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# Major histocompatibility complex class I molecule serves as a ligand for presentation of the superantigen staphylococcal enterotoxin B to T cells

ANDREAS C. HÄFFNER*, KAROLINE ZEPTER, AND CRAIG A. ELMETS†

Skin Diseases Research Center, Department of Dermatology, Case Western Reserve University, University Hospitals of Cleveland, 11100 Euclid Avenue, Cleveland, OH 44106

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**ABSTRACT** **Superantigens, such as staphylococcal enterotoxin B (SEB), elicit a strong proliferative response in T cells when presented in the context of major histocompatibility complex (MHC) class II molecules. We observed a similar T-cell response, when MHC class II-negative epidermal cell lines were employed as antigen-presenting cells. Immunoprecipitation studies indicated that the ligand to which SEB bound had a molecular mass of 46 kDa. Radiolabeled SEB could be immunoprecipitated from isolated membrane proteins on the SCC13 epidermal cell line with a monoclonal antibody directed against the MHC class I molecule, and transfection of the K-562 cell line with MHC class I molecules showed a 75% increased SEB-binding capacity compared with the nontransfected MHC class I- and class II-negative counterpart. In functional studies, antibodies to the MHC class I molecule inhibited T-cell proliferation by at least 50%. From these studies, we conclude that MHC class I molecules on malignant squamous cell carcinomas serve as ligands for SEB, which, given the appropriate costimulatory signals, is sufficient to allow for superantigen-induced T-cell proliferation.**

Superantigens are a heterogeneous group of structurally diverse bacterial exotoxins, viral protein products, and endogenous self-antigens that include staphylococcal exotoxins A–E, toxic shock syndrome toxin, streptococcal pyrogenic toxins, and protein products of *Mycoplasma arthritidis* and the human immunodeficiency virus (1, 2). They are key participants in a variety of diseases and have been implicated in the pathogenesis of such chronic cutaneous disorders as atopic dermatitis and psoriasis (3, 4).

The precise mechanism by which superantigens cause these diseases is still incompletely understood, although much attention has focused on their ability to activate subpopulations of T lymphocytes that bear specific Vβ T-cell antigen receptors (TCRs carrying a specific β-chain variable region) (1, 5–7). This activation process initiates T-cell proliferation and cytokine production (8, 9) and confers non-MHC specific T-cell cytotoxicity against a variety of tumor lines (6, 10, 11). Like conventional antigens, superantigens require antigen-presenting cells (APC) for optimal T-cell activation. In most situations, these potent T-cell stimuli bind to APC through covalent interactions with MHC class II determinants. However, unlike conventional antigens, superantigens adhere to nonpolymorphic regions on the outer face of the molecule rather than to the antigen-binding groove (12, 13). This unique interaction enables APC to bypass the usual MHC haplotype restrictions for T-cell activation.

The relationship between superantigens and class II MHC is well established and, with respect to SEB, has recently been confirmed by x-ray crystallography (14, 15). However, observations in our laboratory (*vide infra*) and reports by other groups (16–19) strongly suggest that ligands aside from MHC class II molecules exist on APC. This is of particular interest because many of the superantigen-presenting cells used to demonstrate MHC class II-independent T-cell activation are tumor cell lines (17–19).

The nature of the alternative ligand on MHC class II-negative cells, has yet to be determined, however. To address this issue, we conducted a series of experiments employing SCC13 cells, a cutaneous squamous cell carcinoma line devoid of MHC class II molecules, and other class II-negative squamous cell carcinoma cell lines (A431 and SCC4) to identify their superantigen-binding structures. Our studies indicate that, on these cells, MHC class I determinants serve as SEB-presenting surface molecules able to initiate strong immunological responses.

## MATERIALS AND METHODS

**Antibodies.** RR-1, a neutralizing monoclonal antibody (mAb) against intercellular adhesion molecule 1 (ICAM-1), was the gift of Robert Rothlein (Boehringer Ingelheim). BB-1, a neutralizing mAb against B7 adhesion molecule was provided by Edward Clark (University of Washington, Seattle). mAbs 9.3F10 (which identifies a common determinant among HLA-DR, HLA-DP and HLA-DQ molecules) and W6/32 (which recognizes MHC class I antigens) were both obtained from the American Type Culture Collection (ATCC). A polyclonal rabbit anti-SEB antibody was obtained from Sigma. Monoclonal anti-Vβ2 and anti-Vβ3 TCR antibodies were obtained from Immunotech. All antibodies were used in purified form.

