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*J Immunol* published online 21 August 2015

http://www.jimmunol.org/content/early/2015/08/20/jimmunol.1500258
Human Mucosa-Associated Invariant T Cells Accumulate in Colon Adenocarcinomas but Produce Reduced Amounts of IFN-γ

Patrik Sundström,* Filip Ahlmanners,* Paulina Akéus,* Malin Sundquist,* Samuel Alsén,* Ulf Yrlid,* Lars Börjesson,† Åsa Sjöling,*‡ Bengt Gustavsson,† S. B. Justin Wong,*§ and Marianne Quiding-Järbrink*

Mucosa-associated invariant T (MAIT) cells are innate-like T cells with a conserved TCR α-chain recognizing bacterial metabolites presented on the invariant MHC-related 1 molecule. MAIT cells are present in intestinal tissues and liver, and they rapidly secrete IFN-γ and IL-17 in response to bacterial insult. In colon cancer, IL-17–driven inflammation promotes tumor progression, whereas IFN-γ production is essential for antitumor immunity. Thus, tumor-associated MAIT cells may affect antitumor immune responses by their secreted cytokines. However, the knowledge of MAIT cell presence and function in tumors is virtually absent. In this study, we determined the frequency, phenotype, and functional capacity of MAIT cells in colon adenocarcinomas and unaffected colon lamina propria. Flow cytometric analyses showed significant accumulation of MAIT cells in tumor tissue, irrespective of tumor stage or localization. Colonic MAIT cells displayed an activated memory phenotype and expression of chemokine receptors CCR6 and CCR9. Most MAIT cells in unaffected colon tissues produced IFN-γ, whereas only few produced IL-17. Colonic MAIT cells also produced TNF-α, IL-2, and granzyme B. In the tumors, significantly lower frequencies of IFN-γ–producing MAIT cells were seen, whereas there were no differences in the other cytokines analyzed, and in vitro studies showed that secreted factors from tumor tissue reduced IFN-γ production from MAIT cells. In conclusion, MAIT cells infiltrate colon tumors but their ability to produce IFN-γ is substantially reduced. We suggest that MAIT cells have the capacity to promote local immune responses to tumors, but factors in the tumor microenvironment act to reduce MAIT cell IFN-γ production.
transcription of Vα7.2 in kidney and brain tumors (16), but the functional capacity of tumor-associated MAIT cells has not been determined.

To investigate the infiltration of MAIT cells into human colon tumors, as well as their potential contribution to the local cytokine environment, we used material from tumors and unaffected tissue obtained during surgery to investigate the presence and functional capacity of tumor-associated MAIT cells. We show that MAIT cells are present in all tumors examined, irrespective of tumor stage or differentiation grade, and that they produce IFN-γ, TNF-α, IL-2, and granulysin B, but little IL-17 upon stimulation. However, the production of IFN-γ is reduced in tumor-associated MAIT cells compared with cells from the unaffected mucosa in the same patients, and we show that tumor-derived factors may act on local MAIT cells to reduce their production of IFN-γ.

Materials and Methods

Patients, volunteers, and sample collection

This study was performed according to the Declaration of Helsinki and approved by the Regional Board of Ethics in Medical Research in west Sweden. All volunteers gave written informed consent before participation. Altogether, 44 individuals undergoing curative resection of colon tumors at the Sahlgrenska University Hospital were included in the studies (27 males and 17 females, aged 43–89 y, median age of 76 y). Additional patient data are presented in Supplemental Table I. None of the patients suffered from autoimmune disease or had undergone radiotherapy or chemotherapy for at least 3 y prior to colectomy. Immediately after colectomy, a section of the tumor tissue encompassing both the center and more peripheral parts of the tumor was collected, as well as unaffected mucosa from at least 10 cm away from the tumor. Biopsies from the resection material were placed in RNAlater (Ambion) for 24 h before freezing at −80˚C and subsequent RNA extraction. The remaining colonic tissue material was transported in ice-cold PBS before immediate isolation of lymphocytes or fixation of tissue in ice-cold conditioned medium within <2 h. Heparinized venous blood was also obtained during surgery. Information about tumor stage, differentiation grade, location, and metastases was retrieved from the pathology report.

