A Transcriptionally Distinct CXCL13⁺CD103⁺CD8⁺ T-cell Population Is Associated with B-cell Recruitment and Neoantigen Load in Human Cancer

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Abstract

The chemokine CXCL13 mediates recruitment of B cells to tumors and is essential for the formation of tertiary lymphoid structures (TLSs). TLSs are thought to support antitumor immunity and are associated with improved prognosis. However, it remains unknown whether TLSs are formed in response to the general inflammatory character of the tumor microenvironment, or rather, are induced by (neo)antigen-specific adaptive immunity. We here report on the finding that the TGFB-dependent CD103⁺CD8⁺ tumor-infiltrating T-cell (TIL) subgroup population expressed and produced CXCL13. Accordingly, CD8⁺ T cells from peripheral blood activated in the presence of TGFB upregulated CD103 and secreted CXCL13. Conversely, inhibition of TGFB receptor signaling abrogated CXCL13 production. CXCL13⁺CD103⁺CD8⁺ TILs correlated with B-cell recruitment, TLSs, and neoantigen burden in six cohorts of human tumors. Altogether, our findings indicated that TGFB plays a noncanonical role in coordinating immune responses against human tumors and suggest a potential role for CXCL13⁺CD103⁺CD8⁺ TILs in mediating B-cell recruitment and TLS formation in human tumors.

Introduction

Immune checkpoint inhibitors (ICIs) targeting programmed death ligand 1 (PD-L1) or its receptor, programmed death 1 (PD-1), have elicited unprecedented long-term disease remissions in advanced and previously treatment-refractory cancers (1–3).

Unfortunately, only a subset of patients currently benefit from treatment. ICIs are more likely to be effective in patients with a preexisting anticancer immune response, most notably a CD8⁺ cytotoxic T-cell response against tumor neoantigens (4).

Responsive tumors harbor significantly more predicted neoantigens (5, 6) and display evidence of a coordinated immune response comprising T cells, dendritic cells (DCs), and B cells (7). In diseases that parallel tumor development, such as chronic inflammatory conditions, this coordinated infiltration by different immune cell subsets is frequently associated with tertiary lymphoid structures (TLSs)—an ectopic form of lymphoid tissue. TLSs exhibit features of regular lymph nodes, including high endothelial venules, a T-cell zone with mature DCs, and a germinal center with follicular DCs and B cells (8). Several studies have reported the presence of TLSs in tumors, which was generally found to be associated with greater immune control of cancer growth and improved prognosis (9, 10). For several malignancies, the combination of TLS presence and high CD8⁺ T-cell infiltration was found to associate with superior prognosis, whereas high CD8⁺ T-cell infiltration alone associated with poor or moderate prognosis (11, 12). These observations highlight the importance of a coordinated immune response, including TLS formation, in anticancer immunity.

To date, the molecular determinants of tumor TLS formation remain incompletely understood. Current data suggest that TLS formation results from a complex interplay between DCs, T cells, B cells, and supporting stromal cells. Interplay among these cells relies on reciprocal cytokine and chemokine signaling, including chemokine [C-X-C motif] ligand 13 (CXCL13), receptor activator...
of nuclear factor κ B (ligand) (RANK/RANKL), lymphotxin αβ (LTαβ), and chemokine (C–C motif) ligand 21 (CCL21; ref. 13). A central role for CXCL13 in this process is suggested by the inability of B cells to home and accumulate into lymphoid aggregates (14) and generate functional lymphoid tissue (15, 16) in CXCL13-knockout mice and the observation that CXCL13 alone is sufficient to generate lymphoid tissues (17–19). Nevertheless, a key outstanding question remains whether tumor-associated TLSs are formed in response to the general inflammatory character of the tumor microenvironment, or rather, are induced by (neo)antigen-specific adaptive immunity. Two studies suggest that a subset of CXCL13-producing CD8+ tumor-infiltrating lymphocytes (TIL) may link (neo)antigen recognition to TLS formation (7, 20).

Here, we report the finding that TGFβ receptor signaling licensed CD8+ T cells to produce and secrete CXCL13 upon concurrent T-cell receptor (TCR) stimulation. Induction of CXCL13 was paralleled by upregulation of CD103, a marker for tissue-resident TILs. Accordingly, bulk and single-cell RNA sequencing identified exclusive expression of CXCL13 in human CD103+, but not CD103−, CD8+ TILs. In line with these data, the presence of CD103+ TILs correlated to B-cell recruitment and TLSs in tumors with a high mutational load. This discovery sheds light on how B cells could be recruited to tumors by CTLs, identifying a noncanonical role for TGFβ in the orchestration of a coordinated immune response against human (neo)antigen-rich tumors. Our findings also identify CD103+ and B cells as potential biomarkers for ICIs in epithelial malignancies.

Materials and Methods

Patients

Tumor tissue from four patients with stage IIC high-grade serous ovarian cancer was collected during primary cytoreductive surgery, prior to chemotherapy, and from one patient with stage IV high-grade serous ovarian cancer during interval debulking upon three cycles of chemotherapy. Written informed consent was obtained from all patients. Selection of patients with uterine cancer was described previously (21). Briefly, uterine cancer tissue was obtained from patients involved in the PORTEC-1 (22) and PORTEC-2 (23) studies (n = 57), the uterine cancer series (n = 67) from Leiden University Medical Center (LUMC, Leiden, the Netherlands), and uterine cancer series (n = 26) from the University Medical Center Groningen (UMCG, Groningen, the Netherlands) in accordance with local medical ethical guidelines (24). Tumor material was fixed in formalin and embedded in paraffin. Tumor material from 119 patients was available for analysis. Mutations in the exonuclease domain of polymerase epsilon (POLE-EDM) and microsatellite instability status were known from previous studies (24). Of the tumors available for this study, 42 tumors were POLE wild-type and microsatellite stable (MSS), 38 were POLE wild-type with microsatellite instability (MSI), and 39 were POLE-EDM. POLE-EDM statuses did not cooccur with microsatellite instability. All cases were of endometrioid histology (EEC), and the number of low-grade and high-grade tumors was spread equally over the three molecular groups. Selection of patients with ovarian cancer and CD103 staining was reported previously (25). Ethical approval for tumor molecular analysis was granted at LUMC, UMCG, and by Oxfordshire Research Ethics Committee B (approval no. 05/Q1605/66).

