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Modulation of CD103 Expression on Human Colon Carcinoma-Specific CTL

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Recent results have shown a correlation between survival and frequency of tumor-infiltrating T cells in colorectal cancer patients. However, the mechanisms controlling the ability of human T lymphocytes to infiltrate colon carcinoma remain unclear. Although, it is known that expression of the integrin CD103/β7 by intraepithelial lymphocytes controls the retention of lymphocytes in epithelial layers, very little is known about the expression of intestinal homing receptors in human T lymphocytes. In particular, it remains unknown whether expression of CD103/β7 by human colon cancer-specific T lymphocytes is controlled by recognition of tumor Ags and is imprinted during T cell priming, facilitating its expression during memory T cell activation. In this study, we demonstrate that expression of CD103/β7 in human colon carcinoma-specific CTL is synergistically enhanced by the simultaneous TGF-β1 stimulation and Ag recognition. These results were confirmed by using a panel of human T cell clones. Finally, we show that priming of naive CD8+ T cells in the presence of TGF-β1 ensures up-regulation of CD103/β7, in recall responses, at concentrations of TGF-β1 significantly lower than those required by memory T cells primed in the absence of TGF-β1. These results indicate a role of TGF-β1 during T cell priming in modulating expression of CD103/β7, and controlling retention of human memory CD8+ T cells into tumor epithelium.

The role of T lymphocytes in controlling the growth of colon carcinoma has been described recently by comparing the percentage of tumor-infiltrating CD3+ lymphocytes and patients’ survival (1, 2). These results have highlighted the importance of analyzing the frequency of tumor-infiltrating T lymphocytes as a prognostic marker in colon cancer patients. However, the trafficking properties of colon cancer-specific T cells remain unclear. Understanding these mechanisms is of importance to clarify the events that control recirculation of colon cancer-specific T cells and to design vaccination strategies capable of maximizing the ability of T cells to infiltrate colon carcinoma.

The local microenvironment plays an important role in providing direct signals to responding T cells and in controlling expression of tissue-specific homing receptors. Dendritic cells (DC) in organized mucosal lymphoid tissues preferentially induce the expression of defined integrins and chemokine receptors on T cells, which results in T cell homing to the intestinal lamina propria (3, 4). Although experiments have been conducted in mouse models, very little is known about the mechanisms that control the expression of tissue-specific homing receptors in human T lymphocytes.

Integrins are heterodimeric glycoproteins that play a prominent role in the trafficking of lymphocytes (5). Experiments conducted in mouse models have elegantly demonstrated that the integrin CD49d/β7 (also named αEβ7) and the chemokine receptor CCR9 help lymphocytes home to the small intestinal mucosa (3, 4). The ligand for CD49d/β7 is the mucosal addressin cell adhesion molecule 1, a vascular addressin present on postcapillary venules in the intestinal lamina propria (6), whereas the chemokine receptor CCR9 recognizes CCL25, a chemokine produced in the small intestines. The integrin CD103/β7 (hereafter referred to as CD103) plays an important role in the localization of lymphocytes in the intraepithelial compartment (7). More recently, it has been shown that CD103 expression on DC can influence the balance between effector and T regulatory cells in the intestine (8). The ligand for CD49d/β7 is the mucosal addressin cell adhesion molecule 1, a vascular addressin present on postcapillary venules in the intestinal lamina propria (6), whereas the chemokine receptor CCR9 recognizes CCL25, a chemokine produced in the small intestines. The integrin CD103/β7 (hereafter referred to as CD103) plays an important role in the localization of lymphocytes in the intraepithelial compartment (7). More recently, it has been shown that CD103 expression on DC can influence the balance between effector and T regulatory cells in the intestine (8). CD103 is found on >90% of intraepithelial lymphocytes and on only 0.5–3% of peripheral blood lymphocytes (PBL) (9). The only known ligand for CD103 is E-cadherin (10, 11), which is expressed on epithelial cells. Although T cells migrating to murine intestines are CD103− and CD49d/β7− (12), CD103 is induced only after their entry into the mucosa (13, 14). The factor known to up-regulate CD103 is TGF-β1 (15), a cytokine produced by many cells, including both normal and malignant intestinal epithelial cells (16–18). The importance of CD103 in controlling homing and retention of CD8+ T cells into the epithelial layers has recently been confirmed by demonstrating that CD103+ CTL, unlike CD103− CTL, play an important role in the destruction during graft-vs-host disease (GVHD) of gut epithelial cells and the epithelial components of renal and pancreatic allografts (19, 20).

