Bacterial Superantigens Induce T Cell Expression of the Skin-selective Homing Receptor, the Cutaneous Lymphocyte-associated Antigen, via Stimulation of Interleukin 12 Production

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Summary

T lymphocyte infiltration is a prominent feature of the skin inflammation associated with infections by toxin (superantigen)-secreting *Staphylococcus aureus* or *Streptococcus* bacteria. The cutaneous lymphocyte–associated antigen (CLA) has been hypothesized to be a homing receptor (HR) involved in selective migration of memory/effector T cells to the skin. Since the expression of this putative skin-selective HR is known to be under strict microenvironmental control, we sought to determine the effect of staphylococcal and streptococcal toxins on T cell expression of CLA. After in vitro stimulation of peripheral blood mononuclear cells with staphylococcal enterotoxin B, toxic shock syndrome toxin-1, and streptococcal pyrogenic exotoxins A and C, there was a significant increase in the numbers of CLA^+ T cell blasts ($p < 0.01$), but not blasts bearing the mucosa-associated adhesion molecule $\alpha e\beta 7$-integrin, compared with T cells stimulated with phytohemaglutinin (PHA) or anti-CD3. Bacterial toxins were also found to specifically induce interleukin (IL) 12 production. More importantly, induction of toxin-induced CLA expression was blocked by anti-IL-12, and the addition of IL-12 to PHA-stimulated T cells induced CLA, but not $\alpha e\beta 7$-integrin, expression. These data suggest that bacterial toxins induce the expansion of skin-homing CLA^+ T cells in an IL-12–dependent manner, and thus may contribute to the development of skin rashes in superantigen-mediated diseases.

Staphylococcus aureus and *streptococcus* produce a large family of exotoxins (1). These toxins cause toxic shock-like syndromes and have been implicated in the pathogenesis of several allergic and "autoimmune" diseases (2, 3). Included within this group of proteins are the staphylococcal enterotoxins (SE), for example, SEB, staphylococcal toxic shock syndrome toxin-1 (TSST-1), group A streptococcal pyrogenic exotoxins (SPE) A, and SPEC. These toxins are prototypic superantigens, which stimulate large populations of T cells expressing particular TCR-β chain variable ($V\beta$) gene segments and activate cytokine release from monocytes/macrophages in an MHC-dependent but unrestricted manner (for review see reference 3).

A prominent feature of diseases thought to be triggered by superantigens, for example, toxic shock syndrome, Kawasaki syndrome, scarlet fever, atopic dermatitis, and guttate psoriasis, is the eruption of skin rashes (2, 4–8). Although it is thought that immunologic mechanisms, particularly T cell effector functions, contribute to the pathogenesis of skin inflammation in such diseases, the actual events are poorly understood. One element of effector T cell function that has not been studied in regard to these diseases is their homing ability. Studies in both animal models and humans have demonstrated clear heterogeneity in the ability of previously activated (memory/effector) T lymphocytes to migrate to mucosal versus nonmucosal (e.g., cutaneous) sites (for reviews see references 9, 10). This tissue-selective homing is regulated in large part at the level of lymphocyte recognition of postcapillary venular endothelial cells via the interaction of differentially expressed lymphocyte homing receptors and their endothelial cell ligands. In humans, the T lymphocyte molecule thought to participate in "skin-selective" lymphocyte homing is called the cutaneous lymphocyte–associated antigen (CLA), which interacts with E-selectin on endothelial cells (9–15).

Skin biopsies from the rashes of patients with superantigen-

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mediated diseases have demonstrated the infiltration of T lymphocytes and the expression of IL-1– and TNF-induced vascular adhesion molecules such as E-selectin (16, 17). To further understand the potential mechanism(s) that results in the migration of T lymphocytes into the skin of patients with superantigen-mediated infection, we assessed the expression of CLA on peripheral blood T cells from normal donors at baseline and after stimulation with various staphylococcal and streptococcal toxins. Our findings indicate that these toxins selectively induce CLA expression on T cells by a mechanism that is, at least in part, IL-12 dependent.