Analysis of TCGA mRNA sequencing data

RSEM-normalized mRNA-seq data and clinical data from uterine corpus endometrial carcinoma (UCEC), ovarian cancer, breast cancer, and lung adenocarcinoma were downloaded from fireBrowse.org on March 13, 2017 (UCEC) and July 14, 2017 (ovarian cancer, breast cancer, lung adenocarcinoma). RSEM mRNA sequencing expression data were log2+1 transformed, and genes with zero reads in all samples were removed. POLE-EDM, MSI, and MSS cases were identified in the endometrial cancer data. The mononucleotide and dinucleotide marker panel analysis status was provided by the Cancer Genome Atlas (TCGA), and mutations in the exome-coding domain of POLE were determined previously (26). Heatmaps were constructed in R (version 3.3.1) with packages ggplot and ggplot2. The gene set of TLSs was reported previously (10, 27). Gene sets for the CD8+CD103+ and CD8+CD103− signatures were derived from the sequencing data (Supplementary Table S1). Spearman correlations between the TLS signature, the CD8+CD103+ signature, and the CD8+CD103− signature were visualized in correlation plots using the Corrplot package (Version 0.77) in R. CXCL13-high vs CD103-high versus CXCL13-low vs CD103-low groups in MSS UCEC were based on median expression of CXCL13 (4.35698) and ITGAE (8.086985). Survival curves were constructed with R packages survival (version 2.41-3) and survminer (version 0.4.3). All analyses were performed in R (version 3.4.0), with exception of the construction of the heatmap in Fig. 3C, which was made in R version 3.3.1.

IHC

To assess the presence of TLSs in uterine cancer, we stained for the B-cell marker CD20 on whole tissue sections. Formalin-fixed, paraffin-embedded (FFPE) slides were deparaffinized and rehydrated in graded ethanol. Antigen retrieval was initiated with a preheated 10 mmol/L citrate buffer (pH = 6) and endogenous peroxidase activity was blocked by submerging sections in a 0.45% hydrogen peroxide solution. Slides were blocked in PBS containing 1% human serum and 1% BSA. Slides were incubated overnight with anti-CD20 (0.63 mg/L; clone L26, catalog number M0755, Dako) at 4°C. Subsequently, slides were incubated with a ready-to-use peroxidase-labeled polymer for 30 minutes (Envision+®/HRP anti-mouse, 2 drops, catalog number K4001, Dako). Signal was visualized with 3,3′-diaminobenzidin (DAB) solution, and slides were counterstained with hematoxylin. Appropriate washing steps with PBS were performed in-between incubation steps. Sections were embedded in Eukitt mounting medium (Sigma Aldrich), and slides were scanned on a Hamamatsu digital slide scanner (Hamamatsu Photonics). The number of CD20+ (dense) follicles in each slide was quantified in NDPview2 software by two independent observers who were blinded to clinicopathologic data.

IHC for CD8 was performed previously in this cohort (24). To assess the survival effect of CD103 infiltration in mismatch repair–proficient (pMMR) cancers, we used a staining for CD103 on tissue microarray slides of uterine cancer (28). FFPE slides were prepared as described above and incubated with rabbit-anti human CD103 (1 mg/L, anti-E7-integran, clone ERPR4166(2), catalog number Ab129202, Abcam), followed by a ready-to-use peroxidase-labeled polymer (Envision+®/HRP anti-rabbit, cat.no. K4003, Dako) and Biotin Tyramide working solution (TSA kit, Perkin Elmer), streptavidin-HRP (TSA kit, Perkin Elmer), and 3,3′-diaminobenzidin/hematoxylin. Positively stained cells were quantified per core and adjusted for core surface. Patients with
at least two cores with a minimum of 20% tumor epithelium were included for analysis. All slides were counted manually by two individuals who were blinded for clinicopathologic data.

Multicolor immunofluorescence

FFPE slide preparation and antigen retrieval were performed as described above. Next, slides were incubated overnight at 4 °C with primary antibody and subsequently incubated with the appropriate secondary antibody for 45 minutes at room temperature (Supplementary Table S2). Specific signal was amplified using the TSA Cyamine 5 (Cy5) detection kit (Perkin Elmer, NEL705A001KT) or the TSA Cyamine 3 (Cy3) and Fluorescein detection kit (Perkin Elmer, 753001KT), according to the manufacturer’s protocols. To allow multiple amplifications on the same slide, primary HRP labels were destroyed between incubations by washing with 0.01 mol/L hydrochloric acid for 10 minutes. Appropriate washing steps with PBS containing 0.05% Tween20 (Sigma-Aldrich) were performed during the procedure. For embedding, Prolong Diamond antifade mounting medium with or without DAPI was used (Invitrogen/Thermo Fisher Scientific, P36962 and P36961). Finally, slides were scanned at room temperature using the TissueFAX acquisition software and microscope (TissueGnostics) with the following specifications: Zeiss EC "Plan-Neofluar“ 40x/1.3 Oil, DIC objective, CMSOS-color camera PL-B623 Pixelink (3.1 Megapixels), EXFO Excite 120 PC fluorescence illumination and Chroma ET Dapi (49000), Chroma ET Cy3 (49004), Chroma ET Cy5 (49006), and Chroma FITC (49011) filter sets. Overlay images were produced using Adobe Photoshop software.

mRNA sequencing

Ovarian tumors from two patients were cut into pieces of <1 mm3 and placed in a T75 culture flask (Nunc EasyFlask Cell Culture Flasks, catalog no. 156499, Thermo Fisher Scientific) with digestion medium, consisting of RPMI (Gibco), 10% FBS (Gibco), collagenase type IV (1 mg/mL, Gibco), and recombinant human DNase (12.6 μg/mL; Pulmozyme, Roche) for overnight digestion at room temperature. After digestion, the suspension was strained through a 70-μm filter and washed with PBS. Cells were centrifuged on a Ficoll-Paque gradient (GE Healthcare Bio-Sciences AB) and lymphocytes were isolated from between the two layers. After a wash with PBS the cells were pelleted. Total cell pellet was suspended in 1 mL FBS with 10% dimethylsulfoxide (Merck) and stored in liquid nitrogen until further use.

Prior to sequencing, tumor digests were thawed on ice, washed with AIM-V medium (Gibco) with 5% pooled human serum (PHS, One Lambda) and centrifuged at 1,000 × g. The total cell pellets were resuspended in AIM-V with 5% PHS, and cells were incubated with CD3-BV421, CD4-PerCP-Cy5.5, CD8α-APC-eFluor780, CD8β-PEcy7, TCRβ-APC, CD103-FITC, and CD56-PE antibodies at 4 °C for 45 minutes (Supplementary Table S3). For gating on CD3+CD4-CD8α+TCRβ+CD56− cells, CD103+ and CD103+ single cells were sorted on a Beckman Coulter Astrios cytometer directly into 4 μL lysis buffer (2 μL of 0.2% Triton X-100, Sigma-Aldrich, catalog no. T9284) with 5% recombinant RNase inhibitor (Westburg-Clontech, catalog no. 2313A), 1 μL of 10 μmol/L barcoded oligo dT primer, and 1 μL 4 × 10 mmol/L dNTP mix in 96-well PCR plates. Each well contained a unique indexed Oligo dT primer (custom designed by P. van der Vlies [UMGC, Groningen, the Netherlands]; Supplementary Table S4), enabling identification of individual cells after pooled RNA sequencing.