**Cell Lines and Culture Conditions.** The human epidermal squamous cell carcinoma cell line SCC13 was maintained in 1:1 (vol/vol) Dulbecco's modified Eagle's medium (DMEM)/Ham's F-12 medium (Whittaker M. A. Bioproducts, Walkersville, MD) containing 10% (vol/vol) heat-inactivated fetal calf serum (FCS; Hyclone Laboratories), 10 μg of insulin per ml, 40 ng of hydrocortisone (Sigma) per ml, 100 units of penicillin per ml, 100 μg of streptomycin per ml, and 2 mM L-glutamine (GIBCO). The human squamous cell carcinoma cell lines SCC4 and A431 were grown in DMEM containing 10% FCS, 100 units of penicillin per ml, 100 μg of streptomycin per ml, and 2 mM L-glutamine. K-562-cells and Epstein–Barr virus (EBV)-transformed B-cell lines were cultured in RPMI 1640 medium containing 10% FCS, 100 units of penicillin per ml, 100 μg of streptomycin per ml, and 2 mM L-glutamine (RPMI

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Abbreviations: SEB, staphylococcal enterotoxin B; MHC, major histocompatibility complex; FACS, fluorescein-activated cell sorting; ICAM-1, intercellular adhesion molecule 1; TCR, T-cell receptor; APC, antigen-presenting cells; V, variable; mAb, monoclonal antibody; RT, room temperature.

*Present address: Department of Dermatology, University of Zurich, Zurich, Switzerland.
†To whom reprint requests should be addressed.

culture medium). Cultures were fed twice a week and maintained at 37°C in 5% $CO_2$/95% air.

T-lymphocytes were isolated by a four-step purification procedure that has been described (20). Briefly, cells were collected from the blood of healthy volunteers. Mononuclear cells were then isolated by Ficoll/Hypaque density gradient centrifugation. The resulting cell population was sequentially incubated on plastic, passed over a nylon wool column, treated with L-leucine methyl ester (Sigma), and incubated with anti-MHC class II monoclonal antibodies (OKIa; Ortho Pharmaceuticals) and complement (PelFreeze). The purified T cells failed to proliferate to SEB or anti-CD3 antibodies without the addition of APC to cultures.

**Proliferation Assays.** Proliferation assays were performed by coincubating $5 \times 10^4$ x-irradiated squamous cell carcinoma cell lines with $1 \times 10^5$ purified T cells and SEB in 200 μl of complete medium consisting of RPMI culture medium in round-bottom, 96-well microtiter plates (Becton Dickinson) for 5 days at 37°C in 5% $CO_2$/95% air. SEB (Sigma) was added to a final concentration of 5 ng/ml. T-cell proliferation was determined by the uptake of [$^3$H]thymidine (ICN), which was added to the cultures 18 hr before termination of the assay. Radioactive measurements were taken on a Beckman LS6000 scintillation counter. For some experiments, antigen-presenting squamous cell carcinoma cell lines were suspended in sterile phosphate-buffered saline (PBS) containing 1% paraformaldehyde and incubated for 30 min at room temperature. They were then washed extensively in PBS, resuspended in complete medium, and allowed to stand for 30 min before being placed in culture.

**Immunoblot (Western Blot) Analysis for MHC Class II Expression.** For each sample, cell membranes of each of $3 \times 10^6$ B cells, epidermal melanocytes, and SCC13 cells were recovered by lysis in 0.5% Triton X-100 buffer containing 50 mM Tris chloride (pH 8.0), 100 mM NaCl, and proteinase inhibitors (2 μg of aprotinin, 2 μg of leupeptin, and 100 μg of phenylmethylsulfonyl fluoride per ml) for 30 min on ice. After ultracentrifugation [at 14000 rpm (50.2 Ti fixed-angle rotor) for 30 min at 4°C], 30 μg of each protein sample was subjected to nonreducing SDS/12% PAGE. It was subsequently electrotransferred to a nitrocellulose membrane (Millipore). Nonspecific binding sites were blocked by incubation in 5% fat-free dried milk with 0.05% Tween 20 for several hours. Staining was carried out by using the mouse monoclonal antibody 9.3F10. To visualize the hybridization, a second alkaline phosphatase-conjugated anti-mouse IgG antibody was applied (Sigma).

