Staphylococcal Enterotoxin B Upregulates Expression of ICAM-1 Molecules on IFN-γ–Treated Keratinocytes and Keratinocyte Cell Lines

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The effects of staphylococcal enterotoxin B (SEB), a *Staphylococcus aureus*–derived bacterial superantigen, on expression of intercellular adhesion molecule-1 (ICAM-1) were examined in cultured normal and transformed (DJM-1 cells) human keratinocytes by flow cytometry, confocal microscopy, digital image processing, and reverse transcriptase–polymerase chain reaction.

SEB significantly upregulated ICAM-1 expression in the interferon-γ (IFN-γ)–pretreated, HLA-DR–positive normal keratinocytes and DJM-1 cells in a dose-dependent manner, but not in the untreated, HLA-DR–negative cells. Other toxins such as diphtheria and pertussis toxins did not have the effect.

The distribution of SEB and HLA-DR molecules was identical on the IFN-γ–treated, HLA-DR–positive DJM-1 cells by confocal microscopy. Digital image processing analysis demonstrated that SEB induced a transient increase of intracellular calcium concentration only in the IFN-γ–treated DJM-1 cells. Pretreatment of the IFN-γ–treated DJM-1 cells with anti–major histocompatibility complex class II monoclonal antibody completely blocked the effect of SEB. Furthermore, ICAM-1 mRNA was detected in the IFN-γ–pretreated, SEB-exposed normal keratinocytes by reverse transcriptase–polymerase chain reaction.

Our results demonstrate that SEB binds to keratinocytes, presumably *via* major histocompatibility complex class II molecules such as HLA-DR, triggers calcium mobilization, and induces the synthesis of ICAM-1 molecules. We speculate that, in various cutaneous disorders, SEB penetrates the epidermis and interacts with HLA-DR–positive keratinocytes to upregulate ICAM-1 expression, thus modulating the course of the inflammatory process. ***Key words: cell adhesion molecule/superantigen/signal transduction/cytokine.***
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Certain inflammatory skin diseases such as atopic dermatitis [1,2] and psoriasis [3] are frequently colonized by *Staphylococcus aureus*. *In vitro* studies have shown that bacterial superantigens such as staphylococcal enterotoxin B (SEB) and exfoliative toxin induce T-cell proliferation in the presence of Langerhans cells and interferon-γ (IFN-γ)–treated, major histocompatibility complex (MHC) class II–positive keratinocytes [4,5]. Cytokines released from these cells and regulated expression of adhesion molecules may in part be responsible for the proliferation, because binding of bacterial superantigens to MHC class II molecules [6] upregulates interleukin-1β and tumor necrosis factor-α (TNF-α) mRNA levels in antigen-presenting cells [7]. These observations suggest that bacterial superantigens penetrate the cutaneous lesions through defective skin barriers, thereby stimulating infiltrating T cells and modifying the course of the disease. However, direct influences of bacterial superantigens on keratinocytes have not been fully examined, except for one report showing that SEB augments the production of T-cell–activating cytokines by keratinocytes [5]. In addition, Nickoloff *et al* [4] suggested that non–MHC-class molecules on cultured human keratinocytes could bind superantigens.

In several cutaneous diseases, intercellular adhesion molecule-1 (ICAM-1) is one of the important adhesion molecules for cell-cell interactions [8] such as leukocyte trafficking [9–12]. Recently, Cornelius *et al* have shown the existence of both tissue- and cytokine-specific responsive elements in the 5′ flanking region of the ICAM-1 gene and the dependence on cellular context for regulatory effects by such elements [13]. In the present study, we examined the effect of SEB on ICAM-1 expression in cultured human keratinocytes. By flow cytometry, confocal microscopy, digital image processing, and reverse transcriptase–polymerase chain reaction, it was shown that SEB actually bound to MHC class II molecules on keratinocytes, mobilized intracellular calcium, and induced surface expression of ICAM-1. These results suggest that bacterial superantigens influence the outcome of cutaneous inflammatory disorders by modulating the expression of cell adhesion molecules on keratinocytes.

MATERIALS AND METHODS

Reagents and Antibodies SEB was purchased from Sigma Chemical Co., St. Louis, MO; biotinylated SEB from Toxin Technology Inc.,

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Abbreviations: PE, phycoerythrin; SEB, staphylococcal enterotoxin B.