In addition to single-cell wells, small bulk populations of 20 cells were sorted per microplate well. Per patient, 40 single CD8+ T cells (20 wells CD103+, 20 wells CD103−) and 20 small bulk 20-25 cells populations (10 wells CD103+, 10 wells CD103−) were sorted. After sorting, the plate was vortexed and spun down briefly, and incubated at 72°C for 3 minutes. After this step, the plate was kept cool. The transcriptomes were amplified by a modified SMART-Seq2 protocol using SmartScribe reverse transcriptase (Westburg-Clontech, LC639537), based on a previously published protocol (29). In brief, custom primers were designed that included a PCR-primer recognition site for pre-PCR, a Unique Molecular Identifier (UMI), a cell barcode (see Supplementary Table S4), and a poly T-stretch. Each cell (or pool) was tagged with an oligo dT primer, including the UMI and cell barcode. A template switching pre-PCR was used to generate cDNA. Pools are made of single cells or pools with unique cell barcodes and 500 pg of the pools was tategnamented and barcoded using a N7xx index and custom P5 hybrid primer: (AAATATACG-GCCAGACACGCTACTCACCCGGTGCGAGAAGCTG-GTATCAACGAGACTG-C) according the Illumina Nextera XT DNA Sample Preparation Kit Protocol (Illumina, catalog no. FC-131-1096). The pools were purified using AMPure beads, in a ratio of 0.6:1, to remove primer dimers. Presence and size distribution of the obtained PCR product were checked on a PerkinElmer LabChip GX high-sensitivity DNA chip. A super pool was created by equimolar pooling (1 nmol/L) of the Nextera products, and the samples were sequenced on Illumina NextSeq500 2500 using 50 bp paired-end reads, one read for the mRNA transcript, and the other for the cell-barcode. The obtained RNA-sequencing data were demultiplexed into individual FASTQ files. The obtained single-end reads were aligned to human reference genome 37 [GRCh37.p13 (GCA_000001405.14), top-level built] using STAR (version 2.5.2).

RNA-SeQC (version 1.1.8) was then used to assess the quality of single cells. Data were visualized and clear low-quality outliers were identified on the basis of number of transcripts, uniquely mapped reads, mapping rate, expression profiling efficiency and exonic rate, and these were removed from further analysis. All cells that did not meet one of the following criteria were removed: <10,000 transcripts detected, <500,000 uniquely mapped reads, <1,000 genes detected, a mapping rate of <0.5, an expression profiling efficiency of <0.4, or an exonic rate of <0.5 (Supplementary Table S5). mRNA expression values for small bulk populations of 20 cells are shown in transcripts per kilobase million (TPM) and for single cells as fragments per kilobase million (FPKM) and log2+1 transformed. Differential expression in the 20-cell populations was analyzed with DESeq2 (version 1.16.1) to obtain insight into the differences between CD103+ and CD103− CD8+ T cells. For this analysis, expression values for each sample have been obtained using RSEM (version1.3.0, with Bowtie 2, version 2.2.5, nonstranded and with the single cell prior activated to account for drop-out genes) and have been computed for the Gencode 19 transcriptome annotation for GRCh37 (reference index built with –polyA activated), Genes with a Benjamini–Hochberg FDR <0.01 and log2 fold change >1 were selected for further analysis. Differentially expressed genes were visualized in a Volcano plot (DESeq2, version 1.16.1). The accession number for the sequencing data reported in this study is GSE127888.

TGFβ Induces CXCL13 in CD103+ Tumor-Infiltrating T Cells
ELISA

TILs from three high-grade serous ovarian cancer digests were stained and sorted as described for mRNA sequencing. The number of sorted T cells for the three patients were 163 \times 10^3, 216 \times 10^3, and 154 \times 10^3 for CD4^+ cells; 82 \times 10^3, 38 \times 10^3, and 83 \times 10^3 for CD8^+ CD103^+; and 207 \times 10^3, 120 \times 10^3, and 146 \times 10^3 for CD8^+ CD103^+ T cells. Sorted T cells remained unstimulated or were activated either with a stimulation cocktail containing phorbol myristate acetate (PMA, 40.5 \mu M) and ionomycin (670 \mu M, 500\times dilution, Invitrogen, 00-4970-93) or with Dynabeads (2 \mu L/1 \times 10^5 cells, T-activator CD3/CD28 beads, 11131D, Gibco).

Peripheral blood CD8^+ T cells were isolated from blood of four healthy volunteers (Sanquin, written informed consent was obtained) by a Ficoll-Paque gradient followed by magnetic-activated cell sorting with a CD8^+ T-cell–negative selection kit (purity >90%, MagniSort Human CD8 T cell Enrichment Kit, catalog no. 8804-6812-74; Thermo Fisher Scientific). Peripheral blood CD8^+ T cells were incubated in 100 \mu L AVM medium with or without Dynabeads (2 \mu L/1 \times 10^5 cells) for activation, recombinant TGF\beta1 (rTGF\beta1, 100 ng/ml, PeproTech), TGF\beta1 receptor inhibitor (10 \mu M), SB431542, Sigma Aldrich/Merck), or a combination of these. Similar experiments were performed with the addition of IL2 (100 \mu IU/ml, Novartis Pharmaceuticals). For the dose–response curve, peripheral blood CD8^+ T cells from three healthy donors were incubated with or without Dynabeads (2 \mu L/1 \times 10^5 cells) for activation and with recombinant TGF\beta1 at doses ranging from 0 to 100 ng/ml (rTGF\beta1, PeproTech). All cells were cultured in AVM medium with 5% pooled human serum (catalog no. A25761, One Lambda) in 96-well plates containing 1 \times 10^5 cells per condition. After 7 days, plates were centrifuged and supernatant was collected for ELISA.