**Immunoprecipitation.** B-cells, epidermal melanocytes, or SCC13 cells ($5 \times 10^6$) were surface-iodinated by using 1 mCi (1 Ci = 37 GBq) of [$^{125}$I]iodine (Amersham) and Iodo-Beads (Pierce) according to the manufacturers' recommendations. After iodination, the cells were washed extensively with PBS to remove unbound iodine and any possible contaminating iodination reagent. They were then incubated with 500 ng of unlabeled SEB in PBS. The soluble membrane fraction was isolated by lysis as described for Western blot analysis (see above). To minimize nonspecific binding effects, supernatants were precleared on a Sepharose column (Pharmacia) and coated with an irrelevant antibody. Column eluates were immunoprecipitated on a CNBr-activated protein A-Sepharose column (Pharmacia) coated with polyclonal rabbit anti-SEB antibody. After an incubation period of 60 min at room temperature (RT), the Sepharose gel was washed extensively with PBS buffer, and proteins bound to the SEB–anti-SEB complex were released by pH-shift elution (alternating washes with 0.1 M NaOAc/0.5 M NaCl, pH 2.0, and 0.1 M Tris chloride/0.5 M NaCl, pH 8.0). Concentrated samples of the column eluate were subjected to SDS/PAGE on 16% Tris glycine gels, and bands of radioactive-labeled proteins were visualized on Kodak XAR film by autoradiography for 48 hr at −70°C.

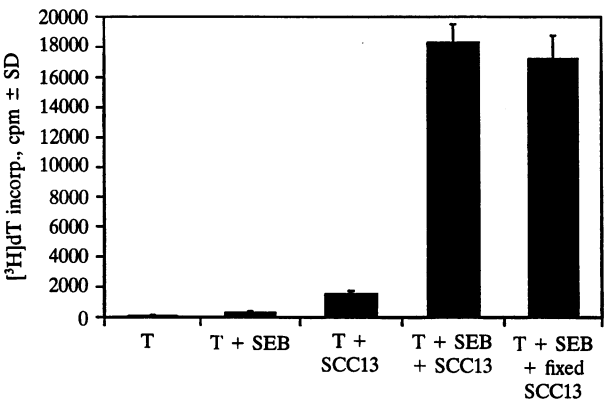


FIG. 1. SCC13 cells (SCC13) present SEB to resting T cells (T) and induce proliferation. Bars indicate cpm of [$^3$H]thymidine incorporation (incorp.) during the last 18 hr of a 5-day incubation period. Cpm are representative of three independent experiments.

A similar protocol was employed to identify the SEB-binding membrane molecule by using untreated cell suspensions of SCC13 and B-cell cultures ($5 \times 10^6$ each) and incubating them with $^{125}$I-labeled SEB (500 ng of SEB; 1 mCi). Membrane solubilization was done as described, followed by immunoprecipitation on an anti-MHC class I antibody (W6/32)-coupled CNBR-activated Sepharose A column. Selective elution by pH shift and SDS/PAGE were conducted as described above.

**Fluorescence-Activated Cell Sorting (FACS) Analysis for SEB Binding and Vβ TCR.** Surface expression of the SEB ligand was assessed by incubation of $1 \times 10^6$ cells with 0.5 mg of SEB per ml for 1 hr at RT. This was followed by an incubation step with 2 μg of polyclonal rabbit anti-SEB antibody and 2 μg of Cy5-labeled goat anti-rabbit mAb per ml at 4°C. Controls included stainings with an unrelated IgG antibody and the Cy5-labeled second antibody only. For Vβ TCR staining, cells were incubated with 2 μg each of mAbs anti-Vβ2 and anti-Vβ3 for 1 hr at RT. They were then incubated with fluoresceinated goat anti-mouse secondary antibodies. FACS analysis was performed on an Epics Elite ESP flow cytometer (Coulter) at 525 nm [fluorescein isothiocyanate (FITC)-conjugates] and 675 nm (Cy5-conjugates).

## RESULTS

**SCC13 Cells Present SEB to Resting T Lymphocytes and Induce Proliferation.** To determine whether squamous cell carcinoma cell lines were able to present antigen to T cells, SCC13 cells were x-irradiated and coincubated with purified, resting T cells in the presence of SEB at 5 ng/ml. Substantial increases in [$^3$H]thymidine incorporation consistently occurred and required the presence of both the tumor cell line and SEB. Failure to induce lymphocyte proliferation in the absence of either SEB or SCC13 cells confirmed that the effect was not caused by contamination of the responder population with APC (Fig. 1). When the antigen-presenting capacity of SCC13 cells for SEB was compared to autologous peripheral blood monocytes, a similar degree of T-cell proliferation was