Additionally, venous blood samples were collected from healthy volunteers (four males and seven females, aged 49–78 y, median age of 63 y).

Cell isolation and stimulation

Lamina propria lymphocytes (LPL) and intraepithelial lymphocytes (IEL) were isolated as previously described (17). Briefly, the tissue samples were washed with PBS and the muscle layers, fat, connective tissue, and blood vessels were carefully removed. The tissue was cut into 5-mm pieces and subjected to four rounds of EDTA/DTT treatment to remove epithelial cells and IEL. The epithelial cell fractions from the second and third EDTA/DTT treatment were filtered and used to determine frequencies of MAIT cells among the intraepithelial cells by flow cytometry. After removal of the epithelium, the remaining tissue was digested with collagenase III (Sigma-Aldrich) together with DNase I (Worthington) for 2 h. The resulting single-cell solution was resuspended in RPMI 1640 (Life Technologies) containing 10% FBS (PAA Laboratories) and 50 μg/ml gentamicin (Life Technologies). Flow cytometry analyses confirmed the distinct composition of IEL and LPL. The iEL fraction had a CD8+/CD4+ T cell ratio of 7.3:1 whereas in the LPL cytometry analyses confirmed the distinct composition of iEL and LPL. The cell isolation followed a 50 ng/ml PMA and 500 ng/ml ionomycin calcium salt (Sigma-Aldrich) for 12 h, or 50 ng/ml IL-12 and 50 ng/ml IL-18 (R&D Systems) for 40 h (18). Protein transport inhibitor (BD GolgiStop, BD Biosciences) was added 4–12 h before harvest of stimulated cells.

Flow cytometry

Single-cell suspensions were stained with CD3–Alexa Fluor 700 (clone UCHT1), TCR γδ–FITC (clone B1), TCR Vα7.2–allophycocyanin (clone 3C10) (all from BioLegend, San Diego, CA), IFN-γ–allophycocyanin–eFluor 780 (clone 4S.B3), IL-17A–PE (clone eBio-D1E2), IL-2–PE (clone GSP1-17H12), CD45–eFluor 450 (clone 30-F11), TNF-α–Alexa Fluor 700 (clone MAb11), and CCR6–PE (clone R6H1; all from eBioscience). CD8a–allophycocyanin-H7, -PE, and -PerC (clone SK1), CD8β–PE (clone 2STs.5H7), CD45RO–allophycocyanin-H7 (clone UCHL1), Ki67–Alexa Fluor 700 (clone B56), granulysin B–PE (clone GB11), CD69–PE (clone FN50), CD4–FITC (clone SK3), CD45-PerC (clone 2D1), CD69–FITC (clone L78), CD45RA–PE (clone HI100), CD44–FITC (clone L344), CD4–FITC–FITC, -FCCITC (clone 12H5 PE), and CD8a from BD Pharmingen, IL-18Rα–PE (clone 70625), and CCR9–PE (clone 112509) (both from R&D Systems). Lymphocytes were identified by their forward and side scatter characteristics, and Live/Dead fixable aqua dead cell stain kit (Molecular Probes) was used to gate out dead cells. FIX&PERM (An Der Grub Bio Research) intracellular staining kit was used for detection of cytokines. Isootype controls were used to determine cut-off levels for positive staining. Data were acquired using a Becton Dickinson LSR II flow cytometer and analyzed by FlowJo software. CD4+ Vα7.2+CD161+ and CD161− T cells were sorted on a FACSAria machine and the cell pellet was immediately frozen before DNA isolation.