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Sarasota, FL; fluorescein isothiocyanate (FITC)–conjugated monoclonal antibody (MoAb) 84H10 (anti-human ICAM-1, mouse IgG1) from Immunotech, Marseille, France; divalent anti-MHC class II MoAb (CR3/43) from DAKO A/S, Glostrup, Denmark; FITC-streptavidin, diphtheria toxin, pertussis toxin, epidermal growth factor, and whole bovine pituitary extract from Gibco BRL, Gaithersburg, MD; recombinant human IFN-γ and TNF-α from Genzyme Corp., Boston, MA; FITC-conjugated mouse IgG1 isotype control antibody, phycoerythrin (PE)–conjugated anti–HLA-DR MoAb (L243) and PE-conjugated mouse IgG2 isotype control antibody from Becton Dickinson, Mountain View, CA; non-labeled anti–HLA-DR MoAb (L243) from Leinco Technologies, Inc., Ballwin, MO; and anti-MHC class I MoAb (B9.12.1) and PE-conjugated anti-mouse IgG goat polyclonal antibodies from Cosmo Bio Co. Ltd., Tokyo, Japan.

Cell Culture DJM-1 cells, a human squamous cell carcinoma line originating from skin [14] (kindly provided by Dr. H. Yaoita, Jichi Medical School, Tochigi, and Dr. Y. Kitajima, Gifu University, Gifu, Japan) and normal human keratinocytes, derived from foreskins (Epipack, Clonetics Corporation, CA), were grown in serum-free keratinocyte growth medium (KC-SFM, Gibco BRL), supplemented with 10 ng/ml of epidermal growth factor and 60 ng/ml of whole bovine pituitary extract in 24-well flat-bottomed culture plates (Nunclon, Nunc, Denmark) in a humidified incubator with 5% CO_2 in air at 37°C. Cells for confocal microscopic analysis and measurement of intracellular calcium concentration were grown on cover glasses.

Because cytokine-mediated modulation of ICAM-1 expression is not different in transformed and normal human keratinocytes [15,16], DJM-1 cells were primarily used in the present study, and normal human keratinocytes were used only when specifically mentioned.

Induction of the ICAM-1 Molecule Cells at 90% confluency were refed with 1 ml of KC-SFM without epidermal growth factor or whole bovine pituitary extract to inhibit proliferation, treated with various concentrations of IFN-γ in the same medium for 48 h, washed in phosphate-buffered saline (PBS, 10 mM sodium phosphate, 133 mM sodium chloride, pH 7.4) to remove IFN-γ, and incubated with various concentrations of SEB for 48 h.

Conditioned Medium DJM-1 cells were pre-treated with 25 U/ml of IFN-γ for 48 h, washed with PBS, and further incubated with 600 ng/ml of SEB for 48 h. Equal volumes of the supernatant obtained from the culture and fresh KC-SFM were mixed and used as "conditioned medium." Although the conditioned medium contains 300 ng/ml of SEB, the biologic activity of SEB required for induction of T-cell proliferation (one of the most sensitive methods to evaluate the activity of SEB [4,5]) was lost during the 48-h incubation (data not shown).

Flow Cytometry Single-cell suspensions were prepared by treatment of the cultured cells with 0.25% trypsin and 1 mM ethylenediaminetetraacetic acid in PBS. After being washed with PBS, 10^5 cells were incubated with 5 μl of FITC-conjugated anti–ICAM-1 MoAb and 5 μl of PE-conjugated anti–HLA-DR MoAb (L243) or mouse isotype control antibodies for 30 min on ice. To analyze SEB binding to DJM-1 cells, we used a previously described method [17] with some modification. In brief, cell suspensions were prepared for two experiments. Cell suspensions were incubated with FITC-conjugated anti-ICAM-1 MoAb and PE-conjugated anti–HLA-DR MoAb (L243) as mentioned above. The other suspensions were incubated with 600 ng/ml of biotinylated SEB for 1 h and then with 2.5 μg/ml of FITC-streptavidin. Binding of SEB to HLA-DR molecules was blocked by the addition of 10 μg/ml of anti-MHC class II MoAb (CR3/43) or anti–HLA-DR MoAb (L243) before incubation of the cells with biotinylated SEB. The stained cells were analyzed in a FACScan (Becton Dickinson). Mean fluorescence intensity on the abscissa was expressed as a logarithm.

