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REGULATION OF THE EPITHELIAL CELL-SPECIFIC INTEGRIN, CD103, BY HUMAN CD8+ CYTOLYTIC T LYMPHOCYTES[1]

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Abstract TOP

Background. The destruction of the graft epithelium by CD8+ cytolytic T lymphocytes (CTL) is an important aspect of organ allograft rejection. Our recent finding in a mouse model that the epithelial cell-specific integrin, CD103, defines a subset of CD8+ CTL potentially sheds new light onto such interactions. The goal of the present study was to assess the relevance of these data to the human system.

Methods. CD103 expression by human T-cell populations generated in mixed lymphocyte cultures or isolated from transplant nephrectomy specimens was quantitated using multiparameter FACS analyses.

Results. CD103 defined a major subset (26-76%) of $CD8^+$ CTL generated in human mixed lymphocyte cultures; cell sorting experiments confirmed that the $CD103^+$ and $CD103^-$ subsets both possess allospecific lytic activity. Anti-transforming growth factor (TGF)-β blocked the appearance of the $CD103^+$ CTL subset, and persistent expression of CD103 by $CD8^+$ CTL was dependent on bioactive TGF-β. Isolated $CD103^+$ and $CD103^-$ CD8 subsets maintained their phenotypic integrity during in vitro expansion, although optimal CD103 expression on the former was TGF-β dependent. Although $CD103^+$ cells were rare among activated CD8 cells in peripheral lymphoid compartments (<10%), analyses of transplant nephrectomy specimens revealed that a major subset (21-61%) of CD8 memory/effector cells that infiltrate rejecting renal allografts express high levels of CD103.

Conclusions. We conclude that CD103 defines a discrete and stable subset of human $CD8^+$ CTL and that CD103 expression by such cells is initiated and maintained by bioactive TGF-β. These data point to the existence of a human effector subset that is uniquely specialized for the destruction of the graft epithelium.

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The functional elements of organ allografts are largely epithelial in origin, and the destruction of such cells by donor-reactive $CD8^+$ cytolytic T lymphocytes (CTL*) is an important aspect of the rejection process (1). For example, the infiltration of the renal tubules by $CD8^+$ lymphocytes of effector phenotype is the sine qua non of clinical renal allograft rejection (2). CTL responses to organ allografts are initiated within host lymphoid compartments in response to donor leukocytes that migrate from the graft site; subsequent to activation, CD8 cells down-regulate the lymph node homing receptor CD62L (L-selectin) and up-regulate integrins such as CD11a (lymphocyte function-associated antigen-1) and CD49d (very late antigen-4) (3, 4). Recent studies implicate CD62L down-regulation in redirecting sensitized CTL precursors away from lymphoid compartments, whereas integrin up-regulation promotes extravasation into the graft site (5). However, little is known of the critical downstream events by which $CD8^+$ CTL gain access to the graft epithelium.

We recently reported that the integrin family protein CD103 (formerly α^E integrin) defines a specialized subset of $CD8^+$ CTL (6). These data potentially shed new light on CTL/epithelial interactions because the known ligand for CD103 is E-cadherin (7, 8), a tissue-specific molecule ubiquitously expressed by cells comprising epithelial layers (9), e.g., including critical graft functional elements such as the renal tubules and glomeruli (10). CD103 was initially identified by its high-level expression on intraepithelial lymphocytes (IEL) in the gut (11, 12), and recent studies implicate CD103/E-cadherin interactions in endowing IEL with their characteristic epitheliotropism. For example, interaction of CD103 has been shown to promote adhesion of IEL to epithelial layers (13, 14) and provide costimulatory signals for IEL proliferation (15) and lytic activity (13). That CD103 may play an analogous role in promoting the interaction of $CD8^+$ CTL with the graft epithelium is supported by our recent demonstration that CD103 can function as an accessory molecule that promotes the lysis of epithelial cell targets by alloreactive $CD8^+$ CTL clones (16).