CXCL13 sandwich ELISA experiments were performed according to manufacturer’s protocol (human CXCL13/BLC/BCA-1 DuoSet ELISA DY801, R&D Systems, Abingdon, United Kingdom). In brief, Nunc MaxiSorp flat-bottom plates (Invitrogen) were coated with a capture antibody, followed by incubation with cell supernatant. Per condition, 70 \mu L of supernatant was diluted with 40 \mu L BSA in PBS, after which 100 \mu L was added to the well. Binding of CXCL13 was detected using secondary antibody, streptavidin–HRP, and TMB 1-Component Microwell Peroxidase Substrate (SureBlue, KPL/SeraCare). Substrate conversion was stopped after 20 minutes with 0.01 mol/L hydrogen chloride. Plates were washed with PBS plus 0.05% Tween20 in-between incubations. Optical density values were obtained using a microplate reader set to 450 nm (Bio-Rad iMark Microplate reader). Finally, the derived CXCL13 concentrations (pg/mL) were multiplied by 1.57 to correct for diluting. AVM medium only was used as a negative control.

Chemokine arrays

CD8^+ T cells were isolated from blood of three healthy donors as described above in the ELISA section. Per condition, 5 \times 10^5 cells were cultured in AVM medium with 5% PHS in a 24-well plate. Cells were either incubated for 7 days in medium alone, with rTGF\beta1 (100 ng/mL, PeproTech), with Dynabeads (2 \mu L/1 \times 10^5 cells, T-activator CD3/CD28 beads, 11131D, Gibco), or with both rTGF\beta1 and Dynabeads. Samples were centrifuged, and supernatants were collected to analyze production of chemokines on chemokine arrays, according to manufacturer’s instructions (31 chemokines using the Proteome Profiler Human Chemokine Array Kit, ARV017, R&D Systems and 38 chemokines using the Human Chemokine Antibody Array-Membrane, ab169812, Abcam; Supplementary Table S6). In brief, chemokine receptor–coated membranes were incubated with supernatant overnight at 4°C. Per condition, 450 \mu L of supernatant was diluted with 1,050 \mu L buffer, provided by the manufacturer, and 1,500 \mu L was added (R&D Systems), or 1,000 \mu L was added undiluted (Abcam). Captured proteins were visualized using chemiluminescent detection reagents, provided by the manufacturers. Appropriate washing steps using wash buffers provided by the manufacturers were performed in-between incubation steps. Membranes were imaged on the Bio-Rad ChemiDoc MP Imaging System, and densitometric analysis of chemokine spots was performed using the Protein Array Analyzer plugin for ImageJ (30).

Statistical analyses

Differentially expressed genes in CD103^+CD8^+ versus CD103^-CD8^+ T cells sorted from human ovarian tumors were determined by DESeq2 for 20-cell populations. Genes with a Benjamini–Hochberg FDR < 0.01 and log2 fold change > 1 were selected for further analysis. Differences in EPKM values of single cells were assessed by a Mann–Whitney U test. Differences in number of CD20^+ follicles on FFPE slides of molecular subgroups of endometrial cancer were determined by a nonparametric Kruskal–Wallis test, followed by Dunn post hoc analysis. We analyzed the TCGA mRNA sequencing data and compared differences in gene expression between molecular subgroups of endometrial cancer with a nonparametric Kruskal–Wallis test and a post hoc Dunn test. Differences in survival were determined by a log-rank test. CXCL13 production was analyzed using a Kruskal–Wallis comparison with a post hoc Dunn test, or, for the dose–response curve, with a two-way ANOVA followed by a post hoc Bonferroni test. The chemokine arrays were analyzed using a Kruskal–Wallis test with a post hoc Dunn test. The survival effect of CD103 in pMMR uterine cancer was assessed by Kaplan–Meier analysis and log-rank test by comparing “above median CD103 expression” and “equal to or below median CD103 expression” (cutoff 16.14) patient groups. Uni- and multivariate analyses were performed by disease-specific Cox regression survival analyses [Enter for univariate and Backward and Forward (both LR and conditional) methods for multivariate analyses]. All statistical analyses were performed using R version 3.4.0 or GraphPad Prism (GraphPad Software Inc.). A P value of <0.05 was used as a cutoff for significance.

Results

Epithelial CD8^+ T cells associate with an activated and exhausted transcriptional signature

We and others have previously shown that intraepithelial CD103^+, but not stromal CD103^+, CD8^+ TILs are promising targets for ICI therapy (25, 29, 31, 32). To understand the underlying transcriptional changes in these two cell populations, we performed mRNA sequencing on single- and 20-cell pools of CD8^+ T cells isolated from human tumors. We chose ovarian cancer as a model tumor because of its large tumor bulk, availability of pretreatment tissue, and a high number of distinct CD103^+ and CD103^- infiltrating CD8^+ T cells. CD8^+ TILs were defined on the basis of a CD3^+/TCR\alpha\beta^+/CD8^+CD56^−/CD4^− phenotype (Fig. 1A). Post hoc t-distributed stochastic neighbor embedding (t-SNE) confirmed the presence of unique CD103^+ and CD103^- CTL populations in these tumors (Supplementary
CD103⁺ CD8⁺ TILs display a distinct phenotype and gene signature characterized by exhaustion genes and CXCL13 expression. 

**Figure 1.**

- **A:** Gating strategy of CD103⁺ and CD103⁻ CD8⁺ T cells according to TCRαβ, CD3, CD56, CD4, CD8α, CD8β, and CD103 expression. One exemplary ovarian tumor is depicted.
- **B:** Volcano plot of up- or downregulated genes in CD103⁺ and CD103⁻ TILs as determined by RNA sequencing, annotated by a set of exhaustion and chemokine-related genes.
- **C:** Expression of exhaustion-related genes (PDCD1 and LAYN) and chemokine CXCL13 in the CD103⁺ and CD103⁻ subsets (n = 20). The lines indicate median Log₂ (TPM+1) values ± IQR.
- **D:** Cell surface expression of CD103 and PD-1 in gated CD8⁺ TILs from exemplary ovarian tumor digests (n = 3) determined by flow cytometry. The red line represents threshold for PD-1 positivity.
- **E:** Cell surface expression of CD103 and PD-1 gated CD8⁺ TIL subsets determined by flow cytometry (n = 8). Graphs indicate median and IQR.
- **F:** Expression of PDCD1 and CXCL13 in single CD103⁺ (n = 12) and CD103⁻ TILs (n = 12). Lines indicate median Log₂ (FPKM+1) ± IQR.
- **G:** Correlation of PDCD1 and CXCL13 expression in CD103⁺ TILs (n = 24). **H:** Secretion of CXCL13 by CD103⁺ TILs and control CD4⁺ TILs after stimulation overnight at 37°C using anti-CD3/CD28 T-cell activation beads (2 µL Dynabeads per 1 × 10⁶ cells) or PMA/ionomycin (500 × dilution according to manufacturer's instructions), determined by ELISA Mean plus SEM of TILs from ovarian tumors (n = 3) are depicted. Significance was determined by Mann-Whitney U test (\( P < 0.05 \), \( ** P < 0.001 \), \( *** P < 0.0001 \)).
Fig. S1A–S1C). The transcriptome of CD103+ CTLs was characterized by an activation and exhaustion signature (Supplementary Table S7) with significant upregulation of GZMB (granzyme B), HAVCR2 (T-cell immunoglobulin and mucin domain 3, TIM3), LAG3 (lymphocyte-activation gene 3), TNRFSF18 (glucocorticoid-induced TNFR-related protein, GITR), KIR2DL4 (killer cell immunoglobulin-like receptor 2DL4), TIGIT (T-cell immunoreceptor with Ig and ITIM domains), and CTLA4 (CTLA attenuator 4) in the 20-cell pools (Fig. 1B). CD103+ CTLs expressed NGCT2 (G protein subunit gamma transducin 2), encoding a G protein gamma family member expressed in lymph nodes and spleen that is involved in GTPase activity (Fig. 1B). The expression of these markers is in-line with our earlier work demonstrating that the intraepithelial CD103+ T cells likely represent CTLs that have undergone activation and/or exhaustion (25, 29). In contrast, CD103+ CTLs displayed a more quiescent phenotype with a high differential expression of the V-set domain-containing T-cell activation inhibitor 1 (VTN1), a known suppressor of T-cell function (Fig. 1B). These cells differentially expressed GAGE12H, GAGE12I, and GPR2 (guanosine monophosphate reductase 2), involved in cell energy metabolism (Fig. 1B). Finally, CD103+, but not CD103−, cells were characterized by expression of genes previously associated (33) with exhausted CD8+ T cells (Fig. 1C).