RNA and DNA isolation and quantitative PCR

Tissue specimens were lysed and homogenized (TissueLyser II, Qiagen) and subsequently treated with QIAshredder (Qiagen) to yield higher purity before total RNA was isolated using the RNaseasy mini kit (Qiagen), including DNA digestion. cDNA was synthesized from the RNA concentration was determined spectrophotometrically (NanoDrop ND-100). The Omniscript kit (Qiagen) was used for cDNA synthesis using 2000 ng RNA as template in a total reaction volume of 20 μl. Each real-time PCR reaction mixture contained 40 ng cDNA, Power SYBR Green master mix (Applied Biosystems) and oligonucleotide primers to detect MR1 forward, 5′-GGTGTCACATGTGCATTCTCA-3′, reverse, 5′-ACATAATGGCACAGACGACG-3′ and 18S RNA forward, 5′-CGGCGTTATTCCTGATGAC-3′, reverse, 5′-AAAGTTACGCTTGTGCAACA-3′ as housekeeping gene. DNA from sorted cells was extracted using the DNeasy blood and tissue kit (Qiagen). Each real-time PCR reaction mixture contained DNA equivalent to 200 cells, Power SYBR Green master mix (Applied Biosystems), and oligonucleotide primers designed to detect the MAIT cell–specific Vα7.2-Jα33 rearrangement, as described by Tilloy et al. (1). MR1 and 18S RNA primers were designed using Primer Express software (Applied Biosystems), and all primers were ordered from Eurofins MWG. Assays were run in duplicates at standard thermal cycling conditions described for the 7500 real-time PCR system (Applied Biosystems). Relative expression was determined by the ΔΔCt method using 18S RNA as endogenous control (19).

Statistical analysis

Statistical analyses were performed using Wilcoxon matched-pairs signed rank test when comparing data obtained from the same individual, and a Mann–Whitney rank test when data were compared between cancer patients and controls. When comparing three groups of matched data, the Friedman test followed by a Dunn posttest was used. The p values <0.05 were considered to be statistically significant.

Results

MAIT cell frequencies in blood of colon cancer patients

We first evaluated the presence of MAIT cells in the circulation of colon cancer patients and healthy volunteers using flow cytometry. MAIT cells were identified as CD45+CD3−CD4−“TCRγδ−”CD161+“Vα7.2+” T cells, as previously established by Dusseaux et al. (3). Fig. 1A shows the gating strategy to identify MAIT cells. We found that MAIT cells were present in the circulation of all colon cancer patients, and there was no significant difference in the frequencies between patients and healthy controls (Fig 1B). Most of the circulating MAIT cells in cancer patients were CD8a+ (84 ± 11%, mean ± SD), as previously described in healthy individuals (3, 20). Circulating MAIT cells from cancer patients also expressed CCR6 and IL-18Rα (CD218a) to a much larger extent than did the total pool of non-MAIT CD8a+ T cells (Fig. 1C–E), as would be expected from previous studies in healthy volunteers (3, 7, 9, 18, 21, 22).
Additionally, IL-18Rα expression was higher in MAIT cells than in the non-MAIT CD8+ T cells (Fig. 1E). Thus, our results indicate that there are no major differences in the circulating pool of MAIT cells in colon cancer patients and healthy subjects.

**Frequencies of colon MAIT cells**

Generally, T cells need to leave the circulation and enter tissues to perform their effector functions, and as MAIT cells respond to bacterial metabolites, their accumulation and function in the intestine may be particularly relevant. To determine MAIT cell frequencies in human intestinal tissues, we used tumor tissue and corresponding unaffected tissue from different locations in the large intestine from patients undergoing surgery for colon cancer. Single-cell suspensions were prepared by enzymatic digestion and analyzed by flow cytometry. Our analyses of unaffected tissue showed that putative MAIT cells were readily detected by flow cytometry in lamina propria from all colonic samples using the same gating strategy as above (Fig. 2A), regardless of their anatomical location, and frequencies ranged from 0.13 to 16.1% of all CD3+CD4-TCRγδ+ T cells in the unaffected tissue. There was no significant difference in MAIT cell frequencies between the different locations along the large intestine (Fig. 2B). Somewhat unexpectedly, the male cancer patients had more MAIT cells in the unaffected mucosa than did females (p < 0.05; Fig. 2C). However, no correlation was seen between the age of the patients and MAIT cell frequencies in this middle-aged to elderly patient material (data not shown).

