigen (1-hydroxy-2-methyl-2-buten-4-yl 4-diphosphate [HDMAPP]) and analyzed by flow cytometry to determine Vδ2 T cell phenotype, cytokine production, and proliferative potential. Circulating Vδ2 T cells expressed gut-homing integrin α4β7 (>70%), which was coexpressed with skin-associated cutaneous leukocyte Ag by up to 15% of the total population. However, Vδ2 T cell activation with HDMAPP and exposure to retinoic acid (signaling via retinoic acid receptor α) increased α4β7 expression and enhanced binding to mucosal addressin cell adhesion molecule-1 in vitro while simultaneously suppressing cutaneous leukocyte Ag, thereby generating a committed gut-tropic phenotype. Confocal microscopy and flow cytometry identified frequent Vδ2 T cells that migrated out of human intestinal biopsies and comprised both CD103+ and CD103− subsets that produced TNF-α and IFN-γ upon phosphoantigen exposure, with more frequent cytokine-producing cells in the CD103+ population. Activated intestinal Vδ2 T cells expressed CD70 and HLA-DR but were unable to drive the proliferation of allogeneic naive CD4+ T cells. Instead, phosphoantigen-activated CD103− Vδ2 T cells increased T-bet expression and enhanced IFN-γ production by autologous colonic αβ T cells via an IFN-γ–dependent mechanism. These data demonstrate that circulating Vδ2 T cells display enhanced gut-homing potential upon microbial activation and populate the human intestinal mucosa, generating functionally distinct CD103+ and CD103− subsets that can promote inflammation by colonic αβ T cells. The Journal of Immunology, 2013, 191: 2752–2763.

The Vγ9Vδ2* (Vδ2) T cell is a type of innate lymphocyte found only in humans and higher primates (1, 2). Although Vδ2 cells typically comprise <5% of total T cells in adult human blood, this population can expand rapidly in response to a wide range of pathogens and is thought to play a key role in human antimicrobial immunity (3–6). The nonpeptide phosphoantigen metabolites that activate Vδ2 T cells are highly conserved among intestinal pathogens and are prevalent across numerous species of bacteria resident within the commensal microbiota (5, 7, 8). Alkylamine Ags derived from edible plants, apples, tea, and wine have also been reported to stimulate Vδ2 T cells (9). Although long presumed to contribute to mucosal barrier protection in man, the mechanisms by which Vδ2 T cells respond to phosphorylated metabolites are only now being uncovered (10), and the role played by Vδ2 T cells in human intestinal immunity remains unknown.

Human γδ T cells constitute only a minor population in the circulation but are substantially enriched in the epithelial layers of barrier organs including the skin, respiratory tract, and intestinal mucosa (2). Although Vδ2+ T cells predominate in blood, the γδ T cell population of the gut epithelium is largely comprised of Vδ1+ cells (11). Vδ1+ T cells play well-documented roles in intestinal homeostasis, epithelial barrier protection, and tumor surveillance (12–14). In contrast, the study of Vδ2 T cells at mucosal barrier sites has been limited by the absence of these cells in rodent models; hence, little is known about Vδ2 T cell function in peripheral tissues. However, because in vivo activation of macaque Vδ2 T cells leads to marked proliferation, IFN-γ release, and mucosal accumulation of these cells (15, 16), it is likely that Vδ2 T cells can also contribute to antimicrobial responses in the human intestine.

Human Vδ2 T cells accumulate in the peritoneal cavity in bacterial peritonitis (17), traffic from the blood to the inflamed skin...
in psoriasis (18), and produce key proinflammatory cytokines including IFN-γ, TNF-α, and IL-17A. Immunohistochemical analyses have previously identified V62T cells in human gastrointestinal lymphoid tissues (19), but a role for these cells in the lamina propria has not been determined. CD4+ qβT cells respond to bacterial metabolites and produce key mediators of intestinal immunity, including IFN-γ, TNF-α, and IL-17A (24, 25), indicates considerable potential for this population to augment conventional T cell responses in the gut (26, 27).

In addition to their capacity to produce proinflammatory cytokines, V62T cells display a striking level of functional plasticity that could potentially shape mucosal immune responses in several unique ways (12). Human blood-derived V62T cells can support the differentiation of proinflammatory dendritic cells (DC) from monocyctic precursors, leading to substantial production of IL-12, IFN-γ, and TNF-α and promoting the Th1/Th17 differentiation of conventional qβT cells in response to bacterial products (17, 28). V62T cells may even migrate to regional lymph nodes and exhibit Ag-presenting potency comparable with DC (19, 29). Human V62T cells have also been reported to enhance the survival of neutrophils that have engulfed phosphoantigen-producing species of bacteria (30), suggesting an additional mechanism by which they might contribute to mucosal barrier protection in man.