Confocal Microscopic Assessment of the Localization of SEB-Binding Sites DJM-1 cells were incubated with 25 U/ml of IFN-γ for 48 h, washed with PBS, and then incubated with 600 ng/ml of biotinylated SEB for 30 min in a CO_2 incubator at 37°C. After being washed with cold PBS, the cells were fixed with methanol at −20°C, air-dried, rehydrated with PBS, and incubated with 2.5 μg/ml of FITC-streptavidin and 5 μl of PE-conjugated anti–HLA-DR MoAb (L243) for 30 min. The specimens mounted in glycerin-PBS were observed in a Biorad MRC600 (Cambridge, MA) confocal laser scanning microscope equipped with an argon ion laser exciting maximally at 488 nm and 514 nm and operated under CoMoS software. Blue (BHS) and red (GHS) high-sensitivity filter blocks contained excitation filters 488 nm DF10 and 514 nm DF10, dichroic filters DR 510 nm LP and DR 540 nm LP, and emission filters 515 nm LP and 550 nm LP, respectively. All images were generated from a frame of 512 × 768 pixels.

Measurement of Intracellular Calcium Concentration DJM-1 cells were either untreated or pre-treated with 25 U/ml of IFN-γ for 48 h and then incubated with 20 μM acetoxymethylester of Fura-2 (Dojin Laboratories, Kumamoto, Japan) in phenyl red-free keratinocyte growth medium (Clonetics Corporation) in the dark for 1 h at 37°C. Following extensive washing, 600 ng/ml of SEB, 10 μg/ml of anti-MHC class II MoAb (CR3/43), or 10 μg/ml of anti-MHC class I MoAb was added to the cells in the same medium. Five minutes later the MoAb-treated cells were exposed to 600 ng/ml of SEB. Fluorescence intensity was measured using a digital image processing system, Argus-100/CA, designed by Hamamatsu Photonics Co. Ltd. (Hamamatsu, Japan).

Reverse Transcriptase–Polymerase Chain Reaction DJM-1 cells and normal human keratinocytes were incubated with 100 U/ml of IFN-γ for 48 h. Cells were washed with PBS and left in medium alone for 48 h to minimize the influence of IFN-γ and then incubated with 800 ng/ml of SEB. Total RNA was isolated 4 h later by the single-step guanidinium thiocyanate method of Chomczynski and Sacchi [18]. After lysing of cells in guanidinium thiocyanate, RNA was isolated by phenol chloroform extraction and ethanol precipitation. Total RNA (1 μg) was reverse transcribed to cDNA in the presence of 2.5 μM of random hexamers, 1 mM of deoxynucleotide triphosphates, and 2.5 U/μl of reverse transcriptase (all from Perkin Elmer Cetus, Norwalk, CT) and then amplified for 35 cycles with a GeneAmp RNA PCR kit (Perkin Elmer Cetus), according to the manufacturer's description. Each cycle consisted of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1.5 min. The sequences of primer pairs, 5′ and 3′, were as follows: ICAM-1, GTGACATGCAGCACC TCCTG and TCCATGGTGATCTCTCCTCA; and β-actin, GATTCCTATGTGGGCGACGA and GTGTCCATCACGATGCCAGT. The product size was 408 bp for ICAM-1 and 314 bp for β-actin. The PCR products and DNA molecular weight marker VI (Boehringer Mannheim GmbH Biochemica, Mannheim) were separated in 2% agarose gels.

Statistical Analysis Data from three independent experiments were analyzed with the Student t test.