The presence and effector functions of MAIT cells in intestinal tumors are virtually unknown, but they could have important implications for antitumor immunity (13). We thus assessed the frequencies of putative MAIT cells in tumor tissues and compared those to the corresponding lamina propria from unaffected mucosa. A representative flow cytometry staining of MAIT cells in unaffected colon lamina propria and tumor is shown in Fig. 2A. To validate that the Vα7.2+CD161high putative MAIT cell population...
did indeed contain the MAIT cell–specific Vα7.2-Jα33 rearrangement, and that tumor-located MAIT cells had not lost their expression of CD161, we sorted CD45+CD3+CD4+Vα7.2+MAIT cells and analyzed Vα7.2-Jα33 rearrangement by quantitative PCR, as previously described by Tilloy et al. (1). These analyses showed a distinct presence of the Vα7.2-Jα33 rearrangement in the CD161high population, but complete absence in the CD161− population (Supplemental Fig. 1). Continued flow cytometry

**FIGURE 2.** MAIT cells in unaffected colon lamina propria and tumors. Single-cell suspensions were isolated from unaffected lamina propria and tumors from colon cancer patients, and the frequencies of MAIT cells were determined by flow cytometry. (A) Gating strategy to identify CD45+CD3+CD4+ TCRγδ−CD161highVα7.2+ MAIT cells in lamina propria and tumor from a representative patient. (B) Frequencies of MAIT cells in lamina propria from unaffected cecum (caec), ascending colon (asc), transverse colon (trans), descending colon (desc), and sigmoid colon (sigm). (C) Frequencies of MAIT cells in lamina propria from unaffected colon tissues from women and men. (D and E) Frequencies of MAIT cells in lamina propria from unaffected colon tissues and tumors, presented as MAIT cells as percentage of CD45+CD3+CD4+ TCRγδ+ T cells (D) or of CD45+ immune cells (E). Symbols represent individual values and horizontal lines the median; n = 39 colon cancer patients analyzed individually. *p < 0.05, ***p < 0.001.
analyses demonstrated significantly higher frequencies of MAIT cells in tumors compared with the corresponding unaffected tissue from the same patient, regardless of whether the frequency of MAIT cells was expressed as a percentage of CD4⁺ T cells or of CD45⁺ immune cells in the tissue (p < 0.05; Fig. 2D) or of CD45⁺ immune cells in the tissue (p < 0.001; Fig. 2E). However, MAIT cell frequencies in tumors were not related to the tumor stage or the tumor location (data not shown).

To investigate MAIT cell presence also in the intraepithelial compartment, we collected the epithelial fraction during lymphocyte preparation and assessed the frequencies of MAIT cells in both unaffected and tumor-associated epithelium. MAIT cells were present also among the T cells, at approximately the same frequencies as in lamina propria (Supplemental Fig. 2A). As in lamina propria cell suspensions, frequencies of MAIT cells tended to be higher in the tumor-associated epithelium than in the epithelium from unaffected tissue; however, owing to a lower number of patients in these analyses, this difference only reached statistical significance when comparing MAIT cell frequencies in relationship to CD4⁺ T cells (p < 0.05). Taken together, these results show that MAIT cells are present in both the lamina propria and ileal compartment at all locations of the large intestine as well as in all tumors analyzed. They also demonstrate that MAIT cells accumulate in colon tumors compared with the unaffected lamina propria of the patients.

**Phenotype of colon MAIT cells**

We then further characterized the colonic and tumor-associated MAIT cells with regard to phenotypic markers to determine differentiation and activation status. The large majority of colonic MAIT cells were CD8⁺, albeit the frequencies of CD8⁺ MAIT cells were lower in the tumors than in the corresponding unaffected tissue (p < 0.001; Fig. 3A, Supplemental Fig. 3). Accordingly, double-negative MAIT cells were more frequent in the tumors than in the unaffected tissues. Of the CD8⁺ MAIT cells, significantly more cells expressed CD8αα in the tumor than in the unaffected tissue (p < 0.05; Fig. 3B, Supplemental Fig. 3), indicating that MAIT cells in tumors are further differentiated than MAIT cells present in the unaffected tissue (12, 23). To further assess differentiation stage, we analyzed the expression of IL-18Rα on MAIT cells. These analyses showed that the large majority of tumor-associated MAIT cells, as well as those in the unaffected mucosa, express IL-18Rα, which has also been described as a maturation marker for MAIT cells (23) (86 ± 8% IL-18Rα⁺ cells in unaffected tissue and 75 ± 16% in tumors; Supplemental Fig. 3).