Translocation of microbial products from the gut into the circulation results in systemic priming of the innate immune system and enhanced antimicrobial immunity even in healthy animals (31). We therefore hypothesized that circulating V62T cells are recruited to the human intestine and contribute to mucosal immune responses as a consequence of exposure to gut-derived microbial products. To test this hypothesis, we assessed the gut-homing potential of blood V62T cells. Allogeneic responder cells were separated from peripheral blood by centrifugation over Ficoll-Paque PLUS or by magnetic separation LPMC released from the biopsies were passed through a cell strainer and washed in Dutch-Modified RPMI 1640 medium (Sigma-Aldrich).

Phosphoantigen stimulation

Intestinal biopsies or cell suspensions were cultured in 24- or 96-well plates and stimulated with 1 nM 1-hydroxy-2-methyl-2-buten-4-y1-diphosphate (HDMAPP) phosphoantigen (7, 33) (tebu-bio, Peterborough, U.K.) and 30 U/ml recombinant human IL-2 (PeproTech, London, U.K.) in the presence or absence of 2 nM all-trans retinoic acid (RA; Sigma-Aldrich), and/or 1 μM RA receptor α (RARα)-selective antagonist Ro41-5253 (Enzo Life Sciences, Exeter, U.K.), with or without 10 μg/ml LEAF-purified anti-human IFN-γ or murine IgG1 isotype control (clones MD-1 and MOPC-21 from BioLegend, Cambridge, U.K.) and then incubated at 37˚C, 5% CO2, for the indicated times. The stimulated cells were labeled with mAb and analyzed by flow cytometry or were reactivated with PMA (10 ng/ml) and ionomycin (2 μg/ml) in the presence of monensin (3 μM) for 4 h at 37˚C, 5% CO2. The reactivated cells were surface-labeled with anti-CD3 and anti-V62 mAb for 15 min on ice and then washed twice in cold FACS buffer for 5 min at 300 × g. Cell pellets were resuspended in 100 μl Leucoperm A (AbD Serotec, Oxford, U.K.) and incubated for 15 min at room temperature. The cell suspensions were washed in FACS buffer, permeabilized in 100 μl Leucoperm B (AbD Serotec), and labeled with 5 μl anti-cytokine mAb for 30 min on ice. The labeled cell suspensions were washed in FACS buffer and fixed in 1% paraformaldehyde.

MLRs

LPMC were labeled with PE-conjugated anti-TCR V62 mAb (B6; Bio-Legend) and depleted of intestinal V62T cells by incubation with anti-PE microbeads followed by magnetic column separation according to the manufacturer’s instructions (Miltenyi Biotec, Bisley, U.K.). The V62+ T cells retained in the column were eluted as a source of enriched mucosal V62T cells. Allogeneic responder cells were separated from peripheral blood by centrifugation over Ficoll-Paque PLUS or by magnetic separation using the Naive CD4+ T cell Isolation Kit II (Miltenyi Biotec) according to the manufacturer’s protocol. Suspensions of responder cells (2 × 105) were labeled with 5 μM CFSE (Invitrogen, Paisley, U.K.) and cocultured with LPMC (5 × 105) for 5 d in 96-well round-bottom plates. Responder cells were labeled with mAb and analyzed by flow cytometry to determine T cell phenotype and proliferation (CFSE dilution).

Flow cytometry

mAbs were CD86-FITC (BU63) from AbD Serotec; PerCP/Cy5.5 and PE/Cy7-conjugated CD8 (SK1 and RPA-T8), CD40-PE (5C3), CD69-PE (L78), CD70-PE (K124), CD103-FITC (Bel-Act8), HLA-DR-PE/Cy7 (L243), and integrin β7-PE (FIB504) from BD Biosciences; PE/Cy5, allopoly-cyanin, and Pacific Blue–conjugated CD3 (UCHT1, H1T3A, and OKT3, respectively), FITC- and allopoly-cyanin-conjugated CD4 (RPA-T4), FITC, PE-, and Alexa Fluor 647–conjugated CD3 (Bio-Act8), CCCR7-Pacific Blue (TGB/CCR7), cutaneous leukocyte Ag (CLA)-FITC (HECA-452), PE- and allopoly-cyanin-conjugated integrin β7 (FIB504), FITC- and PE-conjugated TCR V62 (B6), PE/Cy7- and Alexa Fluor 647–conjugated IFN-γ (4S.B3). TNF-α–PE (Mabab1), IL-17A–PE (BL168), and IL-10–PE (JES3-9D7) from BioLegend, and T-bet-PerCP/Cy5.5 (B810) from eBioscience. Isotype-matched controls were obtained from the respective manufacturers. The CD4+ mAb Act-1 was a kind gift from Prof. Eugene Butcher at Stanford University. Labeled cell suspensions were acquired on FACS Canto II or LSRII flow cytometers using FACS Diva 6.1.2 software (BD Biosciences), and data were analyzed using WinList 6.0 (Verity Software House). Absolute cell numbers were determined by reference to a known quantity of FlowCount Fluorospheres (Beckman Coulter) added to the cell suspensions prior to acquisition.