RESULTS

IFN-γ Induces ICAM-1 and HLA-DR on DJM-1 Cells First, the time course and concentration dependency for the IFN-γ–induced expression of ICAM-1 and HLA-DR were determined. ICAM-1 was constitutively expressed on DJM-1 cells. IFN-γ at 25 U/ml promptly augmented ICAM-1 expression, which reached a maximum intensity after 24 h incubation and then gradually declined to approximately 60% of the peak intensity at 96 h (data not shown). Although not detected in untreated DJM-1 cells, the expression of HLA-DR was gradually induced with 25 U/ml of IFN-γ, peaked at 48 h incubation, and plateaued shortly thereafter with a minimal loss of the intensity by 96 h **(Fig 1*a*)**. In contrast to ICAM-1, HLA-DR was only weakly expressed at 24 h incubation. The intensity of both ICAM-1 and HLA-DR **(Fig 1*b*)** expression increased sharply up to concentrations of 50 U/ml IFN-γ and plateaued at concentrations up to 200 U/ml. These results were consistent with those previously described [19].

SEB Upregulates ICAM-1 Expression on IFN-γ–Treated Keratinocytes ICAM-1 was constitutively expressed on DJM-1 cells even when growth was inhibited by the depletion of epidermal growth factor and whole bovine pituitary extract from the medium **(Fig 2**, experiment *a*). SEB at 600 ng/ml did not augment ICAM-1 expression in cells that were not previously treated with IFN-γ **(Fig 2**, experiment *b*). When the cells were incubated with 25 U/ml of IFN-γ for 48 h and left untreated for an additional 48 h, ICAM-1 expression was still elevated **(Fig 2**, experiment *c*). When the IFN-γ–treated cells were incubated with 600 ng/ml of SEB for 48 h **(Fig 2**, experiment *d*), the fluorescence intensity was further increased when compared with the SEB-untreated cells **(Fig 2**, experiment *c*).

ICAM-1 expression on cells pre-treated with 25 U/ml of IFN-γ for 48 h was augmented even at 200 ng/ml of SEB, increased in a concentration-dependent manner, and plateaued at 600 ng/ml of SEB. Pretreatment of the cells with IFN-γ at 25 U/ml for 24 h instead of 48 h did not augment ICAM-1 expression in the presence of SEB, suggesting that relatively long-term incubation with IFN-γ