Most MAIT cells expressed CD45RO (96 ± 4% in unaffected and 89 ± 13% in tumor tissue; Supplemental Fig. 3), indicating that they were effector or memory cells. Virtually all colonic MAIT cells, both in tumors and unaffected tissue, were recently activated, as judged by their expression of CD69 (Fig. 3C, Supplemental Fig. 3). This was in contrast to the CD8⁺ non-MAIT cells, where there was a significant reduction of activated CD69⁺ cells in the tumor (p < 0.05), as we have previously reported (24). We also analyzed MAIT cell expression of programmed cell death protein 1 (PD-1; CD279), which has been implicated as an attenuating receptor on exhausted T cells, and also on *Mycobacterium tuberculosis*-reactive MAIT cells (25, 26). These analyses revealed a relatively high expression of PD-1 on MAIT cells from both unaffected and tumor tissue (Fig. 3D, Supplemental Fig. 3), whereas CD8⁺ non–MAIT cells in the tumor usually had a higher PD-1 expression than did the corresponding population in unaffected tissue. We could also show that a small fraction of the colonic MAIT cells were proliferating, as judged by their expression of Ki67. In some patients, the tumor-associated MAIT cells proliferated to a larger extent than did those in unaffected tissue, but this difference was not significant (Fig. 3E, Supplemental Fig. 3). Taken together, our results indicate that the MAIT cells in colon tissues are well differentiated and recently activated memory cells, and that MAIT cells in tumors may be further differentiated than MAIT cells in unaffected mucosa.

**Chemokine receptor expression by colon MAIT cells**

Circulating MAIT cells have been reported to preferentially express CCR6, suggesting migration to liver and intestinal tissues (3, 9, 22). To determine some of the migration cues leading to MAIT cell accumulation in colon tissues, we analyzed the expression of chemokine receptors CCR6 and CCR9 on intestinal MAIT cells. CCR6 is the only receptor for CCL20 (MIP-3α), and it has been associated with both Th17 and regulatory T cells, as well as MAIT cells, but it is also ubiquitously expressed on lymphocytes migrating to the gut (27–29).Colon MAIT cells had a varying expression of CCR6, but they expressed significantly more CCR6 than did the non–MAIT CD8⁺ T cells in the colon (p < 0.01; Fig. 4A). However, there was no difference in CCR6 expression between MAIT cells in tumors and unaffected tissues. CCR9 is the sole receptor for the chemokine CCL25 (TECK), which is fundamental for homing of effector T cells to intestinal tissues (30, 31). Many colonic MAIT cells expressed CCR9, as did the non–MAIT CD8⁺ T cells, and there were no differences between the cell populations (Fig. 4B). Taken together, these results show that intestinal MAIT cells express gut-homing chemokine receptors, and that the chemokine responsiveness is relatively similar in both tumor-associated MAIT cells and those in the unaffected mucosa.

**Effector functions of colon MAIT cells**

The local intratumoral cytokine network contributes significantly in determining disease recurrence and patient survival in colon cancer (32). In particular, a high Th1 to Th17 ratio is highly associated with improved patient outcome (13). MAIT cells have been reported to secrete both of these cytokines, as well as the tumor-opposing cytokine IL-22. In contrast, MAIT cells producing IL-22 or IFN-γ were virtually undetectable in the tissues (p < 0.05; Fig. 4B), whereas the median value in tumors was 3.4% (range, 0–10.3%). Most IL-22–producing MAIT cells did not simultaneously produce IFN-γ, whereas many fewer MAIT cells produced IL-17. In the tumors, however, significantly lower frequencies (p < 0.01) of IFN-γ–producing MAIT cells were seen compared with unaffected tissue. The median value of IFN-γ production by MAIT cells in unaffected tissue was 78.5% (range, 49.0–94.0%), whereas the median value in tumors was 53.4% (range, 2.6–87.1%).