Table 1. Comparison of the antigen-presenting function of monocytes and SCC13 cells for SEB

| Days of culture | Autologous monocytes as APC, mean ± SD | SCC13 cells as APC, mean ± SD |
|---|---|---|
| 3 | 13,561 ± 1,533 | 4,880 ± 666 |
| 4 | 15,947 ± 2,769 | 8,360 ± 1,237 |
| 5 | 14,421 ± 1,885 | 17,738 ± 1,885 |

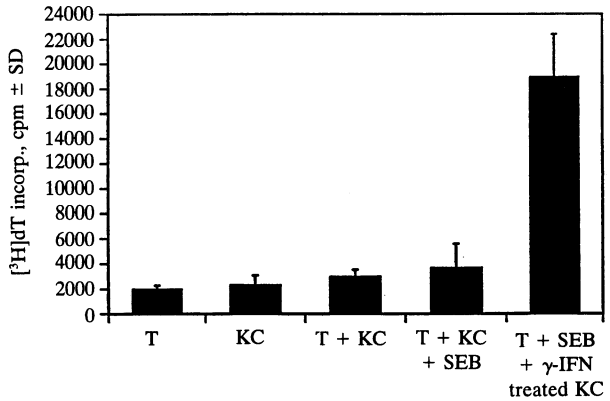


FIG. 2. Normal human keratinocytes (KC) are unable to function as APC for SEB except when pretreated with interferon γ (IFN-γ; 500 units/ml for 12 hr). Bars indicate cpm of [3H]thymidine incorporation (incorp.) during the last 18 hr of a 5-day incubation period. Cpm are representative of three independent experiments.

observed (Table 1). However, the maximum proliferation was first seen on day 3 when monocytes were used, whereas maximum proliferation was typically present on day 5 with SCC13 cells.

In contrast to SCC13 cells, untreated cultured keratinocytes were unable to serve as APC for SEB-induced T-cell proliferation (Fig. 2). It should be noted, however, that pretreatment of cultured keratinocytes with interferon γ, allowed them to present SEB to T cells (Fig. 2), confirming the findings of other investigators (21).

A distinguishing characteristic of the T-cell-activating properties of superantigens is their ability to selectively activate specific subpopulations of T cells that express appropriate *Vβ*-encoded elements on their T-cell receptor (1). In humans, the major Vβ TCR family activated by SEB is Vβ3, whereas the toxic shock syndrome toxin (TSST-1) is proficient at activating T cells that express the Vβ2 TCR (22, 23). To prove that the T-cell proliferative response in this system was truly SEB-mediated, an assessment of the percentage of cells expressing the Vβ2 and Vβ3 TCRs was made. The percentage of T cells bearing the Vβ3 TCR increased from 0.6% at baseline to 7.1% after culture with SEB and SCC13 cells for 5 days. In contrast, Vβ2 TCR-bearing T cells were 0.8% at baseline but were only 1.3% at the end of culture.

**SCC13 Cells Do Not Express Class II MHC Determinants.** To exclude the possibility that SCC13 cells expressed MHC class II molecules, immobilized membrane lysates of SCC13 cells, B cells, and melanocytes were incubated with $^{125}$I-labeled mAb 9.3F10, which recognizes a common determinant shared by HLA-DR, HLA-DP, and HLA-DQ. Bands of ≈65 kDa, corresponding to the size of MHC class II determinants, could be visualized on B cells and melanocytes. Comparable bands were not found in SCC13 extracts (Fig. 3 *Left*). In addition, when SCC13 cells were stained with 9.3F10 and examined by flow cytometry, fluorescence of SCC13 cells remained at background levels (data not shown). B cells and melanocytes served as positive controls, and, when treated with the same reagents, exhibited increased fluorescence intensity.

Because SCC13 cells can be induced to express class II MHC molecules, T-cell proliferation assays were performed in which SCC13 cells were subjected to paraformaldehyde fixation prior to placing them in culture with T cells and SEB. In this way, no up-regulation of class II molecules could occur. Results comparable to those with nonfixed SCC13 cells were observed (Fig. 1). Finally, in some experiments, anti-MHC class II antibodies were added continuously to cultures containing SCC13 cells, T cells, and SEB (Fig. 3 *Right*). No inhibition of T-cell proliferation was observed, providing additional evidence that induced expression of MHC class II molecules on T cells was not responsible for this effect. It should be noted that antibodies employed were able to inhibit T-cell proliferative responses in other systems.