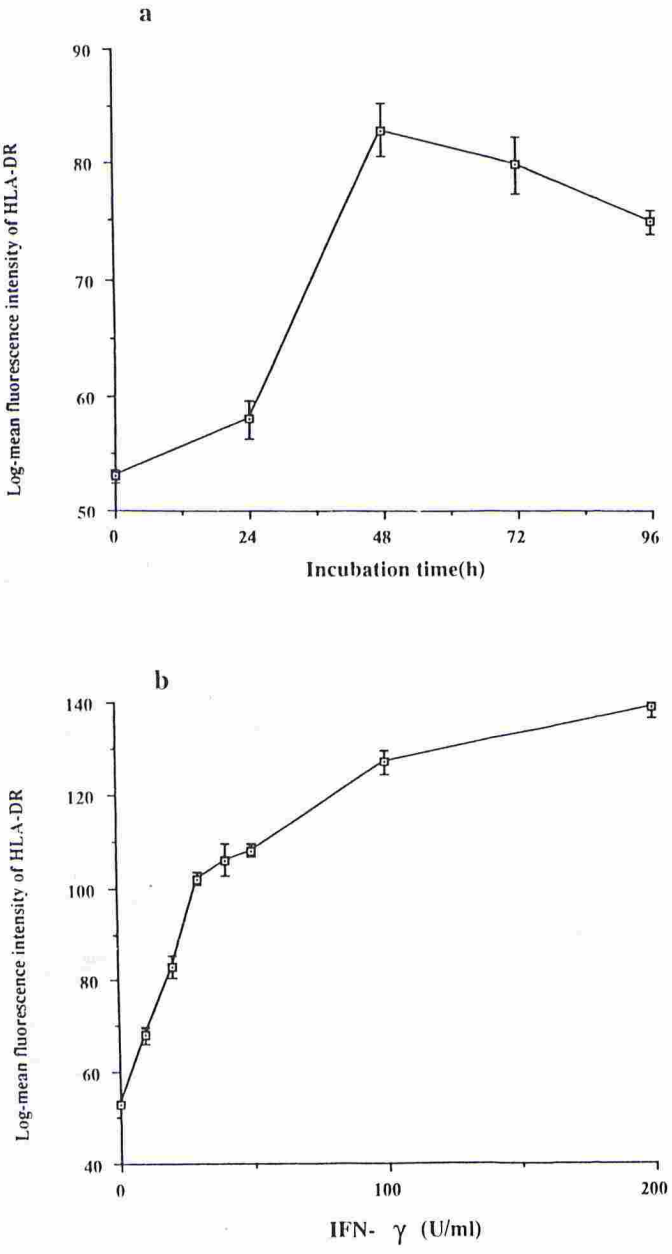

Figure 1. IFN-γ induces HLA-DR molecules on DJM-1 cells. Time course (*a*) and concentration dependence (*b*) of the induction of HLA-DR on DJM-1 cells by IFN-γ were determined by flow-cytometric analysis. Mean fluorescence intensity was expressed as the mean value ± SD from three independent experiments. 25 U/ml of IFN-γ was used in (*a*).

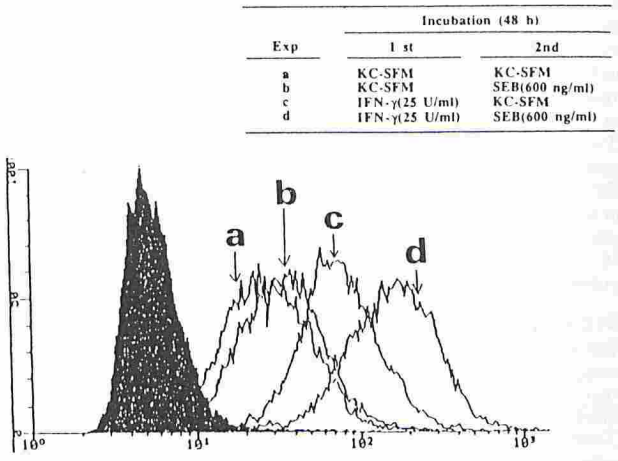

Figure 2. SEB upregulates ICAM-1 expression on IFN-γ–treated DJM-1 cells. The cell number is shown on the ordinate and mean fluorescence intensity (log scale) on the abscissa. The cells were incubated with IFN-γ (25 U/ml) or KC-SFM, washed, and then incubated with SEB (600 ng/ml) or KC-SFM. The *shaded area* represents an isotype-matched control.

was necessary for SEB-related augmentation of ICAM-1 expression. Neither diphtheria toxin nor pertussis toxin at concentrations between 200 and 600 ng/ml affected ICAM-1 expression on the IFN-γ–untreated and IFN-γ–treated DJM-1 cells (data not shown).

In normal human keratinocytes, neither ICAM-1 nor HLA-DR was constitutively expressed **(Fig 3)**. SEB alone did not induce ICAM-1 expression. ICAM-1 expression induced in cells incubated with 100 U/ml of IFN-γ for 48 h was significantly upregulated with exposure to 800 ng/ml of SEB for an additional 48 h ($p < 0.01$). Higher concentrations of IFN-γ and SEB were needed to induce and upregulate ICAM-1 expression in normal human keratinocytes than in DJM-1 cells.

SEB Binds to IFN-γ–Treated DJM-1 Cells To elucidate the binding of SEB to keratinocytes in association with HLA-DR molecules, we double stained DJM-1 cells with biotinylated SEB and PE–anti–HLA-DR MoAb (L243). We stained the cells with biotinylated SEB before fixation to preserve binding sites for SEB and then with PE–anti–HLA-DR MoAb (L243). This procedure was used because a previous report [20] and our preliminary study showed that L243 blocks SEB binding to HLA-DR molecules and because prior incubation with biotinylated SEB did not disturb anti–HLA-DR MoAb binding to DJM-1 cells. No immunofluores-