It has been shown that mouse DC resident in the mesenteric lymph nodes (MLN) and the Peyer’s patches are responsible for inducing CD49d/β7, and CCR9 on naive lymphocytes by secreting retinoic acid (21). Von Andrian and colleagues (22) have shown recently that tissue tropic effector CD8+ T cells that had been
imprinted previously with the ability to migrate to a certain organ remain plastic to further influences by DC and can change their tissue-trafficking abilities. Although these results shed light on how the ability to traffic to the murine small intestine is imprinted on T cells, very little is known about the mechanisms which control homing of human T cells into the gut epithelium. In particular, it remains unclear whether Ag recognition contributes with TGF-β1 to the up-regulation of CD103 and whether the ability of memory T cells to up-regulate CD103 in the presence of TGF-β1 is controlled by events during T cell priming.

Because colon carcinomas are epithelial in origin and express E-cadherin, we analyzed CD103 expression in human colon carcinoma-specific T cells and assessed whether expression of CD103 segregated with their ability to recognize specifically autologous colon carcinoma cells. We then extended these results to a panel of Ag-specific CTL clones to determine whether factors, in addition to TGF-β1 in the tumor stroma, might regulate CD103 expression. The results of our experiments demonstrated that, Ag recognition together with TGF-β1 stimulation, synergistically enhance the proportion of T cells expressing CD103. The presence of TGF-β1 during Ag-specific T cell priming ensures that memory CD8+ T cells express CD103 during subsequent Ag stimulations at concentrations of TGF-β1 significantly lower than concentrations required by memory T cells primed in the absence of TGF-β1.

Materials and Methods

Patients and samples

Blood, colon tumor, MLN, and normal colonic tissues were obtained from patients undergoing surgery for primary colorectal carcinoma (n = 20, mean age 71.3 years, range 50–76 years; 9 females and 11 males). Blood was collected from patients before surgery. Normal colonic tissue was obtained from a macroscopically normal part of the excised colon, at least 10 cm from the tumor margin. None of patients had previously received radiotherapy or chemotherapy. Blood samples from six normal donors were used as controls. The study was approved by the Central Oxford Region Ethics Committee.

Immunohistochemistry

Immunohistochemistry was performed on frozen sections. The sections were fixed in acetone, washed in PBS, and blocked with goat serum before 50 µl of primary Ab (diluted to 5 µg/ml) were added for 30 min at room temperature. The sections were then washed in PBS and incubated with peroxidase-conjugated goat-anti-mouse IgG (Sigma-Aldrich) for 30 min. Bound peroxidase activity was developed using an 3-amino-9-ethylcarbazole kit (Vector Laboratories). The sections were counterstained in hematoxylin.

Abs and tetramers

The following Abs were used to stain ex vivo single-cell suspension or cell lines and in immunohistochemistry analysis: CD3-allophycocyanin (clone UCHL1), CD8-PerCP (SK1), CD8-allophycocyanin (RPA-T8), β2 integrin-R-PE (FIB504), CD103 integrin-PE (Ber-Act8), CD45RO-FITC (UCHL1), and CD45RA-R-PE (HI100) were produced by BD Pharmingen. CD103 integrin-FITC (LF61) and CD49d integrin-FITC (44H6) were produced by Serotec.

Tetrameric HLA-A*0201/peptide complexes were synthesized as described previously (23, 24) with EBV BMLF1 249–257 (25) and Melan A26-35 (1) and Melan A26-35. Cells were stained with tetramers for 20 min at 37°C and then washed in PBS/1% FCS at 37°C, before incubating with anti-CD103-FITC and anti-CD8-allophycocyanin for 30 min on ice. Cells were washed twice in ice-cold PBS/1% FCS.

Flow cytometric analysis was done on a FACSCalibur (BD Biosciences), and data were analyzed using the CellQuest program (BD Biosciences). Lymphocytes were gated according to forward light scatter/side scatter profile, and dead cells were excluded by staining with propidium iodide (Sigma-Aldrich).