Transforming growth factor (TGF)-β plays a critical role in regulating CD103 expression by peripheral CD8 cells. For example, although CD103 is poorly expressed by $CD8^+$ CTL generated in conventional mouse mixed lymphocyte cultures (MLC), exposure of such cells to bioactive TGF-β dramatically induces high-level CD103 expression (6). It is important to note, however, that a subset (40-60%) of $CD8^+$ CTL generated in mouse MLC cultures is apparently committed to a CD103 negative phenotype and thus fails to up-regulate CD103 in response to TGF-β (6).

The goal of the present study was to determine the extent to which the above findings in the mouse are relevant to the human system. In both species, CD103 is expressed as a heterodimer in association with a β7 integrin subunit by >95% of IEL and 40-50% of lamina propria lymphocytes, but is poorly expressed by peripheral T cells (11, 12). CD103 identifies a small subset (0.1-3%) of human peripheral $CD8^+$ T cells with activated/memory phenotype (17), consistent with our finding that CD103 defines a dichotomy among CD8 effectors in the mouse system. However, in marked contrast to the mouse,

CD103 is readily induced on human peripheral CD8 cells after activation with a variety of stimuli (18-21), including alloantigen (i.e., in MLC cultures) (21), and indeed, was initially described as a general marker for T-cell activation in the human system (19). Moreover, in contradiction to the strict TGF-β dependence of CD103 expression by mouse peripheral CD8 cells, its induction on human T cells was recently reported to be unrelated to TGF-β production (21). In an attempt to resolve these discrepancies, we have undertaken an investigation of CD103 expression by $CD8^+$ CTL generated in human MLC cultures. To assess the relevance of these findings to the clinical situation, we examined CD103 expression by CD8 cells infiltrating rejecting human renal allografts.

MATERIALS AND METHODS TOP

Monoclonal antibodies (mAb). Unconjugated mAb to human CD103 (BerAct-8) (18) was purchased from Accurate (Westbury, NY); fluorescein isothiocyanate (FITC)-conjugated BerAct-8 was obtained from Dako Corporation (Carpinteria, CA). Phycoerythrin (PE)-conjugated mAb to CD8 (RPA-T8) and FITC-conjugated mAb to human CD11a (HI111) were purchased from PharMingen (San Diego, CA). Biotinylated mAb to CD49 (15A8) and CD44 (F10-44-2), and FITC-conjugated goat anti-mouse IgG (human absorbed) were purchased from Southern Biotechnology Associates, Inc. (Birmingham, AL). PerCp-conjugated anti-HLA-DR was purchased from Becton Dickinson (San Jose, CA). Neutralizing mAb specific for human TGF-β_{1-3} (22) was purchased from Genzyme (Cambridge, MA).

Mixed leukocyte cultures. Normal human lymphocytes of known HLA phenotype were obtained from the spleens of cadaveric pancreas transplant donors at the University of Maryland Medical System. Splenocytes (SC) were harvested by mincing the tissue, passed through nylon mesh to remove cell clumps, and stored as frozen aliquots at 50×10^6 lymphocytes per vial. Where noted, peripheral blood lymphocytes (PBL) from fully HLA-disparate normal volunteers were substituted for SC. PBL or thawed SC aliquots were centrifuged on Ficoll-Paque (Parmacia LKB, Uppsala, Sweden) before use. For MLC cultures, 2×10^6 PBL or SC responders were cocultured with 10^6 irradiated (3000 R) stimulator SC from a donor mismatched for all HLA-A, -B, and -DR alleles in 2 ml of RPMI-1640 supplemented with sodium pyruvate, nonessential amino acids, penicillin/streptomycin, 10 mM HEPES, 50 •M 2-ME, and 10% fetal calf serum ($RPMI^+$). In some experiments, 0.25×10^6 B lymphoblastoid cell line (B-LCL) cells were substituted as stimulators in human MLC cultures. Human B-LCL were established from cadaver SC by transformation with Epstein-Bar virus and maintained in $RPMI^+$. Where noted, recombinant (mature) human TGF-β1 (R&D Systems, Minneapolis, MN) was added to cultures at a final concentration of 2.5 ng/ml. Total concentrations of human TGF-β1 (latent plus bioactive forms) in MLC culture supernatants were measured by the Cytokine Core Laboratory at the University of Maryland Medical School using a sandwich ELISA. With specificity for human TGF-β1.