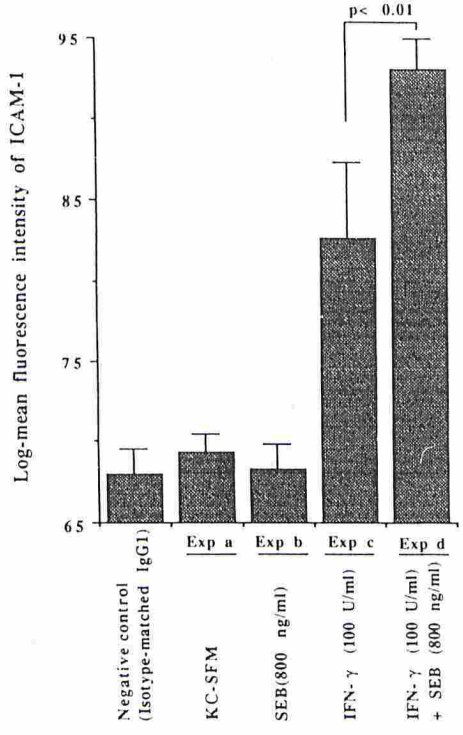

Figure 3. SEB upregulates ICAM-1 expression on IFN-γ–treated normal human keratinocytes. Experiments *a* to *d* were done as in **Fig 2**. Mean fluorescence intensity was expressed as the mean value ± SD from three independent experiments.