CD103+ CTLs differentially express the B-cell recruiting chemokine CXCL13

In addition to the activated and exhausted gene signature, CD103+ CTLs were also characterized by significantly upregulated expression of the TLS-inducing CXCL13 (Fig. 1B and D; P < 0.0001). Although traditionally considered a CD4+ follicular helper T-cell (THF) gene, CXCL13 is also expressed in subsets of CD8+ TILs from hepatocellular carcinoma, melanoma, breast, and non–small cell lung cancer (34–37). In the latter, CXCL13 was identified in a transcriptionally unique PD-1–high (PD-1+CD8+ T-cell population that predicted response to ICI therapy (34). We, therefore, determined whether ovarian tumor-infiltrating CD103+ and PD-1+ CD8+ populations overlapped phenotypically. PDCD1 was differentially expressed in CD103+ over CD103− cells on the mRNA level, although this did not reach statistical significance in the 20-cell pools (Fig. 1B and C). Nevertheless, at the protein level, PD-1+ was expressed almost exclusively on the cell surface of CD103+ CD8+ T cells (Fig. 1E and F), although considerable heterogeneity existed between patients with regards to PD-1 expression (Fig. 1E). Accordingly, analysis of the CD103+ and CD103− CD8+ single-cell mRNA sequencing data revealed that PDCD1 was heterogeneously expressed in CD103+ cells but absent in CD103− cells (Fig. 1G). In contrast, CXCL13 was homogenously expressed in almost all CD103+ CD8+ T cells (Fig. 1G). In line with the above, CXCL13 and PDCD1 transcripts were significantly, although poorly, correlated (Fig. 1H). Finally, we assessed whether CD103+ TILs secreted CXCL13 protein, using CD4+ TILs obtained by flow cytometry–based sorting as controls. CD103+ CD8+ TILs and CD4+ TILs readily secreted CXCL13 upon ex vivo activation with anti-CD3/anti-CD28-conjugated beads or PMA/ionomycin (Fig. 1I).

TGFβ primes cytotoxic CD8+ T cells to secrete CXCL13 in vitro

We next sought to define the molecular mechanism underlying production of CXCL13 by CD103+ CD8+ TILs. Previously, we and others have demonstrated that induction of CD103 on CD8+ T cells is dependent on concurrent T-cell receptor (TCR) and TGFβ receptor 1 (TGFβR1) signaling (25, 38). TGFβ is a reported inducer of exhaustion-related genes such as PD-1 in T cells (39), which is also in-line with the transcriptional and phenotypical profile that we obtained for CD103+ CD8+ TILs. Therefore, we hypothesized that TGFβ might stimulate CD8+ T cells to secrete CXCL13. To investigate this, we activated peripheral blood CD8+ T cells from healthy donors with anti-CD3/anti-CD28–conjugated beads in the presence or absence of recombinant TGFβ1 (rTGFβ1) and measured secretion of CXCL13. Resting CD8+ T cells did not produce or secrete CXCL13 (Fig. 2A), nor did they express CD103 at the cell surface. Activation using anti-CD3/anti-CD28–conjugated beads induced minimal secretion of CXCL13 (Fig. 2A) and a minor upregulation of CD103 (Fig. 2B). Secretion of CXCL13 and expression of CD103 was inhibited by coin cubation with a TGFβR1 inhibitor, suggesting autoinergic TGFβ signaling was required for this induction of CXCL13 (Fig. 2A and B). Accordingly, activation of CD8+ T cells in the presence of rTGFβ1 induced significant CXCL13 secretion (Fig. 2A) and expression of CD103 on the CD8+ T-cell surface (Fig. 2B). Again, induction of CXCL13 and CD103 were inhibited by coinubation with a TGFβR1 inhibitor (Fig. 2A and B). Induction of CXCL13 by TGFβ was dose-dependent and induced CXCL13 secretion at 0.1 ng/ml rTGFβ1 with a peak at 10 ng/ml rTGFβ1 (Fig. 2C). Because IL2 inhibits the secretion of CXCL13 in follicular helper CD4+ T cells (40), we also examined whether IL2 impaired CXCL13 secretion by CD8+ T cells. In contrast to CD4+ T cells, induction of CXCL13 in CD8+ T cells was not inhibited by IL2 (Fig. 2D), suggesting underlying differences in CXCL13 gene regulation and/or IL2 signaling between CD8+ and CD4+ T cells. On the basis of our findings, we concluded that TGFβ was sufficient for CXCL13 induction in activated CD8+ T cells.

Next, we determined whether TGFβ also modulates secretion of other chemokines. Analysis of chemokine mRNA expression in CD103+ versus CD103− TILs revealed a significant upregulation of CCL3, CCL5, and CXCL13 and a trend toward overexpression of CCL3L1, CCL4L2, CCL20, and CXCL9 in CD103+ TILs (Fig. 2E). Subsequent analysis of 47 chemokines secreted from peripheral blood CD8+ T cells revealed activation-dependent production of CCL4, CCL5, CXCL9, and CXCL10 (Fig. 2F and G; Supplementary Fig. S2A–S2C). As before, significantly higher CXCL13 was secreted upon activation of T cells in the presence of rTGFβ1 (Fig. 2H and I). In contrast, rTGFβ1 did not affect the induction of other chemokines (Fig. 2I). Taken together, our data indicated that TGFβ was a specific inducer of CXCL13 in CD8+ T cells and identified a hallmark chemokine pattern for CD103+ CD8+ TILs.