Materials and Methods

Reagents. Purified TSST-1, SEB, SPEA, and SPEC were prepared by P. M. Schlievert as previously described (18, 19). PHA-M was purchased from either Boehringer-Mannheim Biochemicals (Indianapolis, IN) or Wellcome Diagnostics (Greenville, NC). Neutralizing goat antibodies to human IL-12 (a gift from Dr. John Hakimi, Hoffman-La Roche, Little Falls, NJ) were prepared as previously described (20, 21). IL-12 and TGF-β1 were obtained from R&D Systems, Inc. (Minneapolis, MN), as were the anti-TGF-β1 antisera used in this study. IL-2 and anti-IL-4 antisera were obtained from Genzyme Corp. (Cambridge, MA). mAbs Leu4 (CD3; PE; PerCP), Leu3a (CD4; FITC, PE; peridinin chlorophyll protein [PerCP]), Leu2a (CD8; FITC, PE; PerCP); G1CL (mouse IgG1 control; FITC, PE, PerCP), G2GL (mouse IgG2 control; FITC, PE), and streptavidin-PE were obtained from Becton Dickinson Immunocytometry Systems (Mountain View, CA). MAb HML-1 (CD103; αe-integrin; biotin) was obtained from AMAC (Westbrook, ME), and mAb 2H4 (CD45RA; PE) was obtained from Coulter Immunology (Hialeah, FL). MAb HECA-452 (a rat IgM against CLA; FITC), and MECA-79 (used as a rat IgM control; FITC) were prepared as previously described (11). To assess TCR Vβ repertoire, mAbs directed at different Vβ segments were used. These included PE-conjugated mAbs directed to Vβ2, Vβ3, and Vβ17, from AMAC, and mAbs directed to Vβ5.1, Vβ8.1;8.2, Vβ12, and Vβ13.1, from T Cell Sciences, Inc. (Cambridge, MA).

Cell Isolation and Culture. PBMC obtained from normal donors were isolated from heparinized venous blood by density gradient sedimentation over Ficoll-Hypaque (Histopaque; Sigma Chemical Co., St Louis, MO). Cells were then washed three times in HBSS (Gibco Laboratories, Grand Island, NY) and resuspended in medium, as appropriate, for either cell culture or immunofluorescence staining of freshly isolated cells. Purified T cells (>95% $CD3^+$) were isolated from the PBMC preparations by negative selection by use of T cell purification columns (R&D Systems). Purified CLA^- virgin (>99% $CLA^-CD45RA^+$) and memory/effector (>99% $CLA^-CD45RA^-$) T cells were isolated from T cell preparations via fluorescence-activated cell sorting with the HECA-452 (FITC) and 2H4 (PE) mAbs, as previously described (14, 22).

For cell culture studies, PBMC (at 10^6 cells/ml) or T cells (0.5×10^6 cells/ml) were cultured in RPMI-1640 (Bio-Whittaker, Walkersville, MD) supplemented with 10% heat-inactivated FCS (Hyclone Sterile Systems, Inc., Logan UT), 20 mM Hepes buffer, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (all from Gibco Laboratories). Cells were cultured in the presence of either PHA (1 μg/ml), anti-CD3 (20 ng/ml) or staphylococcal or streptococcal toxins (100 ng/ml) for various time periods (usually 3–5 d), washed, and resuspended in staining buffer for immunofluorescence staining (see below). These concentrations of mitogens and superantigens were chosen on the basis of preliminary titration studies indicating they were the lowest concentrations yielding optimal T cell stimulation. In the CLA induction studies, IL-12 and TGF-β1 were used at 0.2 and 5.0 ng/ml, respectively (again, these optimal concentrations were determined in advance with titration studies). These studies also included 10 U/ml IL-2 in the culture media, required to obtain maximal stimulation and cell viability during PHA stimulation of the highly purified T cells. These levels of IL-2 had no specific effect on the expression of either CLA or $\alpha e\beta 7$-integrin by T cell blasts (data not shown). In those experiments that involved analysis of TCR Vβ expression, cells were stimulated for 3 d with bacterial toxin, washed and allowed to grow for an additional day in the presence of IL-2 (20 U/ml) before washing, staining with anti-TCR Vβ mAbs, and flow cytometric analysis, as previously described (23, 24).