**Presentation of SEB to Resting T Cells by SCC13 Is Not Cell Line-Restricted.** To assess whether SEB-mediated T-cell activation was a phenomenon restricted to SCC13 cells, two other epidermal tumor cell lines—A431 and SCC4—were subjected to the same experimental conditions. Both of these cell lines were assayed for MHC class II expression and were found to be negative. Coincubation of paraformaldehyde-fixed A431 and SCC4 cells with SEB and purified T cells for 5 days yielded an identical pattern of results with respect to T-cell proliferation (Fig. 4).

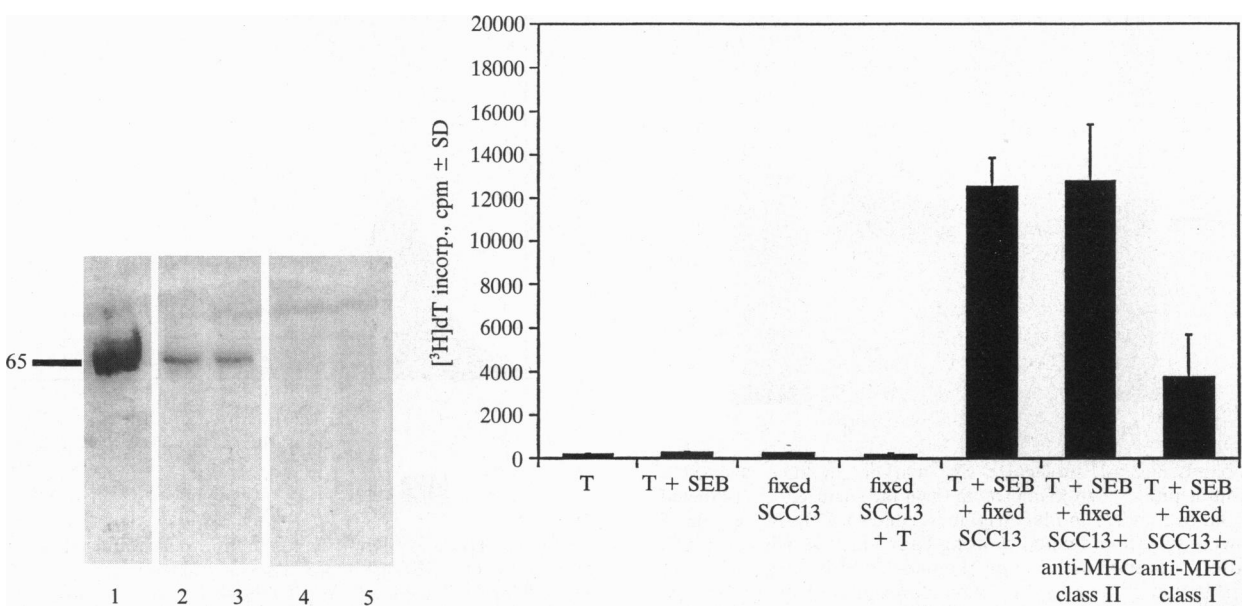


FIG. 3. (*Left*) Nonreducing Western blot of membrane isolates hybridized with an anti-class I mAb. Size is shown in kDa. Lanes: 1, B cells; 2 and 3, cultured melanocytes; 4 and 5, SCC13 cell line. (*Right*) Anti-class I, but not anti-class II, antibodies inhibit the antigen-presenting function of SCC13 cells for SEB. Bars indicate cpm of [3H]thymidine incorporation (incorp.) during the last 18 hr of a 5-day incubation period. Cpm are representative of two independent experiments.