CXCL13+ CD103+ CTLs associated with a high mutation load, B-cell infiltration, and TLSs

CXCL13 is a key driver of B-cell recruitment and TLS formation in cancer and autoimmune diseases (16, 41–44). As such, we speculated the CXCL13+ CD103+ TIL population would be involved in recruitment of B cells and TLS formation across human tumors. We retrospectively analyzed a cohort of 125 (high-grade serous) ovarian tumors for CD103+ cells and CD20+ B cells using a tissue microarray. CD103+ and CD20+ cells were detected in most patients, with some CD20+ cells forming aggregates in close proximity to CD103+ cells in the tumor epithelium (Fig. 3A). Indeed, division of patients by high (>26.3), intermediate (8.6–26.3), or low (<8.6) CD103+ cell infiltration revealed a significant association with the number of B cells (Fig. 3B). Next,
Figure 2.

TGFβ induces CXCL13 in CD103+ Tumor-Infiltrating T Cells

**A** Sorted peripheral blood CD8+ T cells

**B** Sorted peripheral blood CD8+ T cells

**C** Sorted peripheral blood CD8+ T cells

**D** Sorted peripheral blood CD8+ T cells + IL2

**E** RNA Expression tumor-infiltrating CD8+ T cells

**F** Protein secretion by activated peripheral blood CD8+ T cells

**G** Proportion of CD8+ T cells secreting chemokines

**H** Protein secretion by activated peripheral blood CD8+ T cells + TGFβ

**I** Log2 (fold change)

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TGFβ-dependent induction of CXCL13 in CD8+ T cells and CD103+ TIL chemokine profile. **A**, Secretion of CXCL13 by sorted peripheral blood CD8+ T cells after stimulation for 7 days using anti-CD3/CD28 T cell activation beads (2 μL per 1 × 10^6 cells), recombinant TGFβ1 (100 ng/mL), and/or TGFβ1 receptor inhibitor SB431542 (10 μM), determined by ELISA. Depicted are mean plus SEM of CD8+ T cells from 4 donors. Significance was determined using Kruskal–Wallis comparison with a post hoc Dunn test (*, P < 0.05). **B**, Cell surface expression of CD103 on sorted CD8+ T cells after stimulation using anti-CD3/CD28 T-cell activation beads, recombinant TGFβ1, and/or TGFβ1 receptor inhibitor SB431542 as described for A. One exemplary donor is depicted. **C**, Secretion of CXCL13 as determined by ELISA of CD8+ T cells after stimulation using anti-CD3/CD28 T-cell activation beads as described for A and/or varying concentrations of recombinant TGFβ1 as depicted on the x-axis. Mean plus SD from 3 donors is depicted. Significance was determined by a two-way ANOVA followed by a post hoc Bonferroni test (*, P < 0.05; ***, P < 0.0001). **D**, Secretion of CXCL13 by sorted peripheral blood CD8+ T cells after stimulation using anti-CD3/CD28 T-cell activation beads, recombinant TGFβ1, and/or TGFβ1 receptor inhibitor SB431542 (as described for A) in the presence of IL2 (100 IU/mL), determined by ELISA. Depicted are mean plus SEM of CD8+ T cells from 4 donors. Significance was determined using Kruskal–Wallis comparison with a post hoc Dunn test (*, P < 0.05). **E**, Up- or downregulated chemokines between CD103+ and CD103+ TILs determined by differential expression of RNA sequencing of 20-cell pools (n = 20). Dashed red line indicates ≥2-fold change; dashed blue line indicates ≤0.5-fold change. Error bars indicate fold change SE. Significance was determined using Benjamin–Hochberg FDR. **F**, Secretion of chemokines by CD8+ T cells after 7 days stimulation using anti-CD3/CD28 T-cell activation beads (2 μL per 1 × 10^6 cells) determined by a chemokine array kit, n = 3. Shown are log2 fold changes of the mean compared with resting unstimulated CD8+ T cells. Dashed red line indicates ≥2-fold change; dashed blue line indicates ≤0.5-fold change. Error bars indicate SEM. **G**, Exemplary chemokine array data from anti-CD3/CD28-activated CD8+ T cells of one donor depicting chemokines with ≥2-fold and ≤0.5-fold change compared with resting CD8+ T cells from F. **H** and **I**, Secretion of chemokines by CD8+ T cells after stimulation using anti-CD3/CD28 T-cell activation beads and recombinant TGFβ1 (n = 4). Shown are log2 fold changes of the mean compared with resting unstimulated CD8+ T cells (H) or to anti-CD3/CD28-activated CD8+ T cells (I). Dashed red line indicates ≥2-fold change; dashed blue line indicates ≤0.5-fold change. Error bars indicate SEM. Significance in F–I was determined using Kruskal–Wallis comparison with a post hoc Dunn test (*, P < 0.05; ***, P < 0.001; ****, P < 0.0001).
we assessed whether CD103+CD8+ T cells might also link neoantigen-specific T-cell responses to B-cell–driven immune responses. To address this, we determined whether tumors with a high neoantigen load and concomitantly high CD103+ TIL infiltrate were enriched for B-cell– and TLS-associated genes in mRNA sequencing data from The Cancer Genome Atlas (TCGA). Specifically, we analyzed uterine cancer samples stratified by neoantigen load. In brief, four distinct molecular subtypes can be distinguished in uterine cancer: microsatellite stable (MSS), microsatellite unstable (MSI), polymerase epsilon exonuclease domain mutated (POLE-EDM) tumors, and p53-mutant tumors. We have previously demonstrated an increased number of mutations, predicted neoantigens, and (CD103+)+ T cells in POLE-EDM and MSI tumors compared with MSS tumors (24). In-line with the above, MSS tumors mostly lacked B-cell– and TLS-related genes, whereas MSI and POLE-EDM tumors were enriched for these genes (Fig. 3C). To confirm these findings, we further analyzed an independent cohort of MSS, MSI, and POLE-EDM tumors from patients with uterine cancer for the presence of CD20+ B cells by IHC. B cells were predominantly observed in large aggregations in the tumor and surrounding stroma (Fig. 3D and E). In-line with the TCGA data, only 48% (20/42) of MSS tumors were found to have B-cell aggregates, whereas 74% (28/38) of MSI and 92% (33/36) of POLE-EDM tumors contained B-cell aggregates (Fig. 3D). Quantification per tumor revealed a significant increase in the number of B-cell aggregates when comparing MSS to