Mucosal addressin cell adhesion molecule-1 binding assay

PBMCs were incubated for 20 min on ice in PBS containing 2.5 mM EDTA and 3% FCS before being surface-labeled with mAb to identify the constituent cell populations. The cells were washed twice (300 × g, 5 min) in HBSS containing 2 mM NaN3 and 3% FCS, and then incubated on ice for

Materials and Methods

Human intestinal tissue

Biopsies of human terminal ileum and colonic mucosa were obtained from patients undergoing colonoscopy for colorectal cancer screening or for investigation of rectal bleeding but with no significant findings. Additional mucosal tissue was obtained from patients undergoing surgical resection for colorectal cancer or for noninflammatory intestinal motility disorders. The mean age of the study volunteers was 37.8 years (range 5–79), and the population was 55% male. The protocol was approved by the local research ethics committee, and informed consent was obtained from all study participants.

Peripheral blood cells

Human whole blood was obtained by venipuncture into sodium-heparin Vacutainers (BD Biosciences, Oxford, U.K.) and directly labeled with mAbs for 15 min at room temperature. RBCs were lysed by addition of OptiLyse C reagent (Beckman Coulter) for 15 min at room temperature. The resultant cell suspension was washed twice in cold FACS buffer (PBS containing 2% FCS, 0.02% sodium azide, and 1 mM EDTA) and fixed in 1% paraformaldehyde. Alternatively, mononuclear cells were separated from peripheral blood by centrifugation over Ficoll-Paque PLUS (GE Healthcare, Buckinghamshire, U.K.) and washed in Dutch-Modified RPMI 1640 medium (Sigma-Aldrich, Dorset, U.K.).

Lamina propria mononuclear cells

The procedure for obtaining lamina propria mononuclear cells (LPMC) from human intestinal biopsies has been described in detail elsewhere (32). Briefly, biopsies of colonic tissue were collected into cold, Dutch-Modified RPMI 1640 medium (Sigma-Aldrich). Mucus and feces were removed in calcium- and magnesium-free HBSS (Sigma-Aldrich) containing 1 mMol/L DTT (Sigma-Aldrich), and the epithelium was removed using 1 mMol/L EDTA (Sigma-Aldrich) in HBSS at 37°C. The biopsies were incubated in complete medium (Dutch-modified RPMI 1640 medium, 10% FCS, 2 mM l-glutamine, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 25 μg/ml gentamicin) in 24-well plates to allow leukocyte egress from the mucosa. Alternatively, the tissue was rapidly digested in 1 mg/ml collagenase D (Roche Diagnostics, Mannheim, Germany) in RPMI 1640 HEPES medium (Sigma-Aldrich) containing 2% FCS and 20 μg/ml DNase 1 (Roche Diagnostics). LPMC released from the biopsies were passed through a cell strainer and washed in Dutch-Modified RPMI 1640 medium (Sigma-Aldrich).
30 min prior to addition of 10 μg/ml mucosal addressin cell adhesion molecule-1 (MadCAM-1)–Fc (R&D Systems). After incubation, the cells were washed twice with 3% FCS in PBS and labeled with PE-conjugated anti-human Fcγ (eBioscience). After 20 min incubation on ice, the cells were washed twice with 3% FCS in PBS and then fixed in 1% paraformaldehyde. The extent of T cell binding to MadCAM-1 was subsequently assessed by flow cytometry.

**Confocal microscopy**

Cryosections were fixed in ice-cold acetone for 15 min, and nonspecific binding sites were blocked by incubating the sections with 10% donkey serum/PBS for 30 min. Each section was incubated with primary mAb for 1 h at room temperature (anti-CD1 from Santa Cruz Biotechnology, Santa Cruz, CA; anti-TCR Vβ6 from BioLegend). The sections were then washed and incubated with DAPI and Alexa Fluor–conjugated secondary Abs (Invitrogen) for 1 h at room temperature. Specific staining was confirmed using isotype control Abs. Confocal images were acquired using an LSM 510 Meta, Plan-Neofluar 40× oil/0.50 objective (Carl Zeiss, Jena, Germany).