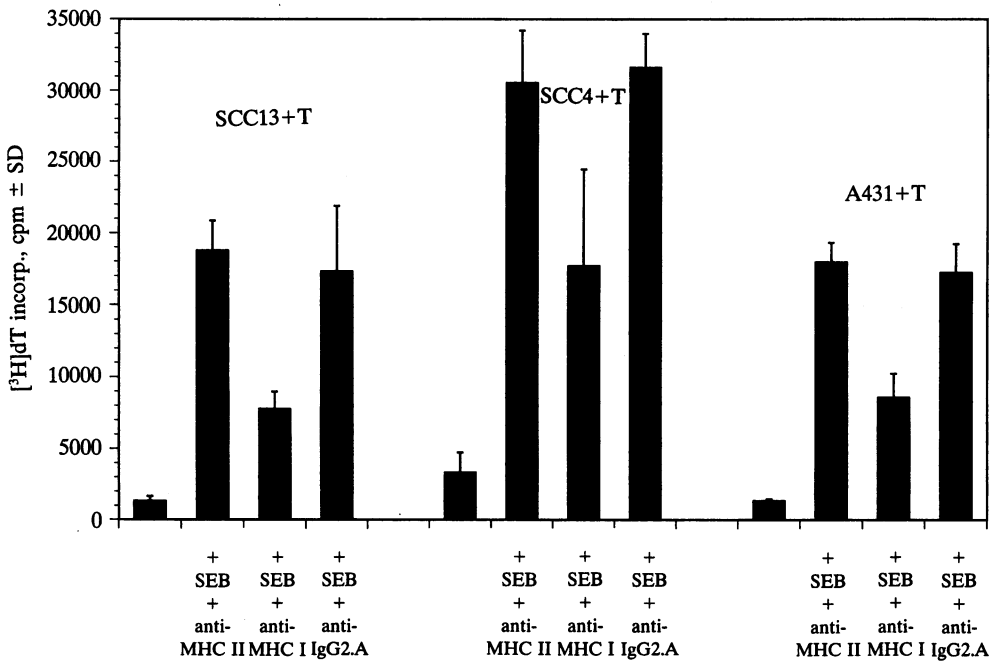


FIG. 4. Different epidermal tumor cell lines are able to present SEB to resting T lymphocytes. Bars indicate cpm of [³H]thymidine incorporation (incorp.) during the last 18 hr of a 5-day incubation period. Cpm are representative of three independent experiments.

**SEB Binds to a 46-kDa Membrane Protein on SCC13 Cells That Can Be Precipitated with a Monoclonal Antibody Against MHC Class I Molecules.** To further characterize the molecule to which SEB binds on MHC class II-negative cells, the surface of intact SCC13 cells was labeled with $^{125}I$. Following incubation with SEB for 1 hr at 37°C, membrane lysates were immunoprecipitated by using an anti-SEB antibody and were separated by PAGE under denaturing conditions. Autoradiographs consistently identified a single band at 46-kDa molecular mass (Fig. 5, lanes 1 and 2) that was not present when a non-cross-reacting control antibody was employed. Additional bands at 28 and 35 kDa—corresponding to the expected size of MHC class II $\alpha$ and $\beta$ chains—were detected in identically treated B-cell lysates (Fig. 5, lanes 3 and 4).

Because the molecular mass of the heavy chain of MHC class I is also in the 46-kDa range, studies were next conducted to

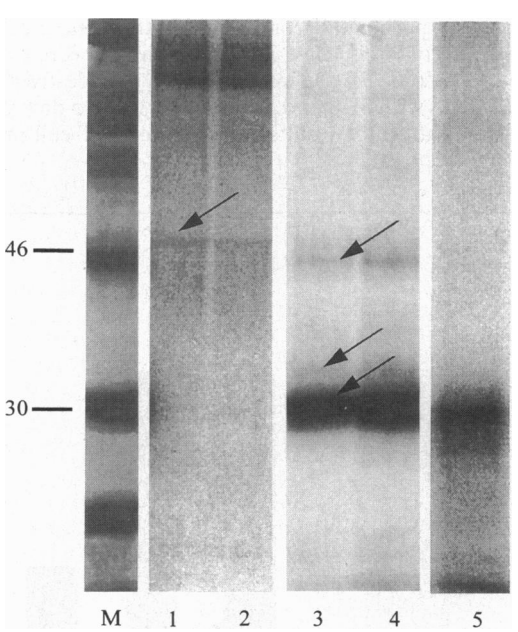


FIG. 5. Lanes: M, molecular size markers in kDa; 1–4, SEB immunoprecipitates a 46-kDa protein from the surface of $^{125}I$-labeled SCC13 (lanes 1 and 2) and B cells (lanes 3 and 4). Cells of each set ($5 \times 10^6$) were surface-iodinated by using Pierce Iodo-Beads and 1 mCi $^{125}I_2$. After incubation with 500 $\mu$g of unlabeled SEB, cells were lysed, and SEB-bound proteins were immunoprecipitated on protein A-Sepharose. In lane 5, the anti-MHC class I antibody W6/32 immunoprecipitates $^{125}I$-labeled SEB from membrane lysates of SCC13 cells. Proteins are separated on denaturing nonreducing 12% Tris glycine gels, and autoradiographs were developed after 48-hr exposure at −70°C on Kodak X-AR film.