Figure 3.
B cells, tertiary lymphoid structures, and correlation to CD103+CD8+ T cells in human tumors. A and B, IHC analysis of CD103+ and CD20+ cells in ovarian cancer. Exemplary image of a CD20+ aggregate in close proximity to CD103+ cells in the tumor epithelium (A) and B-cell infiltration in CD103-low, CD103-intermediate, and CD103-high tumors stratified by tertile (n = 125; B). Significance was determined using Kruskal–Wallis comparison with a post hoc Dunn test (*, P < 0.05; **, P < 0.01; ***, P < 0.001). C, Gene expression of B cell, tertiary lymphoid structure, cytotoxic T cell, CD8+ T cell, and CD4+ follicular helper T-cell–associated genes in TCGA data of uterine cancer (UCEC, n = 546). Tumors were ranked according to molecular subtype and then by CD20 expression. D and E, Prevalence and number of B-cell aggregates (D) in MSS, MSI, and POLE-EDM uterine cancers. E, One exemplary image of each subtype is depicted. Significance was determined using Kruskal–Wallis comparison with a post hoc Dunn test (*, P < 0.01; **, P < 0.001). F, Immunofluorescent analysis of CD20 aggregates in uterine cancer. CD20 aggregate expression of characteristic TLS markers CD11c, PNAD, CD20, CD38, CD79A, CD138, CD3, and CD8, one exemplary image is depicted.
POLE-EDM and MSI to POLE-EDM tumors (Fig. 3D, \(P < 0.001\) and \(P < 0.01\), respectively). To confirm that the observed B-cell aggregates were phenotypically similar to TLSs, we performed multicolor immunofluorescence. Indeed, B-cell aggregates showed the typical characteristics of lymphoid tissues, as determined by the presence of high endothelial venules (HEV), germinal B-cell centers, and DCs surrounded by a rim of T cells (Fig. 3F).

On the basis of the above findings, we speculated that CXCL13\(^+\)CD103\(^+\) CTL genes would associate with TLS genes across human epithelial tumors. To assess this, we analyzed TCGA mRNA expression data of ovarian, uterine, lung, and breast cancer using the differentially expressed genes from CXCL13\(^+\)CD103\(^+\) and CXCL13\(^-\)CD103\(^-\) CTLs identified by our mRNA sequencing. TLS genes correlated with CXCL13\(^+\)CD103\(^+\), but not CXCL13\(^-\)CD103\(^-\), CTL genes across all four tumor types (Fig. 4). Taken together, our data suggested that CXCL13\(^+\)CD103\(^+\) CTLs promote migration of B cells to tumors and the formation of TLSs across tumor types.

CXCL13\(^+\)CD103\(^+\)CD8\(^+\) cells correlate to improved survival irrespective of neoantigen burden

To assess whether CXCL13\(^+\)CD103\(^+\) CTLs were also correlated with improved survival, we analyzed clinical outcomes in two cohorts of patients with uterine cancer. We focused on tumors with a low neoantigen burden because a high neoantigen load is associated with better survival. First, we analyzed TCGA endometrial cancers of the MSS subtype and correlated high CXCL13 and CD103 (ITGAE) gene expression to survival. CXCL13\(^{\text{high}}\) ITGAE\(^{\text{low}}\) MSS tumors had a significantly improved survival as compared with CXCL13\(^{\text{low}}\) ITGAE\(^{\text{low}}\) MSS tumors from TCGA (Supplementary Fig. S3A). To confirm this survival benefit for CXCL13\(^+\)CD103\(^+\) CTLs in neoantigen low tumors, we analyzed FFPE tumor tissue of an independent cohort of pMMR uterine cancers by IHC (Supplementary Table S8). We were unable to detect CXCL13 protein expression in CD103\(^+\) CTLs, consistent with our previous finding that almost all CD103\(^+\) cells express CXCL13 on the mRNA level (Fig. 1B, D, and G), but express and

![Figure 4](https://example.com/figure4.png)

**Figure 4.** CXCL13\(^+\)CD103\(^+\) TLS genes correlate with TLS-associated genes across cancer types. Spearman correlation of genes associated with TLS, CD103\(^+\)CD8\(^+\) cells, and CD103\(^-\)CD8\(^+\) cells in ovarian (\(n = 307\)), uterine (\(n = 546\)), lung (\(n = 517\)), and breast (\(n = 1,100\)) cancer. Correlation plots depict relative correlation of log2 transformed mRNA sequencing data from TCGA.
secretes the protein only after reactivation (Fig. II). Therefore, we analyzed the effect of CXCL13\textsuperscript{+}CD103\textsuperscript{+} CTL infiltration on clinical outcome in this cohort by proxy, using CD103\textsuperscript{+} staining. We observed a significant survival benefit for patients with mismatch repair-proficient tumors infiltrated by a high number of CD103\textsuperscript{+} cells over tumors infiltrated by a low number of CD103\textsuperscript{+} cells (Supplementary Fig. S3B). This effect was independent of other clinical variables, as demonstrated by multivariate analysis (Table 1). As such, our data demonstrated that (CXCL13\textsuperscript{+}) CD103\textsuperscript{+} CTLs are associated with improved clinical outcome, independent of neoantigen burden.

**Discussion**

In this study, we reported on the finding that TGF\textbeta\textsuperscript{1} stimulates activated CD8\textsuperscript{+} T cells to produce CXCL13, a known inducer of TLSs (17–19). This production of CXCL13 was paralleled by the induction of CD103 on the cell surface of CD8\textsuperscript{+} cells in vitro. CD103\textsuperscript{+} CD8\textsuperscript{+} T cells isolated directly from human tumors expressed CXCL13 mRNA and secreted CXCL13 protein upon ex vivo reactivation. The presence of B-cell and TLS genes was increased in mutated, CXCL13 ex vivo protein upon reactivation. The presence of B-cell and TLS genes was increased in mutated, CXCL13-expressing tumors, indicating T cells of liver cancer, breast cancer, lung cancer, and melanoma does support expression of CD103 and B cells as potential biomarkers of interest for cancer immunotherapy.