**Statistical analysis**

Statistical analyses were performed using SigmaStat 3.5 (Systat Software). Normal data were analyzed by t test or paired t test. Mann–Whitney and Wilcoxon signed-ranks tests were used to test nonnormal data. One-way ANOVA was used to determine the effects of phosphoantigen stimulation (or Kruskal–Wallis one-way ANOVA on ranks for nonnormal data). One-way repeated-measures ANOVA was used to assess the effects of the neutralizing anti-IFN-γ mAb, and to test the influence of RA and Ro41-5253 on the generation of gut-homing Vβ2T cells. Correlations between variables were assessed using Pearson product-moment and Spearman rank-order tests.

**Results**

**RA regulates tissue tropism of circulating human Vβ2T cells**

Vβ2T cells are antimicrobial lymphocytes that typically comprise <5% of blood T cells in higher primates, but this population can expand rapidly and traffic to sites of inflammation following activation (15–18). To determine whether circulating Vβ2T cells display potential for migration to the human intestinal mucosa, we assessed the expression of tissue homing receptors by Vβ2T cells and εβT cells in peripheral blood (Fig. 1A). Integrin complex of subunits ε4 and β7 facilitate leukocyte entry into the intestinal lamina propria via interactions with MadCAM-1 (34). In direct ex vivo analyses of human blood, the majority of circulating Vβ2T cells expressed integrin β7 (mean 72.2 ± 1.9%; Fig. 1B), consistent with reports from other investigators (19).CLA identifies T cells in blood that migrate to the skin (35) and was detected on mean 29.7% of circulating Vβ2T cells (Fig. 1B), in line with other reports (18, 35). In contrast with conventional T cells, coexpression of β7 and CLA by Vβ2T cells was relatively common (∼15%; Fig. 1B, 1C), suggesting that a significant number of these cells are capable of homing both to the intestine and to the skin. Together, these data indicated that Vβ2T cells in human blood display a multipotent tissue homing phenotype, but expression of gut-homing integrin ε4β7 predominates.

To determine the effects of activation on the tissue tropism of blood Vβ2T cells, we next examined the expression of homing receptors following stimulation with HDMAP phosphoantigen. Staining with the Act-1 Ab (specific for the ε4β7 integrin heterodimer) confirmed that the majority of circulating Vβ2T cells express the ε4β7 complex that mediates homing to the gut. Unstimulated Vβ2T cells downregulated the expression of integrin ε4β7 over 5-d culture in vitro, whereas exposure to HDMAP in the presence of IL-2 induced these cells to proliferate and up-regulate ε4β7 (Fig. 2A). Vβ2T cells expanded ∼9.5-fold over 5-d culture with HDMAP and IL-2 (p = 0.002), and integrin β7 expression levels (mean fluorescence) increased 4-fold among these cells (p = 0.006). Thus, in addition to increasing total Vβ2T cell frequency, cotreatment with HDMAP and IL-2 generated higher numbers of activated, gut-tropic Vβ2T cells than either HDMAP or IL-2 stimulation alone (Fig. 2B). Consistent with these data, increased β7 expression by HDMAP-activated Vβ2T cells was accompanied by an enhanced capacity of these cells to bind to MadCAM-1 in vitro (Fig. 2C). The extent of MadCAM-1 binding among HDMAP-activated Vβ2T cells was comparable with that observed for conventional εβT cells stimulated with anti-CD2/3/28 activation beads (not shown). These data indicated that exposure to microbial phosphoantigen and IL-2 is sufficient to drive circulating Vβ2T cells to expand and upregulate molecules that mediate trafficking to the human intestinal mucosa.

**Tissue tropism of conventional εβT cells is regulated by RA** (36), so we investigated whether the gut-homing potential of human Vβ2T cells was also regulated by RA. In PBMC cultured for 5 d with HDMAP and IL-2, supplementation with physiological concentrations of RA (37) blocked the generation of CLA+ Vβ2T cells and significantly increased the proportion of β7+ gut-homing Vβ2T cells (Fig. 3A) as well as the level of β7 expressed per cell (mean fluorescence increased ∼2-fold; not shown). RA regulation of Vβ2T cell-homing receptor expression was mediated by signaling throughRARs because the effects of exogenous RA were blocked by addition of Ro41-5253 (a selective antagonist of RARs). Exposure to RA during HDMAP activation increased the ability of Vβ2T cells to bind to MadCAM-1 in vitro, whereas exposure to Ro41-5253 decreased MadCAM-1 binding (Fig. 3B, 3C). The reciprocal effects of RA and Ro41-5253 on binding to MadCAM-1 were consistent between HDMAP-activated Vβ2T cells and conventional εβT cells stimulated with anti-CD2/3/28 activation beads (not shown). Furthermore, when all of the various treatment conditions for all experimental replicates were assessed together, we observed a direct correlation between β7 expression level and the extent of Vβ2T cell binding to MadCAM-1 (r = 0.570; p = 0.033). These data indicated that the gut-homing potential of Vβ2T cells is regulated by RA-dependent mechanisms comparable to those that direct εβT cell trafficking to the intestine.