Cell Staining and Flow Cytometric Analysis. Five-parameter analysis was performed on a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems) with FITC, PE, and PerCP used as the three fluorescent parameters. Immunofluorescence staining for this multiparameter analysis was performed as previously described (14, 22). Methods of cytometer set up and data acquisition have also been described previously (22). List mode multiparameter data files (each file with forward scatter, side scatter, and three fluorescent parameters) were analyzed by use of either the LYSIS II Windows or the PAINT-A-GATE^{Plus} programs (Becton Dickinson Immunocytometry Systems). Day 0 analysis was performed by use of a light scatter gate including only viable small lymphocytes, whereas the analysis of activated populations was performed with the light scatter gate set on the T cell blast population. Negative control reagents were used to verify the staining specificity of experimental antibodies.

IL-12 Assay. The quantitation of human IL-12 p40 by sandwich enzyme immunoassay using 2-4A1 anti-IL-12 p40 as the capture antibody was done as previously described (25) with the following modifications. Captured IL-12 was detected by addition of peroxidase-conjugated 4D6 anti-IL-12 p40 antibody, 250 ng/ml, to each well, followed by incubation for 2 h at room temperature with shaking. The plates were developed with TMB (Kirkegaard & Perry Labs, Inc., Gaithersburg, MD), the reaction was stopped by addition of 1 M H_3PO_4, and absorbency was measured at 450 nm. Bioactive human IL-12 was measured by means of an antibody capture bioassay using Kit225/K6 indicator cells, as described by Zhang et al. (25).

Statistical Analysis. Data are expressed as the mean ± SEM for each group. Statistical comparisons were made by use of unpaired two-tailed *t* tests with the Stat View 4.0 program (Abacus, Berkeley, CA) on a Macintosh IIcx computer. Analysis of variance (ANOVA) was used to test differences in the means of three or more groups. When ANOVA was significant, a Fisher's multiple comparison procedure was used to determine which groups were significantly different at the 5% level. Differences between groups were considered significant at $p < 0.05$.

Results

To determine whether bacterial toxins induce the expansion of CLA^+ T cells, we used multiparameter flow cytometric analysis to examine the expression of CLA on $CD3^+$ T cells within freshly isolated PBMC preparations from seven healthy donors versus their T cell blasts after 3 d of in vitro incubation with PHA, anti-CD3, SEB, TSST-1, SPEA, and SPEC. As shown in Fig. 1 (*top*), the percentage of CLA^+