CXCL13 is generally associated with DCs and TFH (40, 45, 46). Nevertheless, single-cell sequencing of exhausted, tumor-infiltrating T cells of liver cancer, breast cancer, lung cancer, and melanoma does support expression of CXCL13 in TLSs (34–36). We found that TGF\textbeta\textsuperscript{1}, a cytokine mostly associated with immune suppression (40, 47–49), was essential for the induction of CXCL13. Under homeostatic conditions, TGF\textbeta\textsuperscript{1} is abundantly present in epithelial tissue and controls the epithelial localization of resident memory immune subsets, such as the intraepithelial lymphocytes in the colon (30). In epithelial cancers, we suggest that TGF\textbeta\textsuperscript{1} has a similar role in promoting not only recruitment, signaling, and retention of CD8\textsuperscript{+} T cells via CD103 expression (51), but also stimulating immunity via attraction of C-X-C chemokine receptor type 5 (CXCR5\textsuperscript{+}) immune cells through CXCL13 signaling.

CXCL13 is the key molecular determinant of TLS formation (17–19), ectopic lymphoid structures that are thought to enable efficient local priming of T cells by DCs (9). Hereby, the time-consuming migration of DCs and T cells to and from lymph nodes may be circumvented, augmenting local antitumor immunity. In-line with this, characteristic components of TLSs, such as HEVs and B cells, are found to be generally associated with an improved prognosis (10), and plasma B cells in the TLSs are thought to enhance antitumor responses by production and subsequent accumulation of antitumor antibodies, potentially leading to antibody-dependent cytotoxicity and opsonization (12). Thus, TLSs may orchestrate a joint T- and B-cell response to improve antitumor immunity.

Because TLSs were more abundant in tumors with a high mutational load, we postulated that activated CD103\textsuperscript{+} CTLs were involved in the migration of B cells to tumors via production of CXCL13. This is supported by our observations that mutated, CD8\textsuperscript{+} T-cell-rich tumors showed higher expression of CXCL13 and ITGAE (CD103) and that they presented with significantly higher numbers of B cells. In accordance, a higher degree of TCR clonality within CD8\textsuperscript{+} T cells correlates with a higher number of TLSs in non–small cell lung cancer (52). These TLSs may represent an ongoing immune response that is insufficient to halt tumor progression at an early time point. It would, therefore, be of great interest to study the induction and formation of TLSs in...
developing cancer lesions and to determine whether CD8+ T-cell infiltration precedes TLS formation.

In line with previous work (24, 29, 53), CD103+ CTLs from human tumors were also characterized by an activation- and exhaustion-related gene expression signature, with differential expression of granzymes and well-known immune checkpoint molecules, such as CITA4. CD103+ CTLs expressed several additional immune checkpoint genes currently under clinical investigation, such as TIM3, LAG3, and TIGIT. This observed phenotype is concordant with a reported subset of PD-1+ high (PD-1+CD8+ T cells in lung cancer (34). The PD-1+ subset was transcriptionally distinct and characterized by expression of CXCL13. This subset also expressed higher ITGAE, although cell surface expression of CD103 was not examined. Nevertheless, our analysis of the gene expression profile reported for PD-1+CD8+ cells (34) revealed overlapping overexpression of approximately 200 genes with CD103+CD8+ cells, suggesting these populations might represent the same T-cell subset. Importantly, CXCL13+PD-1+ CD8+ cells are associated with response to ICI (34).

The association between CXCL13+PD-1+CD8+ cells and response to ICI is in line with the observation that CD103+ CTLs significantly expand upon treatment with nivolumab or pembrolizumab (anti–PD-1) in tumor specimens of patients with advanced-stage metastatic melanoma (34). Accordingly, an article by Riaz and colleagues demonstrates that tumors from patients who responded to nivolumab treatment differentially expressed genes such as CXCL13, CITA4, TIM3, LAG3, PDCD1, GZMB, and TNF receptor superfamily member 9 (TNFRSF9), all genes overexpressed in CD103+ versus CD103- CTLs (7). Pretreatment, but not on-treatment, CXCL13 was differentially expressed in responders versus nonresponders in this study (7). This may be explained by the low basal CXCL13 secretion we observed in the exhausted, CD103- CTLs freshly isolated from untreated human tumors. In their exhausted state, CTLs might accumulate mRNA encoding several key effector molecules that is translated only upon reactivation (e.g., by ICI). Consistent with this, Riaz and colleagues observed an increase in the number of B-cell–related genes on-treatment in responding patients (7), perhaps hinting at B-cell recruitment and the formation of TLSs in these patients upon ICI-mediated release of CXCL13. This hypothesis is supported by the observed increases in serum CXCL13 and concomitant depletion of CXCR5+ B cells from the circulation in patients treated with anti–CTLA-4 and/or anti–PD-1 (55). Our data, therefore, suggest that ICIs are of particular interest for patients with a high CXCL13+CD103+ CTL infiltration pretreatment across malignancies.

Several combination immunotherapy regimes that promote CD8+ T-cell infiltration and TLS formation may also function via CD8+ T-cell–dependent production of CXCL13. For instance, combined therapy with antiangiogenic and immunotherapeutic agents in mice stimulated the transformation of tumor blood vessels into intratumoral HEVs, which subsequently enhanced the infiltration and activation of CD8+ T cells and the destruction of tumor cells (56, 57). These T cells formed structures around HEVs that closely resembled TLSs (56, 57). One of these studies found that induction of TLSs was dependent on both CD8+ T cells and macrophages (56). However, the exact intratumoral mechanism of action remains unclarified. Because macrophages produce TGFβ in a chronically inflamed environment (40), we hypothesize that the macrophages in these studies may have generated a TGFβ-enriched environment, thus, leading to the production of CXCL13 chemokine by activated T cells and subsequently to the formation of lymphoid structures. TLSs may, therefore, reflect an ongoing CD8+ T-cell response in cancer. As such, TLSs may be used as a biomarker to predict response to ICI, and these structures may be used as a general biomarker for response to immunotherapy because TLSs were found to identify patients with pancreatic cancer who responded to therapeutic vaccination (58).

Taken together, we demonstrated that TGFβ induces coexpression of CXCL13 and CD103 in CD8+ T cells, potentially linking CD8+ T-cell activation to B-cell migration and TLS formation. Our findings provide a perspective on how (neo)antigens could promote the formation of TLSs in human tumors. Accordingly, CD103+ cells and B cells should be considered as a potential predictive or response biomarker for ICI therapy.

Disclosure of Potential Conflicts of Interest

H.W. Nijman reports receiving commercial research funding from Aduro Biotech Europe and BionTech, has ownership interest in ViciniVax, and is a consultant/advisory board member for ViciniVax. M. de Bruyn reports receiving commercial research funding from Aduro Biotech Europe and European Industrial Doctorate (I-Direct). No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, the Department of Health, or the Wellcome Trust.

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A Transcriptionally Distinct CXCL13⁺CD103⁺CD8⁺ T-cell Population Is Associated with B-cell Recruitment and Neoantigen Load in Human Cancer

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