In contrast, the median value of IL-17 production by MAIT cells in unaffected tissue was only 1.8% (range, 0–15.1%) and in tumors was 3.4% (range, 0–10.3%). Most IL-17–producing MAIT cells did not simultaneously produce IFN-γ, but there were also some double-positive cells present (Fig. 5A).

A substantial proportion of colonic MAIT cells also produced TNF-α and IL-2 (Fig. 5A, 5B). TNF-α was usually coexpressed with IFN-γ in the lamina propria MAIT cells, as was IL-2. In contrast, MAIT cells producing IL-4 or IL-10 were virtually undetectable in the tissues (<0.5%), and only few MAIT cells produced IL-5 (0–5%, data not shown). Notably, the reduction in IFN-γ production in the tumors was selective, as there were no significant differences in the frequencies of IL-17, TNF-α, or IL-2–producing cells between unaffected tissues or tumors (Fig. 5B). Freshly isolated MAIT cells from both unaffected and tumor tissue had...
MAIT cells

![Graphs showing the differentiation markers and proliferation of MAIT cells and CD8+ non-MAIT cells in unaffected colon lamina propria and tumors.](http://www.jimmunol.org/)

**FIGURE 3.** MAIT cell differentiation markers and proliferation in unaffected colon lamina propria and tumors. Single-cell suspensions were isolated from unaffected lamina propria and tumors from colon cancer patients, and expression of CD8 (both CD8αα and CD8αβ) (A), CD8 αα only (B), CD69 (C), PD-1 (D), and Ki67 (E) by MAIT cells and CD8+ non-MAIT cells determined by flow cytometry. Symbols represent individual values and horizontal lines the median; n = 6–22 colon cancer patients analyzed individually. *p < 0.05, **p < 0.01, ***p < 0.001.

a low expression of granzyme B, as previously described for circulating MAIT cells (3, 33). Following stimulation, these frequencies rapidly increased (Fig. 5C), suggesting that MAIT cells would readily increase their cytolytic potential also upon ligand encounter.

Taken together, these results show that colonic MAIT cells respond to stimulation with production of Th1-associated cytokines and cytotoxic molecules, but little IL-17 or Th2-associated cytokines. Tumor-associated MAIT cells, alternatively, have a much lower capacity for IFN-γ secretion.

**MR1 expression in colon tumors**

MAIT cells recognize Ags presented in the context of the invariant MR1 (2), and reduced MR1 expression could be one reason for defective IFN-γ responses in the tumors. Surface expression of MR1 is unstable in the absence of stabilizing ligands (8). Therefore, we used quantitative real-time PCR to determine the expression of MR1 in tumors and unaffected colon tissue. MR1A transcripts were readily detected in all tissues examined, and there was no significant difference in the expression between tumors and unaffected
colon tissue (Fig. 6). It would thus appear that lack of MR1 expression in tumor tissue is not the cause of the reduced IFN-γ production in tumor-associated MAIT cells.

Effect of the tumor microenvironment on MAIT cell effector functions

To investigate whether soluble mediators from the tumor microenvironment could influence MAIT cell activation, we used TCM from tumors and unaffected tissue from five different patients and stimulated circulating MAIT cells from healthy donors with IL-12 and IL-18 in the presence of TCM. TCM from each patient was used with PBMC from two different healthy donors. Tumor-derived TCM from four of the five patients tested reduced the frequencies of IFN-γ-producing MAIT cells in stimulated cell cultures compared with TCM from unaffected tissue in both volunteers examined (Fig. 7A). In the fifth patient, the tumor-derived TCM induced 1.5-fold more IFN-γ+ cells than did the TCM from unaffected tissue. All in all, MAIT cells incubated in the presence of tumor-derived TCM had significantly fewer IFN-γ+ cells than did the control cells stimulated without TCM (p < 0.01), whereas TCM from unaffected tissue did not significantly reduce IFN-γ production compared with stimulated cells alone. The incubation with IL-12 and IL-18 induced only few IFN-γ-producing cells among CD4+ T cells (0.8 ± 0.4%) and non–MAIT CD8+ T cells (1.7 ± 2.0%). These frequencies were not significantly altered by the presence of TCM from tumors. In contrast to IFN-γ, coinoculation with TCM from both types of tissue resulted in a significantly higher expression of CD69 on MAIT cells. TCM from tumors did not reduce the CD69 expression induced by MAIT cell activation (Fig. 7B), suggesting that the tumor-derived factors act to specifically reduce IFN-γ production, even though the MAIT cells are activated. Taken together, these results strongly suggest that soluble mediators from most colon tumors act to specifically reduce IFN-γ induction or synthesis in MAIT cells.