Flow cytometry. Cell populations were stained for two-color FACS analyses using anti-CD8-PE in conjunction with mAbs to markers of interest. CD103 and CD3 were detected using unconjugated mAb followed by FITC-conjugated goat anti-mouse IgG or were stained directly with FITC-conjugated BerAct-8. CD3, CD11a, CD44, and CD49d (see above for details) were detected directly using FITC-conjugated mAb or indirectly using either biotinylated mAb followed by streptavidin-FITC (Caltag, San Francisco, CA). For three-color analyses, PerCP-conjugated anti-HLA-DR (Becton Dickinson) was used in conjunction with the above reagents. Species and isotype-matched mAbs of irrelevant specificity were used as controls for nonspecific fluorescence. After staining, cells were fixed with 0.5% paraformaldehyde, and 3×10^4 cells were analyzed by dual-color flow cytometry using a FACScan (Becton Dickinson). Lymphocyte populations were gated by forward scatter/side scatter analysis to exclude dead cells and nonlymphocytes. Windows 3.1 Multiple Document Interface (version 2.1.4) software developed by Dr. Joseph Trotter (Scripps Institute, San Diego, CA) was used for analysis and graphical display of flow cytometry data. Percent positive cells for a given marker and quadrant settings were based on cutoff points chosen to exclude >99% of the negative control population. For cell sorting experiments, cells were stained with anti-CD8-PE and anti-CD103 as described above. $CD8^+CD103^+$ cells and $CD8^+CD103^-$ cells were isolated using a FACStar*Plus* (Becton Dickinson). The purity of sub-populations was confirmed by postsort analysis.

Cytotoxicity assays. Standard Cr-51 release assays were conducted as described previously (6). Briefly, effector cells were added in quadruplicate to the wells of round bottom 96-well culture plates (Costar, Cambridge, MA) containing ^{51}Cr-labeled target cells in a total volume of 0.2 ml RPMI. After 4 hr, culture supernatants were harvested using a Skatron collection system (Skatron, Sterling, VA) and radioactivity (cpm) was measured using a Cobra Auto-Gamma counter (Packard, Downers Grove, IL). Means of quadruplicate values were used to calculate percent specific lysis using the

following formula: (Equation 1) Spontaneous release (*SR*) and maximum release (*MR*) values were determined by incubating target cells in wells containing 0.2 ml of assay media or 1% Triton X-100, respectively.

Equation 1

Transplant nephrectomy specimens. Transplant nephrectomy kidneys were derived from renal allograft recipients who underwent transplantation at the University of Maryland Medical System between 1991 and 1998. Patients DB, DH, ED, and DS were recipients of cadaveric kidneys, OW received a simultaneous kidney/pancreas transplant, and MW received a kidney from a living-related donor. All patients were partially mismatched with their donors for HLA class I and class II alloantigens and received cyclosporine microemulsion, prednisolone, and azathioprine, as maintenance immunosuppression. Specimens were minced and incubated for 30 min in Dulbecco's modified essential medium/F12 (50/50) containing 0.1% collagenase (type IV; Worthington, Freehold, NJ), 0.1% soybean trypsin inhibitor (Sigma, St. Louis, MO), and 0.01% DNase I (Boehringer Mannheim, Indianapolis, IN). Lymphocytes were isolated from the resulting cell suspension by centrifugation on Ficoll-Paque (Pharmacia LKB, Piscataway, NY) and stained immediately for FACS analyses.