Lymphocyte extraction and culture

PBL were isolated by Ficoll density gradient centrifugation. Freshly isolated tissue was collected in wash medium (RPMI 1640 medium supplemented with penicillin/streptomycin). The tissue was washed four times with wash medium before being cut into small pieces using blades. The pieces were digested in 2% FCS RPMI 1640 medium containing 1 mg/ml type IV collagenase (Worthington Biochemical) for 2 h at room temperature. Half of the sample was used for ex vivo FACS staining, and the other half was put into culture medium (RPMI 1640 medium supplemented with 2 mM L-glutamine, penicillin/streptomycin and 5% pooled human serum AB+) supplemented with recombinant human IL-2 (rIL-2) 150 U/ml. The lymphocyte yield was 0.4–0.8 × 10^6 cells and 0.75–3.5 × 10^6 cells per gram of tissue from normal colon and from colon cancer, respectively. After 5–7 days, they were stimulated with purified phytohemagglutinin 20 µg/ml (Murex) and irradiated (50 Gy) allogeneic feeder cells. Tumor-infiltrating lymphocyte (TIL) cell lines were fed with 150 UI/ml rIL-2 twice a week.

Mixed lymphocyte tumor culture (MLTC) was performed as follows: autologous tumor cells were irradiated (150 Gy) and cultured with PBL at a ratio of 1 tumor cell to 10 lymphocytes in RPMI 1640 medium supplemented with 5% human serum and IL-2 (150 U/ml). New batches of irradiated tumor cells were added to the lymphocyte culture at 2-wk intervals.

CD8+ T cell clones, specific for HLA-A*0201-binding EBV BMLF1 249–257 (27) and Her2/neu 978–987 (36) peptides, were generated from normal donors as described previously (23) and were maintained in medium supplemented with IL-2 (150 U/ml). In some of the experiments on CD103 expression, 2.5 × 10^6 T cells were pulsed with the peptide of interest at 1 µM for 1 h. The peptide was then washed off and the cells added to 10^6 unpulsed T cells of the same clone. In other experiments, T cells were pulsed with plate-bound anti-CD3 (OKT3, 10 µg/ml). These experiments were done in the presence or absence of rhTGF-β1 (2 ng/ml; R&D Systems).

51Cr release cytotoxicity assay

Effector CTL are co incubated with 51Cr-labeled autologous tumor or EBV transformed B cells over 5 h. Maximally lysed cells (in 10% Triton X-100) and 51Cr-labeled target cells without effectors (spontaneous release of 51Cr) acted as reference points. Specific lysis of targets was calculated as follows: (sample – spontaneous release)/maximum spontaneous release) %.

ELISPOT assays

ELISPOT assays were performed as described previously (26). Spots were counted using an automated ELISPOT reader (Autoimmun-Diagnostika). Results were calculated by subtracting the mean background spot count from the mean spot count for each peptide or tumor cell line. Blocking assays were done with target cells that had been incubated with an Ab against MHC class I (w6/32, 30 µg/ml; American Type Culture Collection).

TGF-β1 measurement by ELISA

TGF-β1 levels were assayed using the TGF-β1 EIA Immunoassay kit (Promega) according to the manufacturer’s recommendations. Samples were obtained from 48-h culture supernatants of 10^6 tumor cells. Levels of total and active TGF-β1 were determined using a previously described protocol (27).

Generation of monococyte-derived DC and T cell priming

Monocytes were isolated from PBL from a healthy HLA-A2+ donor by positive sorting using anti-CD14-conjugated magnetic beads (Miltenyi Biotec). These were cultured in RPMI 1640 medium 10% FCS supplemented with 1000 U/ml IL-4 and 50 ng/ml GM-CSF (Leucomax; Norvatis) for 5 days. Cells were matured with 1 µg/ml LPS (Salmonella abortus equi; Sigma-Aldrich) over 36 h. DC were determined to be mature by expression of CD80, CD86, and TGF-β expression by FACS analysis.