Discussion

In this study, we show that human colonic MAIT cells have an activated memory phenotype, carry chemokine receptors to direct them to intestinal tissues, and that MAIT cells accumulate in colon tumors. Still, MAIT cell production of the important antimicrobial effector cytokine IFN-γ was selectively reduced in the tumors, possibly due to soluble factors produced in the tumor tissue.

Our initial experiments showed that the frequency and phenotype of circulating MAIT cells was similar in colon cancer patients and healthy age-matched volunteers. Thus, there is no systemic depletion of MAIT cells, as was previously documented in autoimmune diseases and chronic infections with, for example, HIV or M. tuberculosis (7, 11, 21, 22, 34, 35). This confirms a previous study showing that circulating MAIT cell frequencies were not reduced in patients with various solid tumors (7). We then focused our attention to intestinal MAIT cells, and our results substantially advance the knowledge of human colon-resident MAIT cells. First, we show that MAIT cells are present in both the lamina propria and the iEL compartment in all individuals examined, and at all locations along the length of the colon. An early study of human gut tissues reported preliminary results suggesting that gut MAIT cells were restricted to lamina propria (2), but recently small intestinal intraepithelial MAIT cells were reported (6). Our results now confirm that MAIT cells are present also among colonic iEL. Circulating MAIT cell frequencies have been shown to decrease with increasing age, at least in healthy subjects (36, 37), but we could not document such an effect in the colon from this middle-aged and elderly patient material. In contrast to our results of higher MAIT cell frequencies in males, one of the latter studies (36) found no sex difference in circulating MAIT cell frequencies at the ages 50 y and above. The differences between these studies and the present results may be related to differences between the large intestine and the blood, as well as different ethnic populations.

As previously shown (35), most colonic MAIT cells are CD8+, with only a small proportion of double-negative cells in the MAIT cell pool. Furthermore, we also show that colon-resident human MAIT cells express a memory phenotype and are highly activated at steady-state, based on CD69 expression. About 10−20% of ex vivo blood MAIT cells express CD69 (data not shown), but in the gut, the CD69 expression is almost universal among MAIT cells, suggesting that most MAIT cells in the colon are well-differentiated effector cells that have recently encountered their cognate Ag. This is further supported by the large proportion of gut MAIT cells that rapidly respond to polyclonal stimulation by increasing their cytokine production and upregulating granzyme B expression. As previously demonstrated in circulating MAIT cells (3, 33), the proliferation of colonic MAIT cells in situ is very low. We also extend previous studies on blood MAIT cells (3, 9, 22) by showing that most colon MAIT cells express the chemokine receptors CCR6 and CCR9, presumably contributing to their intestinal localization.

We then compared MAIT cell frequencies and function in colon tumors to those in the unaffected lamina propria from the same individuals, and we showed that MAIT cells accumulated to a greater extent in the tumors than in unaffected colonic lamina.