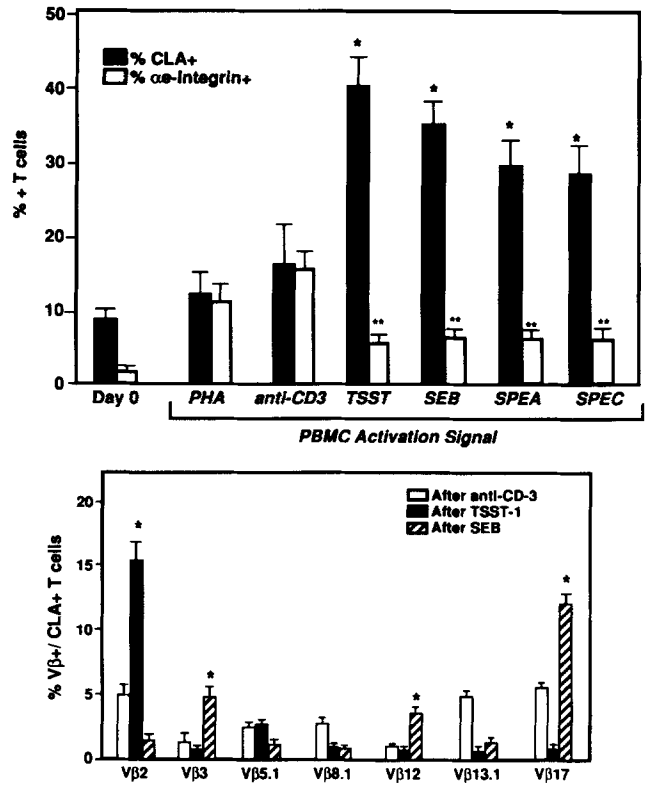

Figure 1. Bacterial toxins induce the expansion of CLA$^+$ T cells. (*Top*) CLA$^+$ T cell expression was analyzed before and after in vitro stimulation with PHA (2 μg/ml), anti-CD3 (20 ng/ml), SEB, TSST-1, SPEA, and SPEC (100 ng/ml for all toxins) in seven normal donors. PBMC stimulated with SEB, TSST-1, SPEA, and SPEC had significantly higher ($^*p$ <0.01, ANOVA) percentages of CLA$^+$ T cells than PBMC stimulation with anti-CD3 or PHA. In contrast, bacterial toxins stimulated significantly lower ($^{**}p$ <0.05) levels of αeβ7 than PHA or anti-CD3. (*Bottom*) PBMC were stimulated with either anti-CD3 (20 ng/ml), TSST-1 (100 ng/ml), or SEB (100 ng/ml) for 3 d, washed, and grown for an additional day in the presence of IL-2 (20 U/ml). TSST-1 induced the selective expansion of CLA$^+$ T cells expressing Vβ2 ($^*p$ <0.01 compared with anti-CD3–stimulated cells), and SEB induced the selective expansion of CLA$^+$ cells expressing Vβ3, Vβ12, and Vβ17 ($^*p$ <0.01).

T cells did not substantially change after 3 d of in vitro stimulation with PHA or anti-CD3. However, the percentage of CLA$^+$ T cells was significantly greater (p <0.01, ANOVA) for PBMC stimulated with SEB, TSST-1, SPEA, and SPEC compared with cells stimulated with PHA or anti-CD3. Kinetic analyses demonstrated that increased CLA expression by bacterial toxin– versus PHA- or anti-CD3–stimulated T blasts first appears at day 3 and is maintained through day 5, in keeping with previous kinetic studies of CLA induction (14). Both the CD4 and CD8 T cell subsets participated in this bacterial toxin–selective induction of CLA, showing three- and fourfold increases in CLA-expressing T blasts at day 3 over day 0, respectively. In contrast, the activation-dependent induction of the mucosa-associated adhesion molecule αeβ7 (26) by bacterial toxins was significantly less than by PHA or anti-CD3 (p <0.05).

Since bacterial superantigens are known to expand T cells in a Vβ-specific manner (e.g., Vβ2 for TSST-1; Vβ3, Vβ12, and Vβ17 for SEB) (3), we also examined the T cell repertoire of CLA$^+$ T cells before and after stimulation with TSST-1 or SEB (Fig. 1, *bottom*). Within the CLA$^+$ T cell subset, TSST-1 induced a selective expansion of Vβ2-expressing T cells, but not T cells expressing Vβ3, Vβ5.1, Vβ8.1,8.2, Vβ12, Vβ13.1, or Vβ17 TCRs. Similarly, SEB induced selective expansion of Vβ3-, Vβ12- and Vβ17-expressing T cells within the CLA$^+$ subset, compared with T cells with Vβ2, Vβ5.1, Vβ8.1, Vβ8.2, or Vβ13.1 TCR variable regions.