**Phosphoantigen-responsive Vβ2T cells populate the human intestinal mucosa**

We next assessed whether Vβ2T cells are present in the healthy human intestinal lamina propria, where they could contribute to mucosal immune responses. Using confocal microscopy to examine frozen sections of colonic biopsies, Vβ2T cells were readily identified in the intestinal mucosa (Fig. 4). Colonic Vβ2T cells were relatively frequent and widely interspersed throughout the lamina propria. Accordingly, Vβ2T cells could also be identified by flow cytometry in LPMC that migrated out of colonic and small intestinal biopsy tissue (32). Total lamina propria lymphocytes contained a distinct subset of Vβ2T cells (Fig. 5A) that comprised ∼0.2–2% of the total intestinal T cell population and uniformly expressed the archetypal Th1 transcription factor T-bet (Fig. 5B). Mucosal Vβ2T cells were largely CD27+ (mean 76.2%, range 74.4–78.1%; not shown) but lacked CD45RA. In mice, CD27 expression identifies γδ T cells that are committed to robust production of IFN-γ (12), whereas in humans, CD27 expression identifies a population of Th1-biased Vβ2T cells with increased proliferative potential and enhanced survival characteristics (38). Further analysis revealed that Vβ2T cells derived from intestinal tissue comprised distinct subsets of CD103+ and CD103− cells, whereas blood Vβ2T cells uniformly lacked CD103 (Fig. 5C, 5D) (18, 19).

To assess whether human intestinal Vβ2T cells are responsive to microbial phosphoantigen, we cultured LPMC with HDMAP and
IL-2, either alone or in combination, and monitored population numbers by flow cytometry. Intestinal V82T cells proliferated rapidly following stimulation with microbial phosphoantigen in the presence of IL-2, expanding up to 30-fold over 6 d of culture (Fig. 5E). Mucosal biopsy-derived V82T cells continued to expand even after 30 d of continuous culture in the presence of HDMAPP and IL-2 (not shown). Taken together, these data indicated that V82T cells populate the human intestinal mucosa and comprise both CD103+ and CD103− subsets that can respond to microbial phosphoantigens.

**V82T cells expanded from human intestinal tissue lack Ag-presenting function**

V82T cells in human blood exhibit a striking level of functional plasticity (12, 30, 39), and may display DC-like APC function (19, 29). Our ability to expand small numbers of V82T cells isolated from human gut biopsies (Fig. 5E) enabled us to assess the APC potency of intestinal V82T cells.

Intestinal V82T cells cultured for 24 h in the presence of IL-2 alone included a low proportion of cells that expressed HLA-DR (mean ± SEM: 28.4 ± 6.0%) or costimulatory ligand CD70 (17.7 ± 2.1%; data not shown), perhaps indicating low-level exposure to endogenous/bacterial phosphoantigens in the biopsy cultures. In contrast, intestinal V82T cells exposed to exogenous HDMAPP in the presence of IL-2 included a high frequency of HLA-DR− expressing cells after 24 h (60.8 ± 6.28% HLA-DR−) (Fig. 6A) that further increased over the course of culture (75.5 ± 9.9% HLA-DR− after 5 d; not shown). Phosphoantigen-stimulated intestinal V82T cells also frequently expressed CD70 (50.8 ± 9.7% CD70+) on day 1, Fig. 6B; 74.8 ± 4.1% CD70+ at day 5, not shown), which has been implicated in the APC function of V82T cells (38) and reportedly drives the proliferation and local differentiation of T cells in the mucosa (40). On day 6, the expanded V82T cells were immunomagnetically sorted to >95% purity (Fig. 6C, 6D), but they did not stimulate allogeneic naive CD4 T cells in MLRs (Fig. 6E). In contrast, V82-depleted lamina propria cells from the same donors, replete in conventional APC, stimulated marked proliferation of naive CD4+ T cells when assessed in parallel (Fig. 6F). Similar data were obtained using allogeneic CD8+ T cells or unseparated PBMC as responder populations (26.1 ± 6.4% of the responder cells had divided after stimulation by V82-depleted lamina propria cells, compared with only 0.6 ± 0.1% when cultured with mucosal V82T cells alone, p = 0.029; not shown). Consistent with these data, V82T cell expression of the conventional costimulatory markers CD40 and CD86 was negligible after 6-d culture with HDMAPP and IL-2. It is possible that intestinal V82T cells display APC function that falls short of that displayed by the autologous DC in these stringent assays, but under the conditions tested in this study, we could not find evidence of Ag presenting activity among cultured intestinal V82T cells. CD70 and HLA-DR expression by V82T cells in mucosal tissue may instead represent markers of increased proliferative potential, enhanced cell survival, and efficient Th1 cytokine production, as has been previously reported for their blood counterparts (38).