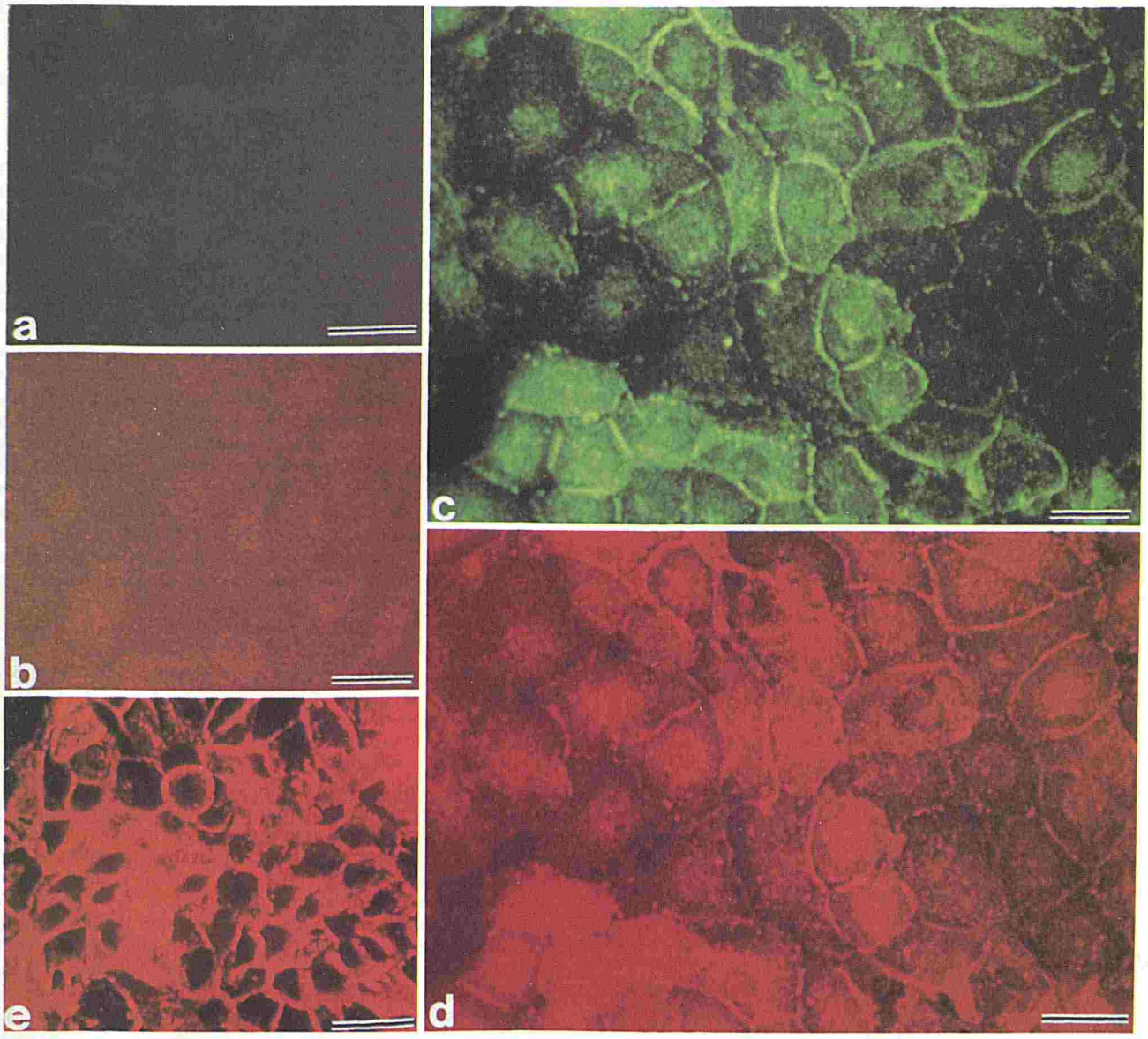

Figure 4. SEB and HLA-DR are identically distributed on DJM-1 cells. DJM-1 cells treated with (*c,d*) or without (*a,b*) IFN-γ (600 ng/ml) for 48 h were sequentially double stained with biotinylated SEB plus FITC-streptavidin and then PE–anti–HLA-DR MoAb. Neither SEB (*a*) nor HLA-DR (*b*) was detected on untreated cells. SEB (*c*) and HLA-DR (*d*) were detected on IFN-γ–treated DJM-1 cells. MHC class I molecules on IFN-γ–treated DJM-1 cells were visualized by anti–MHC class I MoAb plus PE-conjugated anti-mouse IgG (*e*). *Scale bar*, 20 μm (*a,b,e*) or 10 μm (*c,d*).

cence for SEB and HLA-DR molecules was observed in the untreated DJM-1 cells **(Fig 4*a,b*)**. On the other hand, both SEB and HLA-DR were observed in IFN-γ–treated DJM-1 cells **(Fig 4*c,d*)**. Staining patterns with the two molecules were indistinguishable **(Figs 4*c,d*)**. In contrast, an irrelevant mouse MoAb, anti–MHC class I, showed an intense and continuous staining pattern on IFN-γ–treated DJM-1 cells **(Fig 4*e*)**, apparently distinct from that of SEB and anti–HLA-DR MoAb. These results indicated the close, if not identical, binding sites of SEB and anti–HLA-DR MoAb.

We then compared the expression of ICAM-1 and HLA-DR as well as SEB binding to DJM-1 cells by flow cytometry **(Fig 5)**. The mean fluorescence intensity for SEB, HLA-DR, and ICAM-1 was significantly increased in the IFN-γ–treated DJM-1 cells when compared with the untreated DJM-1 cells. Because SEB is able to bind not only HLA-DR, but also other MHC-class II molecules such as HLA-DQ [20], and because IFN-γ induces both molecules on keratinocytes [21], we used not only anti–HLA-DR MoAb (L243) but also anti–MHC class II MoAb (CR3/43) as blocking antibodies for SEB binding to DJM-1 cells. Although both MoAbs significantly inhibited the SEB binding ($p < 0.01$) and CR3/43 inhibited SEB binding more effectively than L243, they did not inhibit SEB binding completely.

Because SEB is reported to stimulate keratinocytes to produce cytokines such as TNF-α [5], which is capable of inducing ICAM-1 expression on these cells, the effect of keratinocyte-derived soluble factors on ICAM-1 expression was examined. First, DJM-1 cells treated with 10 ng/ml of TNF-α neither expressed HLA-DR nor had an affinity for SEB despite the high level of expression of ICAM-1 **(Fig 5)**. In addition, SEB could not augment ICAM-1 expression on TNF-α–pretreated keratinocytes (data not shown). Second, the incubation of either IFN-γ–treated or untreated DJM-1 cells with conditioned medium for up to 48 h did not upregulate ICAM-1 expression. These findings confirmed that HLA-DR molecules were critical for SEB binding to keratinocytes and SEB-mediated upregulation of ICAM-1 expression on keratinocytes and also that