The observation that bacterial superantigens (known to have stimulatory effects on immune accessory cells such as macrophages), but not T cell mitogens, induced CLA$^+$ T cell expansion suggested that an accessory cell product may be involved in this process. We therefore examined the potential role of TGF-β1 and IL-12 in the induction of CLA$^+$ T cells. TGF-β1 has been previously shown to induce CLA expression on mitogen-activated T cells (14), whereas IL-12 had not been previously tested for this activity. As shown in Fig. 2, a neutralizing antiserum to TGF-β1 did not significantly inhibit CLA$^+$ T cell expansion, suggesting that this cytokine did not play a role in bacterial toxin–induced expansion of CLA-expressing T cells. In contrast, the expansion of the CLA$^+$ T cell subset by TSST-1 was significantly reduced by neutralizing antibodies to IL-12 (p <0.05), nearly down to the percentage of CLA$^+$ T cells observed in PHA-stimulated cultures. The addition of anti-TGF-β1 to anti-IL-12 provided no further decrease in the number of CLA$^+$ T cells.

To further examine the potential role of superantigen-induced IL-12 production in the stimulation of CLA$^+$ T cell expansion, we examined IL-12 production in PBMC stimulated by PHA, SEB, and TSST-1. As demonstrated in Fig. 3, both SEB and TSST-1 synthesized significantly more immunoreactive and bioactive IL-12 (p <0.01, ANOVA) than PBMC stimulated with PHA.

Fig. 4 examines the ability of exogenous IL-12 to regulate CLA and αe-integrin expression on PHA-stimulated purified

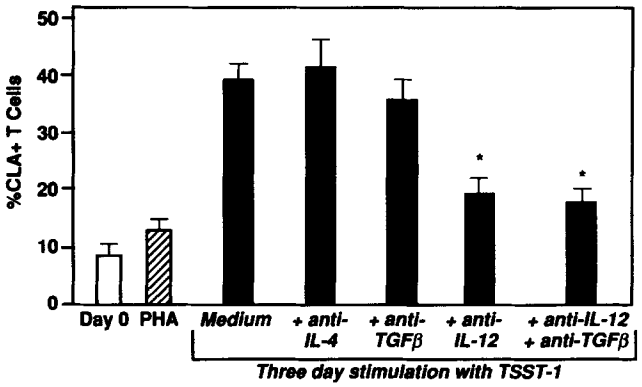

Figure 2. CLA$^+$ T cell expression induced by TSST-1 is dependent on IL-12, but not TGF-β. Neutralizing antibody to IL-12 (1 μg/ml) and antibody to TGF-β (1 μg/ml) were added to TSST-1–stimulated PBMC. The addition of anti-IL-12 caused a significant inhibition of TSST-1–induced CLA$^+$ T cell expansion ($^*p$ <0.01).

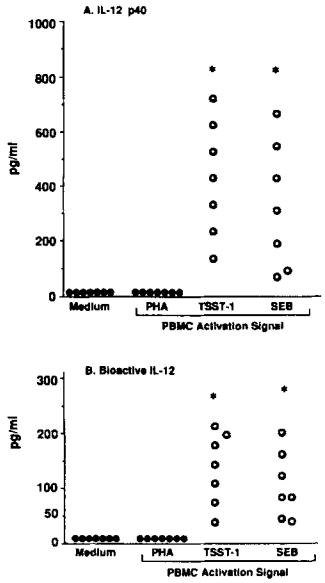

Figure 3. Bacterial toxins, but not PHA, induce IL-12 synthesis by PBMC. PBMC from seven different donors were incubated for 2 d in the presence and absence of TSST-1 or SEB (100 ng/ml). PBMC stimulated with SEB and TSST-1 synthesized significantly more IL-12 than PBMC stimulated with PHA (*p <0.01, ANOVA).