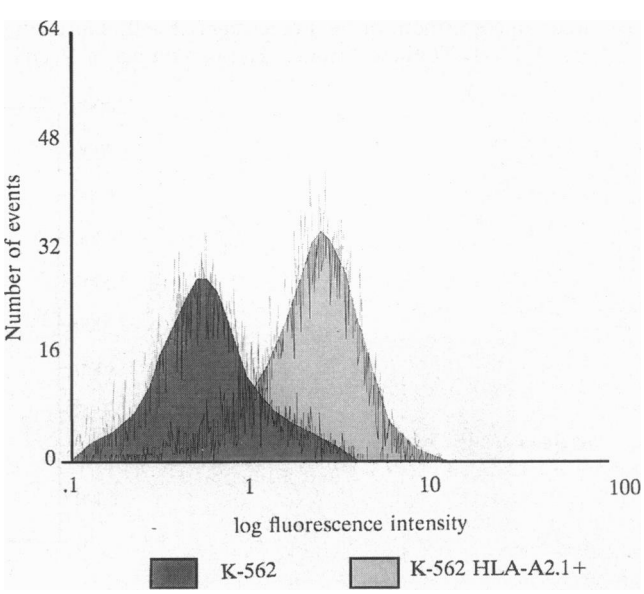


FIG. 6. HLA A2.1-transfected K-562 cells bind SEB. Surface expression of the SEB ligand was assessed by incubation of $1 \times 10^6$ cells with 0.5 mg of SEB for 1 hr at RT followed by an incubation step with polyclonal rabbit anti-SEB antibody and Cy5-labeled goat anti-rabbit mAb at 4°C. Controls included staining with an unrelated IgG antibody and the Cy5-labeled secondary antibody only. The fluorescence intensity of the stained samples was determined by flow cytometric analysis.

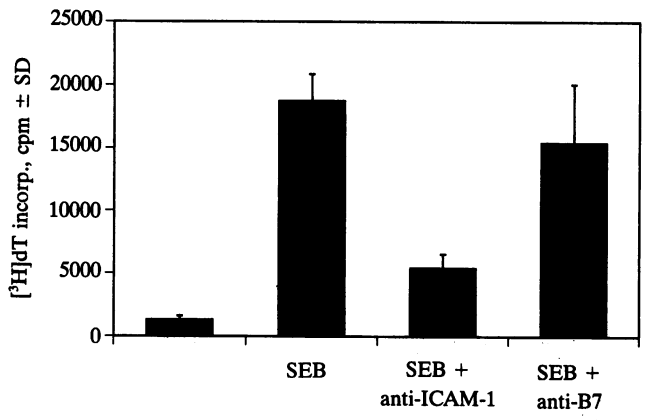


FIG. 7. Anti-ICAM-1 antibodies efficiently block presentation of SEB to resting T cells by epidermal tumor cells. T-cell proliferation is not significantly affected by neutralizing antibodies to the adhesion molecule B7. Bars indicate cpm of [$^3$H]thymidine incorporation (incorp.) during the last 18 hr of a 5-day incubation period. Cpm are average values of three independent experiments.

determine whether it was the ligand to which SEB bound on SCC13 cells. For this experiment, SCC13 cells were incubated with $^{125}$I-labeled purified SEB. The cells were lysed, and isolated membrane proteins were subjected to immunoprecipitation with a mAb directed against MHC class I molecules. After gel-electrophoretic separation, a single band at 28 kDa (Fig. 5, lane 5), equaling the molecular mass of SEB, was identified.

**MHC Class I-Expressing Cells Display Enhanced SEB-Binding Capacity Compared with Class I-Negative Controls.** In the next series of experiments, the K-562 erythroleukemia cell line, which is both class I- and class II-negative, was transfected with an expression vector containing cDNA for HLA-A2.1. The ability of these MHC class I-positive and class II-negative cells to bind SEB was compared to their class I- and class II-negative counterparts. The cells were first incubated with SEB, then stained with anti-SEB antibody and a fluoresceinated secondary reagent, and subjected to FACS analysis. In comparison with the parental K-562 population, HLA-A2.1-expressing K-562 cells displayed a 75% increased capacity to bind SEB on their surface (Fig. 6). The flow cytometric pattern of empty vector-transfected K-562 cells was identical to nontransfected cells (not shown).

**SEB-Induced T-Cell Activation by Squamous Cell Carcinoma Cells Is Inhibited by Anti-MHC Class I and Anti-ICAM-1 Antibodies.** The functional relevance of SEB bound to MHC class I on squamous cell carcinoma cell lines, as far as antigen presentation was concerned, was examined by adding purified, neutralizing anti-class I mAbs to T-lymphocyte proliferation assays containing purified T cells; class II-negative SCC13, SCC4, or A431 cells; and SEB. [$^3$H]Thymidine uptake by T cells was inhibited by >50% in cultures containing purified neutralizing anti-class I MHC mAbs compared with cultures to which the mAbs had not been added (Fig. 3 *Right*). A similar inhibitory effect of anti-class I antibodies was observed when paraformaldehyde-fixed SCC13 cells were preincubated with the antibody, washed, and then placed in culture with T cells and SEB (Fig. 4).