**CD103+ human intestinal V82T cells exhibit a distinct cytokine production profile**

To better determine the role played by intestinal V82T cells in the human lamina propria, we next investigated the profile of cytokines produced by these cells. V82T cells that migrated out of human mucosal biopsies in the presence of HDMAPP and IL-2 contained a high frequency of cells capable of producing proinflammatory cytokines TNF-α and IFN-γ, but few that produced IL-17A (Fig. 7A, 7B), whereas regulatory cytokine IL-10 was undetectable (not shown). The observed high frequency of IFN-γ+...
FIGURE 2. Gut-homing Vδ2T cells proliferate following microbial activation. After 5-d culture in the presence of microbial Ag HDMAPP and IL-2, blood-derived Vδ2T cells had proliferated markedly and substantially upregulated the expression of the gut-homing integrin α4β7 heterodimer. (A) The absolute frequencies of CD3⁺Vδ2⁺ cells per well (cpw) are indicated with the corresponding α4β7 expression profiles shown directly below each plot (unfilled histograms indicate staining with an isotype-matched control mAb). Both the proportion of gut-homing Vδ2T cells and the level of α4β7 expression (mean fluorescence intensity [MFI]) were enhanced by cotreatment with HDMAPP and IL-2. In contrast, Vδ2T cells cultured in medium only or stimulated with either HDMAPP or IL-2 alone displayed far lower levels of α4β7 expression and poor cell proliferation (representative of n = 3 independent experiments). (B) Grouped data demonstrating that blood Vδ2T cells cultured with HDMAPP and IL-2 proliferated efficiently (*p = 0.002; n = 8) and significantly upregulated expression of the β7 chain (**p < 0.001 versus CM or IL-2 only conditions; n = 8) and activation marker CD69 (p = 0.040 for the effects of the different treatments; n = 3). (C) Upregulation of β7 by activated Vδ2T cells was accompanied by a corresponding increase in binding to a chimeric MaDCAM-1 fusion protein in vitro. The proportion of MaDCAM-1⁺ cells and net MFI of binding to MaDCAM-1 were significantly increased upon Vδ2T cell activation with HDMAPP. MaDCAM-1 binding was assessed by flow cytometry, and net MFI was calculated by subtracting the mean fluorescence of the control histogram (gray fill) from the mean fluorescence of the MaDCAM-1 binding histogram (unfilled trace). Data shown are from n = 4 independent experiments, with the symbols indicating different individuals.
FIGURE 3. RA regulates the gut-homing potential of Vδ2T cells. PBMC were cultured for 5 d in the presence of HDMAPP and IL-2 in complete medium (CM) with or without 2 nM RA and/or 1 μM Ro41-5253 (Ro41), a specific antagonist for RARα. (A) Generation of gut-homing β7+ Vδ2T cells was significantly enhanced by exposure to exogenous RA, but this effect was blocked by addition of Ro41. Conversely, Vδ2T cell expression of CLA was strongly suppressed by exposure to RA, but expression of this skin-associated marker was recovered by treatment with Ro41. (B and C) Exposure to RA during HDMAPP activation of Vδ2T cells significantly increased high-level binding to MadCAM-1, whereas exposure to Ro1-5253 decreased the proportion of MadCAM-1hi cells detected. Data in (C) show percentage of MadCAM-1hi Vδ2T cells expressed as a ratio relative to the CM only condition. Data shown are from n = 3 independent experiments (A) or n = 5 independent experiments (B, C). *p < 0.05, **p = 0.001 indicate significant difference from both the CM and RA/Ro41-5253 conditions.

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Phosphoantigen exposure significantly enhanced the prevalence of CD103 by autologous colonic ab activate mucosal V cells in the presence or absence of nonpeptide phosphoantigen HDMAPP to test this hypothesis, we cultured human colonic biopsies in IL-2 in the presence of conventional T cell responses in the gut. To test this hypothesis, we cultured human colonic biopsies in IL-2 in the presence or absence of nonpeptide phosphoantigen HDMAPP to activate mucosal V62T cells before assessing cytokine production by autologous colonic αβT cells in the same cultures.