Mature DC were pulsed with Melan A26–35 ELAGIGILTV peptide at 1 µM for 2 h at 37°C in serum-free medium. The peptide was then washed off, and the DC were incubated with autologous PBL at a 1:10 ratio for 12 days in RPMI 1640 medium 5% human serum. No IL-2 was added for the first 4 days. IL-2 was then used at 20 U/ml for 5 days then at 500 U/ml for the last 3 days. Priming took place in the absence or presence of TGF-β1 2 ng/ml and all-trans-retinoic acid 10 nM (Sigma-Aldrich).

Recall assay

PBL that had been primed previously with Melan A26 and rested for 4 wk were used. A total of 0.5 × 10^6 T cells was stimulated with Melan A26–35 (1 µM) for 7 or 10 days. Cells from the same batch that were not stimulated with peptide were used as controls. Cells were fed with 150
IU/ml rhIL-2 twice a week. Cultures with TGF-
1 were fed with the cytokine every other day at concentrations of 2, 0.5, and 0.2 ng/ml.

Intracellular cytokine staining
Intracellular cytokine staining for IFN-
was done as described previously (28). Briefly, lymphocytes were stimulated with 20 
M peptide for a total of 6 h. Brefeldin A was added in the second hour of stimulation at a final concentration of 5 
g/ml (Sigma-Aldrich). TGF-
1 at 2 ng/ml was added in some assays. The lymphocytes were washed and stained for CD8 and CD103 before they were fixed with 2% paraformaldehyde and permeabilized using FACS permeabilizing solution (BD Pharmingen). IFN-
was detected using FITC-conjugated IFN-
Ab (BD Pharmingen). Lymphocytes that had been either stimulated with PMA (10 
M; Sigma-Aldrich)/ionomycin (1 
g/ml; Sigma-Aldrich) or not stimulated at all were used as positive and negative controls, respectively.

Results
Expression of CD103 by colon carcinoma-specific CD8 + T cells
To study the mechanisms that control CD103 expression in colon carcinoma-specific T cells, a human tumor cell line was established from a colon carcinoma patient, (hereafter referred to as OX-CO-1), and has been used to stimulate in vitro autologous PBL. After two rounds of stimulation, we obtained a colon carcinoma-specific T cell line that was capable of killing in vitro the autologous tumor cells and producing IFN-
(see Fig. 1, A and B). As a control, we showed that OX-CO-1-specific T cells failed to recognize autologous B cells (Fig. 1A) and the NK target K562 cells (data not shown). Although a small proportion (1.5%) of the patient’s CD8 + PBL expressed CD103 in ex vivo FACS staining (data not shown), a significant increase of CD103 + CD8 + T cells (28%) was observed in the colon carcinoma-specific T cell line (Fig. 1C). Consistent with the observation that a large proportion of OX-CO-1-reactive T cells were CD8 positive, we showed that incubation of OX-CO-1 cells with the anti-MHC class I Ab W6/32 inhibited their recognition by autologous CD103-positive OX-CO-1-specific T cells (data not shown). In contrast, incubation of OX-CO-1 cells with an anti-CD103-blocking Ab failed to inhibit recognition by autologous CD103-positive OX-CO-1-specific T cells (data not shown).

FIGURE 1. Recognition of colon carcinoma line by autologous CD103 + T lymphocytes. A, Killing of the tumor cell line OX-CO-1 by an autologous MLTC line (>). Autologous EBV-transformed B cell line was used as negative control (■). B, IFN-γ ELISPOT assay demonstrating the ability of the tumor cell line OX-CO-1 to sensitize IFN-γ secretion by autologous MLTC line. Numbers of MLTC cells per well are indicated. The highest E:T ratio was 1:1. C, FACS staining with anti-CD8 and anti-CD103 Abs of the MLTC line. D, T cells were sorted according to the expression of CD103 and ELISPOT assay was performed using CD103 + and CD103 - fractions. Target cells are shown.

FIGURE 2. Tumor-specific response of mixed lymphocyte tumor culture line to OX-CO-3. Ability of a second MLTC derived from TIL stimulated with the autologous tumor line OX-CO-3 to recognize the autologous tumor line. The MLTC line specific for OX-CO-3 was sorted according to the expression of CD103. ELISPOT assay was conducted using CD103 + and CD103 - fractions incubated with the autologous tumor line OX-CO-3. Autologous fibroblasts and K562 cells were used as negative controls.
To assess whether CD103 expression segregated with tumor-reactive T cells, CD103\(^{+}\)/H11001 cells were sorted and tested for their ability to specifically recognize OX-CO-1 (Fig. 1D). The results of these experiments demonstrated a striking correlation between the expression of CD103 and the presence of tumor-specific T cell responses. These results were confirmed using a second tumor line, OX-CO-3 (from a different colon carcinoma patient), and an autologous MLTC derived from TIL (Fig. 2).