T cells. TGF-β1, which induces T cell expression of both CLA and $\alpha e\beta 7$-integrin (14, 26), was also examined in these experiments for comparative purposes. These data demonstrate the ability of IL-12 to selectively increase T blast expression of CLA (p <0.001, ANOVA), but not $\alpha e\beta 7$-integrin (p = NS, ANOVA), compared with blasts generated in PHA+IL-2 alone (Fig. 4, *top*). This effect was observed for both $CD4^+$ and $CD8^+$ T cells; compared with day 5 mitogen-stimulated T cell cultures without IL-12, T cell cultures with IL-12 demonstrated a mean 2.1- (±0.18; n = 8) and 2.3- (±0.28; n = 6) fold increase in CLA^+ cells among the $CD4^+$ and $CD8^+$ subsets, respectively (p = NS). The lack of an IL-12–mediated $\alpha e\beta 7$-integrin upregulation was not due to a nonspecific lack of responsiveness, as TGF-β1 mediated a profound upregulation of $\alpha e\beta 7$ on the same cells (p <0.002, ANOVA). Finally, we show in the lower panel of Fig. 4 that IL-12 mediates expansion of CLA^+ T cells among highly purified CLA^- virgin ($CD45RA^+$) and memory/effector ($CD45RA^-$) T cells, including both the $CD4^+$ and $CD8^+$ ($CD4^-$) T cell subsets. These findings indicate that the predominant, if not exclusive, mechanism of IL-12 action was the induction of CLA expression, rather than selective expansion of preexisting CLA^+ T cells.

Discussion

Skin infiltration with activated T cells is frequently associated with toxin-producing *S. aureus* and streptococcal infections, and is thought to contribute to the pathology of the disease caused by these organisms (2, 4–8). It is now well established that staphylococcal and streptococcal toxins engage class II MHC antigens on mononuclear phagocytes to induce the release of cytokines such as IL-1β and TNF-α (27)