The subset balance of CD103+/CD103− V62T cells was a significant influence on cytokine production by conventional colonic T cells in biopsies exposed to phosphoantigen during culture (Fig. 8A). Although it was not possible to isolate the individual V62T cell subsets due to their low frequency in endoscopic biopsies (combined with the potential for prolonged culture to alter subset function), the total V62T cell population produced higher levels of IFN-γ if comprised primarily of CD103− cells (p = 0.002; not shown), and there was a clear relationship between the prevalence of CD103− V62T cells in the biopsies and the level of IFN-γ produced by autologous αβT cells (p = 0.713; p = 0.00801). Phosphoantigen exposure significantly enhanced the proportion of IFN-γ+ colonic αβT cells detected (from median 22.7–34.8%; p = 0.023), but only in cultures that contained frequent CD103− V62T cells (≥50% of the total V62T population). No such effect was observed in cultures in which numbers of CD103− V62T cells were low (median IFN-γ+ αβT cells, 29.1% in the absence of phosphoantigen, 24.8% after exposure to HDMAPP; p = NS). Having previously observed that CD103− V62T cells were themselves robust producers of IFN-γ (Fig. 7), we hypothesized that HDMAPP activation of this subset might increase the overall IFN-γ concentration in the biopsy cultures, thereby enhancing the Th1 differentiation of autologous αβT cells. Consistent with this hypothesis, addition of a neutralizing anti–IFN-γ mAb to the HDMAPP-stimulated biopsy cultures was sufficient to abolish CD103− V62T cell enhancement of αβT cell responses (Fig. 8B, 8C). Furthermore, the increased production of IFN-γ by colonic αβT cells was accompanied by increased STAT1 phosphorylation (not shown) and upregulation of T-bet (Fig. 8D; p = 0.002), which are consistent with enhanced IFN-γ signaling.

Taken together, these data suggest that phosphoantigen-activated V62T cells can enhance the proinflammatory responses of lamina propria αβT cells, most likely by increasing the Th1 polarization of these cells via production of IFN-γ, and that the subset balance of mucosal V62T cells alters the magnitude of this effect. These data indicate that in addition to their direct effector functions, mucosal V62T cells have the capacity to influence human intestinal immunity by shaping conventional lymphocyte responses in the lamina propria.

**Discussion**

This study demonstrates for the first time, to our knowledge, that proinflammatory V62T cells can populate the human colon and small intestinal mucosa. Lamina propria V62T cells are phenotypically distinct from their counterparts in blood, are highly responsive to microbial phosphoantigens, produce proinflammatory cytokines including IFN-γ and TNF-α, and undergo rapid expansion following activation. Human mucosal V62T cells incorporate at least two separate subpopulations, characterized by differential expression of CD103 and distinct patterns of cytokine production. The majority of blood V62T cells expressed gut-homing integrin α4β7, and upregulation of this dimer after phosphoantigen activation or exposure to RA enhanced V62T cell binding to MadCAM-1 in vitro, suggesting that these cells may be similarly recruited to the human intestine in vivo. In addition, our data indicate that IFN-γ production by phosphoantigen-activated V62T cells can enhance the Th1 polarization of colonic αβT cells, suggesting that the direct proinflammatory functions of V62T cells may be amplified via effects on conventional lymphocytes in the lamina propria.

The majority of circulating V62T cells expressed gut-homing integrin α4β7, and between 30 and 50% of circulating V62T cells expressed skin-homing marker CLA (18, 35). Activation with microbial phosphoantigens lead to significant upregulation of α4β7, indicating increased potential for trafficking to the intestine (15, 16). Increased β7 expression was associated with enhanced V62T cell binding to MadCAM-1 and could be further enhanced by exposure to physiological concentrations of exogenous RA (37) signaling in an RARα-dependent manner, indicating that gut-homing potential is regulated by similar mechanisms in both V62T cells and conventional αβT cells (36). Even in the absence of RA addition, V62T cell stimulation with HDMAPP selectively upregulated α4β7, which likely reflects the low nanomolar concentrations of RA already present in serum (37). In light of recent data implicating CLA-dependent trafficking of V62T cells to the inflamed human skin (18), the trafficking of CLA+ V62T cells in patients with cutaneous manifestations of inflammatory bowel diseases merits further investigation.