To assess the role of the adhesion molecules ICAM-1 and B7 in this system, purified neutralizing mAbs to those molecules were added to proliferation assays. [$^3$H]Thymidine uptake was inhibited by 60–70% in the presence of anti-ICAM-1 antibodies, whereas antibodies directed against B7 had little or no effect on T-cell proliferation (Fig. 7). It should be noted that the anti-B7 antibody we employed can bind to epidermal tumor cell lines (24) and can inhibit the antigen-presenting function of activated B cells.

## DISCUSSION

Superantigens, such as SEB, are exceptionally potent T-cell-activating agents that induce strong proliferative responses in lymphocytes belonging to distinct TCR V$\beta$ families (1, 25–27). Like most other immunological stimuli, superantigens require the presence of APC to mediate T-cell activation (1, 27–30) but bypass conventional antigen processing (1, 31). A number of studies have clearly demonstrated that many superantigens, including SEB, bind to the surface of APC via MHC class II molecules (32–36) and that this interaction plays a key role in T-cell activation. While not to deny its significance, several studies cast doubt that it is the only molecular pathway that allows T-cell activation to proceed. For example, staphylococcal enterotoxin A (SEA)- and SEB-induced lymphocyte activation can occur when class II-negative colon carcinoma cell lines are used as APC (37, 38). Moreover, MHC class II negative "knockout" mice retain their ability to elicit a T-cell proliferative response to selected staphylococcal enterotoxins (39). In both of these situations, the superantigen binding molecule has not been identified. We were prompted to address this issue by experiments that indicated squamous cell carcinoma lines lacking MHC class II molecules act as proficient APC for SEB-induced T-cell activation. Our studies show that MHC class I molecules on their surface bind the superantigen and enable them to serve as APC.

A band of 46-kDa molecular mass was consistently identified when SEB-bound SCC13 cell membrane extracts were immunoprecipitated with anti-SEB antibodies; radiolabeled SEB could be immunoprecipitated by anti-MHC class I antibodies; and increased SEB binding occurred in cell lines transfected with MHC class I determinants compared with their MHC class I- and class II-negative counterparts. In functional studies, addition of anti-MHC class I antibodies to assays with paraformaldehyde-fixed class II-negative SCC13 cells as the antigen-presenting population inhibited T-cell proliferation *in vitro*.

In contrast to our findings in various squamous cell carcinomas lines (SCC13, SCC4, and A431), normal keratinocytes have been shown to be poor APC for SEB (21, 40). This raises the question of why keratinocytes (and several other cell lines)—despite expression of MHC class I molecules—fail to act as superantigen-presenting cells. One possible explanation is that MHC class I molecules on malignant epidermal cells are structurally different from those on normal keratinocytes and permit increased binding of SEB to the tumor cell. If that were the case, then the finding that several epidermal cell lines are proficient at presenting superantigen to T cells would suggest that they all contain a common mutation in their class I molecule. An alternative explanation is that SEB binds equally well to normal keratinocytes and squamous cell carcinoma cell lines, but that expression of costimulatory signals on tumor cell lines but not on normal keratinocytes allows the former but not the latter cell type to activate T cells. Supporting the latter hypothesis, cultured keratinocytes—in contrast to all tumor cell lines investigated—lack adhesion molecules necessary for antigen presentation to T cells. Both ICAM-1 (41, 42) and B7 (43, 44) have been shown to be critical costimulatory signals in T-cell–target cell interactions. In our experiments, neutralizing antibodies to ICAM-1 had a substantial inhibitory effect on SEB-induced T-cell proliferation in our system. SCC13 cells and other malignant epidermal cell lines strongly express ICAM-1 on their surface (45, 46). Normal keratinocytes remain negative for this adhesion molecule, even under prolonged culture conditions (47, 48).

While the biological significance of SEB binding to class I MHC determinants for chronic diseases is unknown at this

time, our findings suggest that superantigens might be valuable immunotherapeutic agents for the management of squamous cell carcinoma of the skin and other tissues. Either local or systemic administration of SEB or other superantigens might act to encourage an antitumor T-cell response, which in turn would lead to tumor regression. Superantigens could also be used *ex vivo* to expand populations of tumor-infiltrating lymphocytes for tumor immunotherapy.

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