RESULTS TOP

Regulation of CD103 expression in human MLC cultures. As shown in Figure 1, CD103 was expressed by a major subset of CD8 cells generated in human MLC cultures. Figure 1A shows a typical experiment in which MLC cultures were generated using SC from HLA-disparate cadaver donors; experiments using fresh PBL from HLA-disparate normal volunteers gave similar results (Fig. 1B). The proportion of $CD103^+$ cells at day 7 ranged from 26.0% to 76.4% of total $CD8^+$ lymphocytes for different responder/stimulator combinations (n=8). $CD103^+$ cells were detected as early as day 3 of MLC culture; the proportion of such cells peaked by approximately day 5 and reached a plateau or declined slightly by day 7 (data not shown). Consistent with previous reports, <5% of unstimulated $CD8^+$ SC (Fig. 1C) and ~1% of $CD8^+$ PBL (Fig. 1D) expressed CD103. $CD103^+$ cells were also present at a low frequency (<5%) among CD8 cells in human tonsil (not shown).

Figure 1. CD103 expression by CD8 cells in human MLC cultures. Normal human SC (A and B) or PBL (C and D) were stained without culture (B and D) or were cocultured with fully HLA-disparate stimulators of like cell type in one-way MLC cultures (A and C). Cultures were harvested at day 6 and stained for two-color FACS analyses with anti-CD103 mAb (solid line) or an isotype-matched negative control (gray peak) in conjunction with anti-CD8-PE. Data shown are for electronically gated $CD8^+$ lymphocytes.

As shown in Figure 2, optimal CD103 expression was substantially lost during propagation and expansion of alloreactive $CD8^+$ CTL lines by conventional methods, i.e., by restimulation with allogeneic SC or B-LCL stimulators in media supplemented with interleukin (IL)-2. Thus, CD103 expression in secondary (not shown) or tertiary MLC cultures (Fig. 2C) declined sharply from that of the original (primary) culture (Fig. 2A).

Figure 2. The role of TGF-β in regulating CD103 expression in human MLC cultures. (A) Primary MLC cultures were treated with a neutralizing anti-TGF-β mAb (dashed line) or an isotype-matched negative control mAb (solid line). (B) Primary MLC cultures were untreated (solid line) or were treated with exogenous TGF-β (2.5 ng/ml) at day 5 of culture (dashed line). (C) The primary MLC culture shown in panel B was restimulated twice at weekly intervals with allogeneic B-LCL stimulators in media supplemented with 20 U/ml IL-2 with (dashed line) or without (solid line) 2.5 ng/ml exogenous TGF-β added at day 5 of the final restimulation. Cultures were harvested at day 7 and subjected to two-color FACS analyses as described in the legend to Figure 1. Data shown are for electronically gated $CD8^+$ lymphocytes. The same responder/stimulator combination was used for experiments shown in panels A-C.

CD103 defines a discrete subset of activated human CD8 cells. CD103 has been described as an activation marker for human T cells (18, 19). To directly examine this issue, three-color FACS analyses were used to quantitate CD103 expression on HLA-DR$^+$ (activated) CD8 cells. As shown in Figure 3, CD103 defined a discrete subset of DR$^+$ CD8 cells in MLC cultures (Fig. 3A), whereas other markers associated with T-cell activation, including lymphocyte function-associated antigen-1, very late antigen-4, and CD44, were uniformly expressed (Fig. 3B). Thus, human CD103 is a differentiation marker that identifies a subset of activated CD8 cells.

Figure 3. Phenotypic analyses of activated CD8 cells in human MLC cultures at day 6 (A and B) or freshly isolated cells from human tonsil (C) or spleen (D). Cells were stained for three-color FACS analysis with anti-HLA-DR and anti-CD8 in conjunction with mAb to CD103 (panels A, C, and D) or to CD49d, CD11a, and CD44 (panel B only). Data shown are for electronically gated HLA-DR$^+$CD8$^+$ lymphocytes.