Demonstration that expression of CD103 by CD8\(^{+}\)/H11001 T lymphocytes correlated with their ability to recognize autologous colon carcinoma lines prompted us to assess the expression of CD103 in situ on colon cancer-infiltrating T lymphocytes. Although in mice CD103 can be found on the cell surface of both CD4 and CD8 T cells (8, 29), in human colon carcinoma samples, the majority of CD4\(^{+}\)/H11001 T cells are CD103 negative (Figs. 3 and 4). We showed that CD8\(^{+}\)/H11001 T lymphocytes are mainly localized in the tumor epithelium (Fig. 3C) and are CD103 and CD49d/\(\beta_7\) positive (Fig. 3, E and F). In contrast, CD4\(^{+}\) cells, which are mainly found in the tumor stroma, rather than in the epithelial layer (Fig. 3B), are only CD49d/\(\beta_7\) positive (Fig. 3F). To further analyze the expression of CD103 by tumor-infiltrating CD4\(^{+}\) and CD8\(^{+}\)/H11001 T lymphocytes, ex vivo FACS staining was performed on colon carcinoma samples (Fig. 4). These results demonstrated that expression of CD103 is restricted to a proportion of CD8\(^{+}\)/H11001 T lymphocytes (32.3%) (Fig. 4). These results confirm previously published data (9) and are consistent with the results shown in Fig. 1C.

\textbf{CD103 expression on T lymphocytes is enhanced by simultaneous TGF-\(\beta_1\) stimulation and recognition of cognate peptide epitope}

It has been shown previously that the effect of TGF-\(\beta_1\) in enhancing CD103 expression is greater on phytohemagglutinin-stimulated T cells (30, 31). Because OX-CO-1 secretes active TGF-\(\beta_1\) at a level of 0.17 ng/ml per 10\(^5\) cells (data not shown), we reasoned that the correlation between CD103 expression and tumor reactivity observed in Fig. 1 may be consistent with simultaneous recognition of the cognate peptide epitope(s) expressed by OX-CO-1 tumor line and TGF-\(\beta_1\).

To assess the role of TGF-\(\beta_1\) and Ag recognition either alone or in combination to up-regulate CD103 expression, we set up an in vitro model using a Her2/neu\(_{369-377}\)-specific CTL clone (23) (Fig. 5A). Although rhTGF-\(\beta_1\) alone or cognate peptide alone induced CD103 expression on a small proportion of cells (ranging from 53.6% to 56.4%), co-stimulation with rhTGF-\(\beta_1\) and cognate peptide increased CD103 expression to 78.3% and 83.8% for CD8\(^{+}\)/H11001 and CD4\(^{+}\)/H11001 cells, respectively (Fig. 5B). These results confirm previous findings (9) and are consistent with the results shown in Fig. 1C.

\textbf{FIGURE 3.} In situ analysis of CD103 expression by TIL. Immunohistochemical analysis of the expression of CD4, CD8, CD45RO, CD49d, and CD103 on TIL within colon carcinoma sections (magnification, \(\times 400\)). NA, necrotic area; LP, lamina propria; TE, tumor epithelium.

\textbf{FIGURE 4.} CD103 and CD49d/\(\beta_7\) expression in colon cancer-infiltrating lymphocytes. Ex vivo FACS analysis of the T cells in a colon carcinoma sample. Cell acquisitions were gated on CD3\(^{+}\)CD8\(^{+}\) cells or CD3\(^{+}\)CD8\(^{+}\) subpopulations (for CD103 or CD49d/\(\beta_7\) analysis). Percentages of CD8\(^{+}\) cells were calculated on the whole CD3\(^{+}\) population. Percentages of integrin-positive lymphocytes were calculated in the CD3\(^{+}\)CD8\(^{+}\) or CD3\(^{+}\)CD8\(^{+}\) lymphocytes.
0.23 to 4.5%), simultaneous exposure of the Her2/neu369–377 T cell clone to both TGF-β1 and Her2/neu369–377 peptide resulted in a significant increase of the proportion of CD103+ cells (86%). Similar results were obtained by culturing the Her2/neu369–377 CTL clone with the cognate peptide in a Transwell separated from the TGF-β1-secreting OX-CO-1 tumor line (Fig. 5B). This last result demonstrated that the tumor cell line was able to produce enough TGF-β1 to promote CD103 expression on T cells activated with their cognate Ag.