Many constituents of the intestinal microbiota employ isoprenoid biosynthetic pathways that predict the production of phosphoantigens that can potently activate V62T cells (5). In this study, we show that human mucosal V62T cells are highly responsive to phosphoantigens in vitro and may therefore be capable of responding to gut-derived phosphoantigens either locally or systemically in vivo. V62T cells are key early sensors of microbial infection (28, 30, 39), and translocation of bacterial products can drive systemic priming of the innate immune system even in healthy animals (31), thus accumulation of V62T cells in the gut mucosa may be a consequence of Ag exposure and induction of
FIGURE 5. Human intestinal Vδ2T cells respond to microbial phosphoantigens. Lymphocytes that migrated out of intestinal biopsies contained a substantial population of Vδ2+ T cells (A) that were comparably frequent in both colonic and small intestinal mucosal tissue. Intestinal Vδ2T cells uniformly expressed the archetypal Th1 transcription factor T-bet (B), and incorporated two major cell populations identified by differential expression of CD103 (C). Although Vδ2T cells in human peripheral blood do not express CD103 (C) (18, 19), a high proportion of intestinal Vδ2T cells expressed this integrin that is implicated in interactions with the mucosal epithelium (59) (D, n = 3). Intestinal Vδ2T cells exposed to microbial phosphoantigen (HDMAPP) in the presence of IL-2 proliferated rapidly and expanded between ∼3- and 30-fold over 6-d culture in vitro (E), indicating a capacity for these cells to respond to phosphoantigens generated by the gut microbiota. Unfilled histograms indicate staining with isotype-matched control mAbs.
a gut-tropic phenotype. Vδ2T cells have previously been identified in GALTs (19), and T cell activation in these RA-rich environments would be predicted to generate a committed gut-tropic profile (41). In nonhuman primates, HDMAPP activation of blood Vδ2T cells has been shown to promote mucosal recruitment of these cells (15, 16). Because the pathogenesis of Crohn’s disease (CD) is associated with increased gut permeability (42, 43), microbial dysbiosis (44), and altered innate responses to bacteria that cross the gut barrier (45, 46), these changes may also lead to altered activation and trafficking of Vδ2T cells that could contribute to disease in human patients.

Confocal microscopy revealed that Vδ2T cells populate the human colonic lamina propria, and CD103+ Vδ2T cells were detected by flow cytometry in intestinal tissue but not in blood (18), confirming that the cells isolated from gut tissue are not simply blood contaminants. The remarkable functional plasticity displayed by Vδ2T cells in human blood suggests that their roles in the gut may be similarly diverse (12). Activated Vδ2T cells can augment the proinflammatory activities of monocytes (17), neutrophils (30), and DC (47). Vδ2T cells can also express CCR7, mediate cross-presentation, and exhibit Ag-presenting potency comparable with DC (19, 29, 48). In our assays, activated intestinal Vδ2T cells expressed HLA-DR and costimulatory ligand CD70, which has been implicated in the APC function of Vδ2T cells (38), and reportedly drives the proliferation and local differentiation of mucosal T cells (40), but this phenotype did not confer the ability to activate allogeneic naive T cells. However, CD70 has also been reported to enhance the phosphoantigen-
induced activation of Vδ2T cells in human blood, leading to increased proliferation, cell survival, and Th1 cytokine production (38), and might therefore be expected to mediate similar proinflammatory effects in the human gut.

Phosphoantigen-activated CD103⁺ Vδ2T cells produced large quantities of IFN-γ, and a high frequency of these cells in biopsy culture was associated with increases in conventional T cell expression of IFN-γ (B), phosphorylation of STAT1, and upregulation of Th1 transcription factor T-bet. These data suggest that CD103⁺ Vδ2T cells may be capable of enhancing the Th1 commitment of colonic αβT cells, although we did observe a striking increase in IL-17A⁺ αβT cells, although we did observe a striking increase in IL-17A⁺ αβT cells in mucosal tissue obtained from a pediatric healthy control. Data shown in (B) (representative example) and in (C) (grouped data) are from n = 4 independent experiments (*p = 0.013). Unfilled histograms indicate staining with an isotype-matched control mAb.
Vδ2T cells can increase IFN-γ production by conventional T cells in human gut tissue. Modulation of intestinal Vδ2T cell function could thus present a novel strategy for the eradication of intestinal pathogens. Human Vδ2T cells have also shown therapeutic promise as cytotoxic tumor-killing cells in various preclinical and clinical settings (55–57). Activation of intestinal Vδ2T cells in colitis-associated cancer might therefore be another potential outlet for the clinical exploitation of these cells. Therapeutic targeting of Vδ2T cell responses in addition to conventional lymphocyte responses may increase the efficacy of interventions that aim to reduce intestinal inflammation. Indeed, our preliminary data indicate that Vδ2T cells are selectively ablated in the blood of CD patients receiving azathioprine therapy (58). Further investigation of how Vδ2T cell trafficking and activation in the human intestinal mucosa can be modified by environmental factors may lead to the development of novel therapies for CD.

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