Interestingly, CD103 was poorly expressed among activated CD8 cells present in peripheral lymphoid compartments. Figure 3C shows CD103 expression by activated (HLA-DR$^+$) CD8 cells present within the inflamed tonsil of a patient with chronic tonsillitis. Figure 3D shows CD103 expression by activated CD8 cells within a cadaver donor spleen. Note that in both cases, the vast majority (>90%) of CD8$^+$DR$^+$ cells failed to express CD103.

Critical role of TGF-β in regulating CD103 expression by CD8 $^+$ CTL. CD103 expression in human MLC cultures has been reported to occur independently of TGF-β production (21). However, as shown in Figure 2A, treatment of human MLC cultures with an mAb known to neutralize TGF-β activity dramatically blocked the appearance of the CD103$^+$ CD8 subset. Although the addition of recombinant human TGF-β to MLC cultures at day 0 (not shown) or day 5 (Fig. 2B) only slightly increased CD103 expression, such treatment dramatically rescued CD103 expression by CD8$^+$ T-cell lines maintained by repeated stimulation with alloantigen plus IL-2 (Fig. 2C). Based on these data, we conclude that, similar to the mouse system, CD103 expression by human CD8 cells is controlled by TGF-β.

Consistent with the findings of Brew et al. (21), human MLC cultures contained high levels of total TGF-β (700-900 pg/ml) at days 1, 3, 5, and 7 of culture; indeed, even the culture media itself contained high levels of TGF-β (800 pg/ml). Although these data seem to contradict the results of the anti-TGF-β blocking studies noted above, it is important to note that TGF-β is secreted as a biologically inactive complex (latent TGF-β) that must be proteolytically processed to exert its diverse functional activities (23) and, moreover, that processed (active) TGF-β has an exceedingly short half-life (<3 min) (24). Thus, processing rather than production of TGF-β is likely the limiting factor controlling CD103 expression in human MLC cultures.

Stability and functional capability of the CD103 $^+$ CD8 subsets. To examine the functional capabilities of the above subsets, CD8 cells from human MLC cultures were sorted into CD103$^+$ and CD103$^-$ subsets, and assayed for lytic activity in standard Cr-51 release assays. Figure 4 shows that both subsets exhibited strong lysis of B-LCL targets expressing the stimulating alloantigens but failed to lyse third-party B-LCL targets. These data demonstrate that the CD103$^+$ and CD103$^-$ CD8 subsets in human MLC cultures both represent functionally viable effector populations.

Figure 4. Cytolytic activity of CD8 effector subsets in human MLC cultures. Purified CD8 cells from cultures generated against an HLA-3 disparity were sorted into CD103$^+$ and CD103$^-$ subsets, and tested for reactivity to HLA-A3+ (•) and HLA-A3- (•) B-LCL targets in standard Cr-51 release assays. The left and right panels show two independent experiments.

To examine the stability of the CD8 subsets defined by CD103, sorted CD103$^+$ and CD103$^-$ CD8 subsets (Fig. 5A) were

restimulated with alloantigen in media supplemented with IL-2. As shown in Figure 5, both subsets maintained their phenotypic integrity after restimulation: sorted $CD103^+$ CD8 cells remained $CD103^+$ (Fig. 5B), whereas sorted $CD103^-$ CD8 cells remained predominantly $CD103^-$ (Fig. 5C). Although a small proportion of sorted $CD103^-$ CD8 cells were induced to express CD103 by exogenous TGF-β, it is possible that these represent naive CD8 cells that failed to encounter alloantigen in the primary MLC and therefore are uncommitted with regard to the CD103 phenotype. Taken together, these data indicate that the $CD103^+$ and $CD103^-$ subsets both represent discrete and stable lineages of $CD8^+$ CTL. Note, however, that optimal CD103 expression by sorted $CD103^+$ CD8 cells during restimulation required the inclusion of exogenous TGF-β in the culture media (Fig. 5B).