To further assess whether TGF-β1 secreted by the colon carcinoma cells can induce CD103 expression on third-party T cells, we cocultured the OX-CO-1-specific MLTC line with an EBV BMLF1259–267 CTL clone in the presence or absence of OX-CO-1 (Fig. 5C, panel c). A small proportion of EBV BMLF1259–267 T cells express CD103 in the presence of either the cognate peptide alone (Fig. 5C) or OX-CO-1 tumor cells (Fig. 5C, panel b). However, consistent with results shown in Fig. 5A, coincubation of the EBV BMLF1259–267 T cells with OX-CO-1 tumor cells and the cognate peptide epitope resulted in a significant increase of the proportion of CD103+ cells (51%) (Fig. 5C, panel d).

The results of these experiments indicate that bystander secretion of TGF-β1 by colon carcinoma cells can result in the expression of CD103 by third-party T cells in the presence of antigenic stimulation.

Role of TGF-β1 in the expression of CD103 during priming of naive CD8+ T cells
It has been demonstrated that the ability of murine T cells to home into the gut is controlled by events during T cell priming (3, 4). It has been shown that in mice, secretion of retinoic acid by MLN-derived DC imprints on T cells the ability to express integrins CD49d/7 and the chemokine receptor CCR9, which in turn enable lymphocytes to home to the intestine (21). However, it remains unclear whether up-regulation of CD103 may also be controlled by events during T cell priming.

To assess whether exposure of naive T cells to TGF-β1 during priming controls CD103 expression, we studied the expression of CD103 on in vitro-primed Ag-specific CD8+ T lymphocytes. We have demonstrated previously that Melan-A26–35-specific CTL precursors with functional and phenotypic markers of a naive population can be expanded from buffy coat of healthy...
volunteers with mature DC (28), providing the opportunity of characterizing the optimal conditions to expand in vitro tumor-specific CTL.

LPS-matured monocyte-derived DC were used to prime naive Melan A26-35-specific T cells from healthy donors in the presence or absence of TGF-β1 (H9251). The results of these experiments demonstrated that priming in the presence of TGF-β1 ensures the expansion of a larger proportion of CD103-positive cells, compared with the proportion of CD103-positive cells primed in the absence of TGF-β1 (H9251). No difference in the percentage of Melan A26-35 tetramer-positive T cells was observed in the cell populations primed in the presence or absence of TGF-β1 (H9251), demonstrating that the presence of TGF-β1 during T cell priming did not affect proliferation of Melan A26-35-specific T cells. Addition of retinoic acid during Melan-A26-35-specific T cell priming, either alone or in combination with TGF-β1, did not affect CD103 expression on T cells, indicating differences between the mechanisms inducing CD103 expression in human cells and those controlling expression of CD49d/β7 or CCR9 in mouse T cells (data not shown).

Melan A26-35-specific T cells were rested in the absence of exogenous TGF-β1 until CD103 expression had decreased to background levels (Fig. 6B). Memory T cells were then restimulated with Melan A26-35 tetramer-positive T cells that express CD103. Results from one of three experiments are shown. E, Memory Melan A26-35-specific T cells, primed without TGF-β1 (DC only) or with TGF-β1 for 48 h (DC + 48H TGFβ1) or 12 days (DC + 12 days TGFβ1) were restimulated with 1 μM peptide in the presence of different concentrations of TGF-β1. Percentage of CD8+ Melan A26-35 tetramer-positive T cells that express CD103. Results from one of two experiments are shown.

Discussion
CD103 is expressed by a large proportion of CD8+ IEL, compared with <3% of CD8+ PBL (9). Unlike the integrin CD49d/β7,
which facilitates migration of lymphocytes into the mucosa lamina propria, CD103 is essential for the retention of T cells into the mucosa epithelium layer (19). Our results, demonstrating that TGF-β1 stimulation and TCR engagement enhance the expression of CD103 on Ag-specific T cells, confirm and extend this model by highlighting a mechanism to retain in the epithelial layer T cells specific for Ags expressed by epithelial cells.