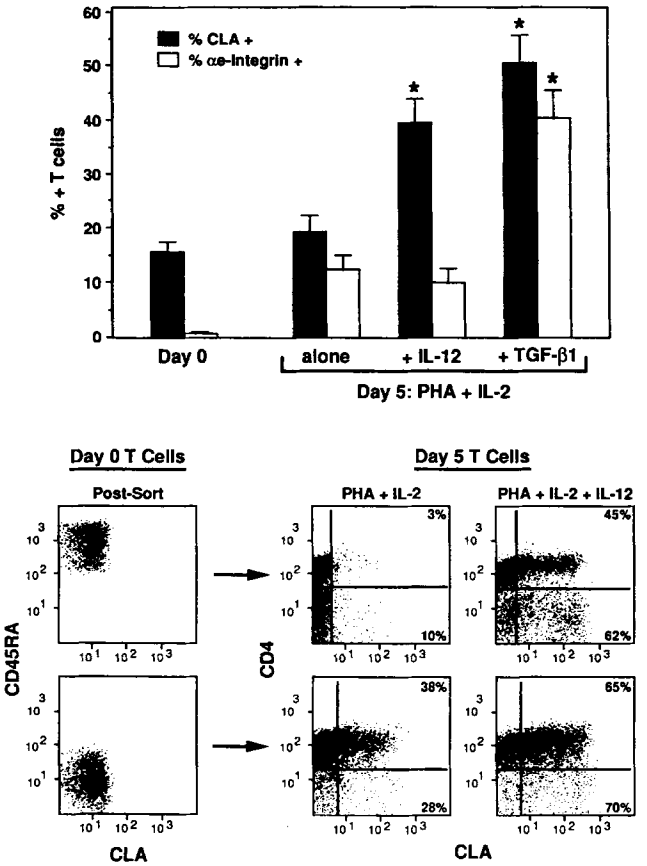

Figure 4. Cytokine regulation of T cell CLA induction in vitro. (*Top*) Purified peripheral blood T cells were incubated for 5 d with PHA and IL-2 in the presence of IL-12 (0.2 ng/ml), TGF-β1 (5 ng/ml), or no additional cytokines, and then assessed for their expression of CLA (seven experiments) and αe integrin chains (five experiments). The results are presented as the percentage (±SEM) of CLA- or αe integrin–expressing T cell blasts at day 5 compared with the same determination among freshly prepared small T cells (day 0). T cells stimulated with mitogen in the presence of either IL-12 or TGF-β1 demonstrated significantly higher percentages of CLA expressing cells at day 5 than T cells stimulated in the absence of these cytokines (*p <0.001, ANOVA). IL-12 had no significant effect on the percentage of day 5 T blasts expressing αe integrin, whereas TGF-β1 increased the percentage of αe integrin–expressing cells almost fourfold (significant at p <0.002, ANOVA). Low levels of IL-2 were included in these experiments to maximize the PHA responsiveness of the purified T cells; IL-2 by itself does not significantly influence the expression of either of the molecules studied (14 and Picker, L. J., data not shown). (*Bottom*) Highly purified $CLA^-CD45RA^+$ (virgin) and $CLA^-CD45RA^-$ (memory/effector) T cells were prepared by flow cytometric cell sorting (*left column*) and then stimulated for 5 d by PHA + IL-2 in the presence or absence of IL-12. The staining characteristics of these day 5 T blasts (essentially all $CD3^+$) with regard to CLA versus CD4 are indicated in the profiles shown, with the quadrants delineating the cutoffs for positive and negative staining. The percentage of $CD4^+$ and $CD4^-$ cells that are positive for CLA are given in the upper and lower corners, respectively, of each profile. Among the CLA^- virgin T cells, pronounced CLA upregulation was observed only in the presence of IL-12. Among the CLA^- memory/effector T cells, a low level of CLA upregulation (low in terms of both percentage positive and staining intensity) was observed in the absence of IL-12, but CLA expression by these cells was greatly potentiated by the addition of IL-12 to the cultures.

and act as superantigens that powerfully stimulate T cells expressing specific TCR Vβ gene segments (3). Here, we provide evidence for yet another mechanism of bacterial toxin–mediated modulation of the immune response: induction of the skin-selective homing receptor (HR) CLA on toxin-activated T cells.

Our data indicate that bacterial toxins, for example, SEB, and TSST-1 simultaneously stimulate T cells and upregulate their expression of CLA. Similar upregulation was not seen for $\alpha e\beta 7$-integrin, an adhesion molecule thought to selectively mediate T cell adhesion to mucosal epithelium (10, 28), and previous studies have shown that bacterial toxins tend to downregulate T cell expression of the peripheral lymph node–selective HR L-selectin (29 and Picker, L. J., unpublished data). Thus, the available data suggest that T cell activation with bacterial toxins appears to selectively alter the expression pattern of tissue-selective HR/adhesion molecules on the responsive T cells. Since a wide variety of data have implicated the interaction between CLA and its vascular ligand E-selectin as a critical, specificity-determining step in T cell extravasation at cutaneous sites of chronic inflammation (8–15), such alteration may well act to selectively increase the skin-homing properties of toxin-responsive T cells, thereby enhancing the ability of these T cells to participate in cutaneous inflammatory processes (see below).

CLA is not expressed by CD45RA^{hi}/RO^{lo} virgin T cells, and its presence on a skin-homing CD45RA^{lo}/RO^{hi} memory/effector T cell subset appears to be initiated by a microenvironmentally controlled induction during the antigen-induced virgin to memory/effector T cell conversion in secondary lymphoid tissue (11, 14). At least part of the regulation of this induction is tissue specific, as we have previously demonstrated that CLA expression on developing memory/effector T cells in peripheral lymph node is fivefold higher than that in mucosal-associated secondary lymphoid tissues (14). While the particular microenvironmental factors operating to regulate CLA induction in vivo are not understood, we previously implicated TGF-β1 as a potent inducer of CLA on activated virgin and memory/effector T cells (14), and therefore initially hypothesized that this pleiotropic cytokine might be involved in bacterial toxin–dependent CLA induction. To our surprise, we found that (*a*) bacterial toxins selectively induce IL-12 production by PBMC (likely by non-T cells; 30, 31); (*b*) anti-IL-12, but not anti-TGF-β1, antibodies significantly inhibit bacterial toxin–mediated CLA induction; and (*c*) exogenous IL-12 is a potent upregulator of CLA on PHA-stimulated T blasts. These data strongly suggest that toxin-dependent CLA induction is mediated in large part by IL-12, with TGF-β1 having no discernable role. We do not rule out a role for TGF-β1 in the regulation of CLA in other physiologic circumstances (i.e., situations not involving bacterial toxins). However, it is interesting to note that TGF-β1 is a potent upregulator of both CLA and $\alpha e\beta 7$, whereas IL-12 only upregulates CLA (Fig. 4). Since among in vivo T cell populations CLA and $\alpha e\beta 7$ expression is usually mutually exclusive (15), IL-12 may well be a physiologically more common regulator of CLA than TGF-β1. IL-12 also has a well-documented capability to influence T cell cytokine synthesis profiles (32–34), making it possible that this cytokine acts indirectly (i.e., via intermediates) to upregulate CLA. However, we have previously demonstrated that a wide variety of T cell and non-T cell cytokines have little (IL-6) or no (IL-1, -2, -3, -4, -5, -7, -8, -10, TNF-α, GM-CSF, and IFN-γ) ability to upregulate CLA (14 and Picker, L. J., unpublished data), suggesting that either other, perhaps unknown, cytokines act as intermediates, or that IL-12 acts directly to influence T cell CLA expression.