Figure 5. Stability of CD8 subsets in human MLC cultures. (A) Prestimulation analysis (48 hr postsorting) of CD103 expression by sorted $CD103^+CD8^+$ cells (solid line), $CD103^-CD8^+$ cells (dashed line), and stained but unseparated $CD8^+$ cells (gray peaks). (B-D) CD103 expression by sorted subsets after restimulation for 7 days with allogeneic B-LCL stimulators in media containing IL-2 without exogenous TGF-β (solid line) or with exogenous TGF-β added at day 5 of culture (dashed line). Cells were stained for two-color FACS analyses as described in the legend to Figure 1. Data shown are for electronically gated $CD8^+$ lymphocytes.

CD103 expression on CD8 cells infiltrating renal allografts. The fortuitous availability of clinical transplant nephrectomy specimens allowed an examination of CD103 expression by human CD8 cells that infiltrate renal allografts. Such specimens yielded adequate numbers of graft-infiltrating lymphocytes for FACS analyses without the usual need for ex vivo expansion, which our in vitro data indicated can profoundly bias CD103 expression (Fig. 2).

As shown in Figure 6, CD103 was expressed at high levels by a major subset of graft-infiltrating CD8 cells. Figure 6A shows the results obtained for patient DB who withdrew herself from immunosuppressive medication at 2 years after transplantation and subsequently experienced acute-on-chronic rejection; in this case, 61% of graft-infiltrating CD8 cells were $CD103^+$. Figure 6B shows the results obtained for a kidney from patient DH undergoing acute rejection at 1 year after transplantation; in this case, 21% of CD8 cells were $CD103^+$. Expression of CD103 on graft-derived cells from both patients was strongly biased toward the CD8 subset with <5% of CD8 cells expressing CD103 (Fig. 7, left panels). CD8 cells comprised ~50% of graft-infiltrating lymphocytes, and these were predominantly medium- to large-sized $CD3^+$ cells (Fig. 7, middle and right panels). The bimodal expression of CD103 on graft-infiltrating CD8 cells contrasted with the uniform expression of other integrins (CD49d and CD11a; Fig. 6). Note that the CD11a peak of $CD8^+$ graft-infiltrating lymphocytes coincided with the $CD11a^{high}$ peak characteristic of CD8 cells activated in vitro (i.e., see Fig. 3B). Importantly, histopathological examination of these specimens confirmed that the kidneys were under-going cellular rejection at the time of removal (not shown).

Figure 6. Integrin expression by the $CD8^+$ graft-infiltrating lymphocytes derived from transplant nephrectomy specimens. Lymphocytes were isolated by collagenase treatment of nephrectomy specimens of the indicated patients and immediately stained for FACS analyses with anti-CD8 in conjunction with mAbs to CD103 (gray peak), CD49d (dashed line), or CD11a (solid line). The posttransplantation interval at which the respective nephrectomy was performed is shown under the patient name. Data shown are for electronically gated $CD8^+$ lymphocytes. Staining by negative control mAb overlapped with the low intensity CD103 peak and is not shown for the sake of clarity.

Figure 7. Additional analyses of $CD8^+$ cells isolated from clinical transplant nephrectomy specimens of patients DB (A-C) and DH (D-F). Lymphocytes were isolated by collagenase treatment of kidneys and immediately stained for FACS analyses with anti-CD8 in conjunction with mAbs to CD103 (A and E) or CD3 (B and F). Data shown are for electronically gated total lymphocytes; quadrants are set to exclude >99% of negative control staining, or in the case of panels C and F, to exclude >99% of resting (small) $CD8^+$ PBL.

Similar results were obtained for nephrectomy specimens from patient ED (3 months after transplantation), patient OW (7 months after transplantation), and patient DS (2 months after transplantation) whose grafts were undergoing mild cellular rejection at the time of removal; CD103^{+} cells comprised 32%, 17%, and 13%, respectively, of graft-infiltrating CD8 cells. In these cases, CD8 cells comprised <28% of total graft-infiltrating lymphocytes. Parallel treatment of a severely necrotic specimen removed at 2 months after transplantation from patient MW and a native (nontransplanted) kidney of patient KK with end-stage renal disease and a normal cadaver donor did not yield sufficient cells for analysis.