Recent papers in mice have clarified some of the mechanisms involved in the homing of murine T lymphocytes into the small intestinal mucosa after priming in either the MLN or the mucosa-associated lymphoid tissue (3, 4, 21). T cells primed in the alimentary tract express the integrin CD49d/β1 and CCR9, the receptor for TECK/CCL25 (3, 4). The ability to express CD49d/β1, and T cells that had been primed in TGF-β1 for 12 days and restimulated in TGF-β1 at 2 ng/ml. Percentage of IFN-γ secreted by Melan A26-35-specific T cells in the presence or absence of peptide is shown. B. Percentage of Melan A26-35-specific T cells used for the intracellular staining assay shown in A as measured by A2 tetramers containing the Melan A26-35 peptide.

FIGURE 7. Melan A26-35-specific T cells that were primed and restimulated in TGF-β1 express IFN-γ in intracellular cytokine staining assays. A. Intracellular staining with IFN-γ-specific Ab of Melan A26-35-specific T cells after 10 days restimulation with the Melan A26-35 peptide. The FACS plots show those for T cells that had been primed and restimulated without TGF-β1, and T cells that had been primed in TGF-β1 for 12 days and restimulated in TGF-β1 at 2 ng/ml. Percentage of IFN-γ secreted by Melan A26-35-specific T cells in the presence or absence of peptide is shown. B. Percentage of Melan A26-35-specific T cells used for the intracellular staining assay shown in A as measured by A2 tetramers containing the Melan A26-35 peptide.

The mechanisms controlling the lower threshold of TGF-β1 required for the expression of CD103 by memory T cells primed in the presence of TGF-β1 remain unclear. One possibility is that the presence of TGF-β1 during T cell priming may increase the density of TGF-β receptors on primed lymphocytes, which would result in an increased sensitivity of these cells to TGF-β1, when subsequently exposed to lower doses of TGF-β1. Alternatively, priming in the presence of TGF-β1 may influence the transcription of both the CD103 and β1 genes, lowering the threshold of TGF-β1 required to express these gene products.

Although TGF-β1 is better known as an immunosuppressive cytokine (18), the observation that TGF-β1-treated Melan-A26-35-specific T cells are capable of proliferating and releasing IFN-γ, when treated with the cognate peptide, is consistent with previously published results (31, 33, 34). In vitro experiments have shown that the duration and intensity of stimulation (33), the presence of CD28 costimulation (31, 33, 34) modulate the effects of TGF-β1 on lymphocytes. It has also been shown that lymphocytes stimulated in the presence of TGF-β1 in the primary culture proliferated more efficiently and secreted more abundant IL-2 and IFN-γ when restimulated in the presence of TGF-β1, as compared with cells which had not previously exposed to TGF-β1 (31–33). Furthermore, in mice, TGF-β1-dependent expression of CD103 on effector T cells is essential for intestinal GVHD (19), and for the destruction of renal and pancreatic allografts (20, 35). T cells from CD103 knockout mice, and T cells from dominant-negative TGFβ2R-transgenic mice, which are unable to express CD103, are less able to migrate into the epithelial components of intestines, renal allografts, or pancreatic allografts (19, 20). Such CD8+ effector T cells were much less capable of causing intestinal GVHD, and were not able to reject renal and pancreatic allografts. These results are consistent with a model in which T cells activated in the presence of TGF-β1 retain some of their properties and acquire the ability to express CD103.

In conclusion, we have analyzed the expression of CD103 on human colon cancer-specific T cells and described the importance for the up-regulation of CD103 of both TGF-β1 and TCR stimulation of Ag-specific T cells. The observation that CD8+ T cell priming in the presence of TGF-β1 modulates the ability of CD8+ T cells to up-regulate CD103 is of importance to provide important insights into the mechanisms which control T cell retention in the intestinal epithelium and to optimize the design of vaccination strategies capable of expanding tumor-specific T cells capable of homing into colon carcinoma.

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Disclosures
The authors have no financial conflict of interest.

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