This finding of a distinct, potent regulator of CLA expression underscores the complexity of the regulation of memory/effector T cell function, which is thought to involve not only the interplay of immunoregulatory cytokines, but perhaps also the nature of the costimulatory signals available on different accessory cell subsets and the nature of the antigenic stimulus itself (10, 32, 35, 36). As mentioned above, IL-12 has been implicated as a potent modulator of the cytokine synthesis potential of memory/effector T cells, promoting the development of effector T cells with a Th1 cytokine synthesis pattern (32–34). Thus, bacterial toxins would appear to have the ability to activate and expand large subsets of the peripheral T cell compartment and simultaneously—via their IL-12–releasing activity—modulate both the homing and effector functions of the responding virgin, and perhaps also memory/effector, T cells.

These mechanisms may play a major role in the manifestation of pathologic sequelae in disorders associated with toxin-producing staphylococci or streptococci. In the case of diseases associated with local skin infection such as atopic dermatitis (7), staphylococcal exotoxins secreted at the skin surface could penetrate inflamed skin and stimulate epidermal macrophages or Langerhans cells (or perhaps other cell types) to produce IL-1, TNF, and IL-12. Local production of IL-1 and TNF would induce the expression of E-selectin on vascular endothelium (37, 38), allowing an initial influx of CLA$^+$ memory/effector cells. Local secretion of IL-12 could increase CLA expression on those T cells activated by antigen or superantigen and thereby increase their efficiency of recirculation to the skin, perhaps including areas with only low levels of vascular E-selectin and minimal inflammatory activity. IL-12 secreted by toxin-stimulated Langerhans cells (39), which migrate to skin-associated lymph nodes (and serve as APC therein; 40) could upregulate the expression of CLA and influence the functional profile of virgin T cells activated by the toxins, thereby creating additional skin-homing memory/effector T cells. Together, these mechanisms would tend to markedly amplify the initial cutaneous inflammation and perhaps also create conditions favoring progression of the staphylococcal skin colonization.

In systemic infections, such as staphylococcal or streptococcal toxic shock syndrome or Kawasaki syndrome, increased production of IL-1 and TNF by circulating macrophages (17, 41) and, in the case of Kawasaki syndrome, widespread induction of cytokine-inducible adhesion molecules on the vascular endothelium (including postcapillary cutaneous venules; 17), have been reported. If IL-12 is produced in similar fashion, it is possible that generalized T cell activation with concomitant CLA induction could lead to diffuse extravasation of

activated T cells at sites of E-selectin induction, particularly the skin, contributing to widespread organ damage.

In summary, this study provides new insights into the potential mechanisms that determine the pathogenesis of skin inflammation in superantigen-triggered diseases. Moreover, the bacterial toxin system provides a useful paradigm of memory/effector T cell function in which a single "antigenic" stimulus leads to production of functionally specialized effector T cells via its ability to simultaneously activate T cells and—via signaling a specific accessory cell response—alter the local microenvironment supervising the differentiation of the responding cells.

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