DISCUSSION TOP

Our recent finding that CD103, a receptor for an epithelial cell-specific ligand, defines a subset of CD8^{+} CTL in the mouse potentially provides new insight into the mechanisms by which CD8^{+} CTL interact with epithelial layers (6). However, the relevance of these findings to the clinical situation was unclear due to previous reports that CD103 expression by peripheral T cells in humans is regulated by mechanisms distinct from those in the mouse (18-21). We herein demonstrate that, similar to the mouse, CD103 defines a dichotomy among CD8^{+} CTL elicited in vitro in response to alloantigen, and that CD103 expression on such cells is regulated by TGF-β. We further demonstrate that CD103^{+} CD8 cells of the effector/memory phenotype are present at the site of renal allografts, consistent with a role for such cells in the rejection process.

CD103 is readily induced on human peripheral CD8 cells after activation with mitogens, specific antigen, or alloantigen, and is broadly considered to represent a general marker of T-cell activation in humans (18-21). We herein extend these findings by demonstrating that CD103 defines a discrete subset of activated (HLA-DR^{+}) CD8 cells in human MLC cultures (Fig. 3). We further show that the CD103^{+} and CD103^{-} CD8 subsets both exhibit allospecific lytic activity (Fig. 4) and, moreover, maintain their respective phenotypes after restimulation (Fig. 5). These data are in accord with our earlier finding in the mouse system that CD103 defines discrete and stable subsets of CD8^{+} CTL (6). Although earlier studies had noted that CD103 identifies a subset of human CD8 cells elicited in response to mitogens (18, 21) and alloantigen (21), the present study provides the first demonstration that the CD103^{+} and CD103^{-} subsets both represent stable and functionally viable effector populations.

The present data clearly implicate TGF-β as a critical factor in regulating CD103 expression by human CD8^{+} T cells. Neutralizing mAb to TGF-β dramatically blocked the appearance of the CD103^{+} CD8 subset in human MLC cultures (Fig. 2A), and persistent expression of CD103 on CD8 cells during in vitro expansion required the continued presence of active TGF-β (Fig. 2C). These data parallel findings in the mouse that exogenous TGF-β induces de novo CD103 expression in mouse T-cell activation cultures (6, 16) and also are in agreement with reports that TGF-β is required for the maintenance of CD103 expression by human IEL populations during ex vivo culture (25). The decline of CD103 expression in secondary and tertiary CD8^{+} T-cell lines (Fig. 2C) may reflect the loss of a cell population that efficiently processes TGF-β; macrophages, for example, are abundantly present in primary human MLC cultures and are efficient processors of TGF-β (26), but are depleted from restimulation cultures due to adherence to culture vessels.

The origin of the CD103^{+} CTL subset remains unclear. Figure 1 demonstrates that although CD103^{+} precursors are virtually undetectable (0.6%) among normal CD8^{+} PBL (Fig. 1D), such cells give rise to CD8 effectors that are >60% CD103^{+} within 3 days after activation in MLC cultures (Fig. 1C). Taken together with the recognition that TGF-β regulates CD103 expression in such cultures, these data strongly suggest that TGF-β induces new CD103 expression on previously negative precursors. Although these data do not exclude a rapid preferential expansion of the preexisting CD103^{+} subset, the data shown in Figure 5 indicate that the CD103^{+} and CD103^{-} CTL subsets undergo comparable degrees of expansion after restimulation irrespective of the presence or absence of TGF-β. Thus, these data argue against a significant role for TGF-β in promoting selective expansion or survival of the CD103^{+} subset.