**FIGURE 4.** Chemokine receptor expression in colonic MAIT cells. Single-cell suspensions were isolated from unaffected lamina propria and tumors from colon cancer patients, and expression of CCR6 (A) and CCR9 (B) by MAIT cells (○) and CD8+ non–MAIT cells (●) was determined by flow cytometry. Symbols represent individual values and horizontal lines the median; n = 8–9 colon cancer patients analyzed individually. **p < 0.01.
propria from the same patient. Notably, MAIT cells were present in all tumors, despite differences with regard to tumor stage and localization, degree of tumor differentiation, and lymphocyte infiltration. The accumulation of lymphocytes in a given tissue could result from increased recruitment, increased proliferation, or increased retention. The low proliferative activity of tumor-associated MAIT cells would speak against the possibility that increased intratumoral proliferation is a key mechanism for MAIT cell accumulation in the tumors. As invasive colon tumors produce more CCL20 and less CCL25 than do surrounding tissue (38, 39), the CCL20/CCR6 axis might be more important than the CCR9/CCL25 axis in mediating MAIT cell recruitment to intestinal tumors. However, other factors such as endothelial adhesion molecules and additional chemokines are probably involved, as CCR6 expression

**FIGURE 5.** Cytokine and granzyme B expression in colonic MAIT cells. Single-cell suspensions were isolated from unaffected lamina propria and tumors from colon cancer patients, stimulated with PMA and ionomycin, and analyzed by flow cytometry to detect intracellular cytokines or granzyme B. (A) Representative staining of indicated cytokines in MAIT cells isolated from unaffected colon lamina propria and tumor. (B) Cytokine production in unaffected colon lamina propria and tumor. (C) Granzyme B expression in untreated and stimulated MAIT cells isolated from unaffected colon lamina propria and tumor. Symbols represent individual values and horizontal lines the median; n = 6–10 colon cancer patients analyzed individually. **p < 0.01.
immune defense against the tumor (14). However, based on our promotion of local Th17 responses that may be detrimental to this results in increased bacterial colonization and invasion, which in rectal tumors compared with the unaffected mucosa (14, 15), and once activated. The intestinal barrier function is impaired in colo-
tissues (16). Based on their prompt secretion of IFN-
g the present study. In contrast, MAIT cell production of IFN-\gamma was actually reduced in the tumor compared with the unaffected mucosa. This did not appear to be caused by altered MR1 expression in the tumors, or by increased PD-1 expression by tumor-infiltrating MAIT cells. Instead, soluble mediators released from the tumor itself or from tumor-infiltrating lymphocytes may reduce IFN-\gamma secretion from MAIT cells. However, these factors do not seem to include IL-18-binding protein. In vitro, there was a selective effect on IFN-\gamma production by MAIT cells by TCM from tumors, as there was no general inhibition of MAIT cell activation determined by CD69 expression. If reproduced in vivo, reduced IFN-\gamma production would potentially act to reduce antitumor immunity, especially as the balance between Th1 and Th17 effector functions is pivotal for the final patient outcome in colon cancer (13). A re-
duction in MAIT cell production of IFN-\gamma has also been reported in patients with active tuberculosis or the autoimmune disease systemic lupus erythematosus, which harbor MAIT cells that respond poorly to *Escherichia coli* stimulation compared with healthy controls (25, 34). However, tuberculosis patients and controls have similar cytokine responses following maximal PMA/ionomycin stimulation, which contrasts with the reduction of IFN-\gamma expression stimulated by PMA/ionomycin that is observed in MAIT cells from colon tumors. In contrast to our results, a recent study by Serriari et al. (40) showed that circulating MAIT cells from pa-
tients with inflammatory bowel disease had increased capacity to produce IL-17 compared with MAIT cells from healthy controls. Taken together, these studies indicate that MAIT cells have the ca-
pacity to adjust their effector cytokine program depending on en-
vironmental cues in different intestinal diseases.

In conclusion, our findings show that colon-resident MAIT cells have the potential to positively influence antitumor immunity based on their cytokine production and cytotoxic potential. We also demon-
strate accumulation of MAIT cells in colon tumors, but local factors in the tumor microenvironment may act to selectively reduce Th1-type responses by MAIT cells. Based on our results, we propose
that MAIT cell accumulation may be important for local antitumor responses. Therapy to overcome the local modulation of their Th1-like effector functions will likely need to capitalize on their full antitumor potential in colon cancer.

Acknowledgments

We thank all patients and volunteers for participation in this study and Hillevi Björkqvist and Ann-Louise Helminen for valuable assistance with collection of clinical samples.

Disclosures

The authors have no financial conflicts of interest.

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