Our current understanding of CTL characteristics is largely derived from studies using T-cell lines or clones expanded and maintained by restimulation with antigen plus IL-2 as the sole growth factor. Even in vivo-derived T cells, such as those derived from rejecting renal allografts (27, 28) or tumors (29, 30), are typically expanded in IL-2-containing media for

extended periods before functional and phenotypic analyses. A salient implication of the present data is that such methods may fail to detect an important component of CD8-mediated immunity.

That the present data are relevant to in vivo events is supported by our finding that both the CD103+ and CD103- subsets are present at the site of rejecting human renal allografts **(Fig. 6)**. The highest frequency of such cells (61% of total CD8) was noted in a patient undergoing late (acute-onchronic) rejection, whereas lower but still substantial frequencies (21% and 32%) were observed in kidneys undergoing classical acute rejection. Such cells were exclusively medium-to-large CD8+CD3+ cells **(Fig. 7)** that expressed elevated levels of CD11a **(Fig. 6)**, consistent with the expected in vivo phenotype of CD8+ effector/memory cells **(31)**. Intriguingly, CD103+ CD8 cells were relatively rare in human lymphoid compartments such as spleen, tonsil, and peripheral blood; such cells comprised <5% of total CD8 cells **(Fig. 1)** and <10% of activated CD8 cells **(Fig. 3)**. CD103+ CD8 cells were also poorly represented in some nephrectomy specimens removed at early posttransplantation intervals, e.g., 13% and 17% at 2 months and 7 months, respectively (**Fig. 6**, D and E). These data suggest that CD103+ cells are poorly represented among CD8 cells that initially infiltrate renal allografts, but that such cells selectively accumulate within the graft, possibly due to the unique presence of bioactive TGF-β at such sites. These data are in accord with our prior observation that CD103+ CD8 cells with activated/memory phenotype accumulate in the kidney but not the spleen in a mouse model of graft-versus-host disease **(6)**.

It will be important to determine whether the appearance of CD103+ CD8 cells is a common occurrence at peripheral sites of inflammation, or alternatively, if this phenomenon is unique to rejecting renal allografts. The latter hypothesis is plausible given that the common immunosuppressive agent, cyclosporine, is known to enhance intrarenal TGF-β production **(32, 33)**. Furthermore, chronically rejecting organ allografts are associated with significantly higher levels of TGF-β than acutely rejecting allografts **(34-36)**; thus, the striking abundance of CD103+ CD8 cells in the case of acute-on-chronic rejection noted above may reflect this difference. However, the interpretation of these observations is clouded not only by the unavoidably small sample size but also by innumerable clinical variables, including the potential for concurrent CD8 responses (i.e., to pathogens or from recurrence of original end-stage renal disease) and differences in levels of immunosuppressive medication (or lack thereof).

Our recent demonstration that murine CD103 can serve as a CTL accessory molecule that promotes lysis of renal epithelial cell targets **(16)** is consistent with a unique role for CD103+ CTL in the destruction of the graft epithelium. In addition, studies of IEL **(7, 8, 13-15)** provide a clear precedent that CD103 may facilitate CTL homing/retention within epithelial microcompartments and deliver costimulatory signals that promote intragraft CTL activation and expansion. The present findings thus potentially provide new insight into mechanisms of organ allograft rejection and provide a plausible basis for the preferential targeting of epithelial layers in organ allograft rejection **(1, 2, 37)** and graft-versus-host disease pathology **(38, 39)**. The development of mouse transplantation models with which to systematically investigate these issues is currently in progress. The present data validate the relevance of such models to clinical events by demonstrating that the regulation of CD103 expression by peripheral CD8 cells in the human system is similar, if not identical, to that in the mouse.

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* Abbreviations: B-LCL, B lymphoblastoid cell line; CTL, cytolytic T lymphocytes; FITC, fluorescein isothiocyanate; IEL, intraepithelial lymphocytes; IL, interleukin; mAb, monoclonal antibody; MLC, mixed lymphocyte cultures; PBL, peripheral blood lymphocytes; PE, phycoerythrin; SC, splenocytes; TGF, transforming growth factor. [Context Link]

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