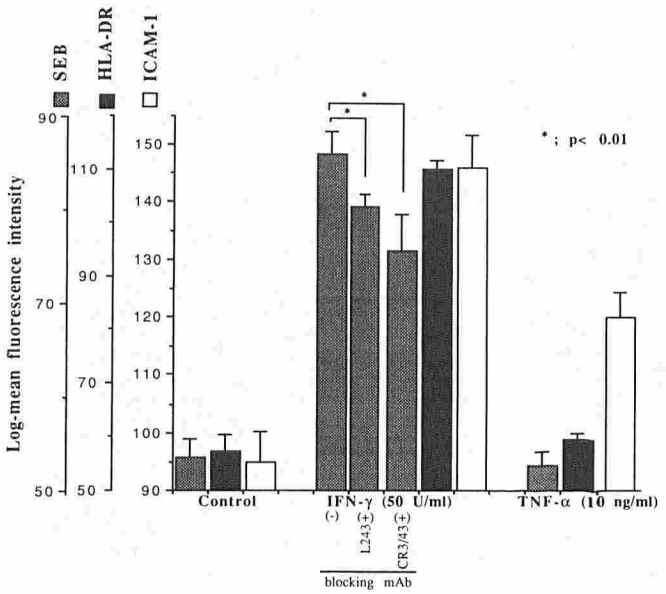

Figure 5. SEB binds to DJM-1 cells pre-incubated with IFN-γ but not TNF-α. After 48 h incubation with 50 U/ml of IFN-γ or 10 ng/ml of TNF-α, cell suspensions from the same well were divided into two samples. One sample was stained with biotinylated SEB (800 ng/ml) plus FITC streptavidin and the other was stained with FITC–anti–ICAM-1 MoAb and PE–anti–HLA-DR MoAb. Some IFN-γ–treated cells were incubated with anti–HLA-DR MoAb, L243 or anti–MHC–class II MoAb, CR3/43 before staining. Mean fluorescence intensity was expressed as the mean value ± SD from three independent experiments.

soluble factors present in the conditioned medium were not responsible for the upregulation.

SEB and Anti–MHC Class II MoAb Mobilize Intracellular Free Calcium We examined the effect of SEB on mobilization of intracellular calcium in DJM-1 cells, which was based on the idea that A23187, well known to increase intracellular free calcium, strongly augments ICAM-1 expression on keratinocytes [19]. In the cells that expressed HLA-DR molecules after 48 h exposure to IFN-γ, the addition of SEB at 600 ng/ml or anti–MHC-class II MoAb at 10 μg/ml led to a transient increase in the level of cytoplasmic free calcium (**Fig 6*a*,*b***, respectively). The levels of cytoplasmic free calcium in untreated DJM-1 cells were not altered by SEB (**Fig 6*a***) or anti–MHC class II MoAb (data not shown). Five minutes after calcium mobilization by the addition of anti–MHC-class II MoAb, the IFN-γ–pretreated cells did not respond further to the addition of SEB (**Fig 6*b***). Prior calcium mobilization by SEB also inhibited the effect of anti–MHC-class II MoAb (**Fig 6*c***). In addition, anti–MHC class I MoAb could neither mobilize intracellular free calcium nor disturb calcium mobilization induced by SEB (**Fig 6*d***). These results suggested that SEB triggered the calcium mobilization, possibly *via* binding to MHC–class II molecules.

ICAM-1 mRNA Is Induced by SEB Because ICAM-1 molecules were constitutively expressed on DJM-1 cells, ICAM-1 mRNA was detected by reverse transcriptase–polymerase chain reaction (data not shown) in all samples including non-treated, IFN-γ–treated (both short incubation for 4 h and long incubation for 48 h followed by medium alone for 52 h), and IFN-γ– and SEB-treated DJM-1 cells. Thus, it is difficult to compare mRNA levels between SEB-treated and non-treated DJM-1 cells by this method. Next, we used normal, ICAM-1–negative keratinocytes to investigate whether SEB induced ICAM-1 mRNA. As shown in **Fig 7**, mRNA for ICAM-1 was detected in IFN-γ–pretreated/SEB-stimulated (incubated with IFN-γ for 48 h and then left untreated for 48 h followed by the incubation with SEB for 4 h) cells (**Fig 7*b***), but not in IFN-γ–pretreated/SEB–non-stimulated (incubated with IFN-γ for 48 h and then left untreated for 52 h) cells (**Fig 7*c***) or control cells (**Fig 7*a***). In contrast, mRNA for ICAM-1 was detected in keratinocytes cultured with IFN-γ for 4 h (**Fig 7*d***). mRNA for β-actin was detected at the comparative level in all groups (**Fig 7*e–h***).

DISCUSSION

Our results demonstrate that SEB modulates the expression of ICAM-1 by binding to MHC class II molecules on keratinocytes, because 1) fluorescence images of the binding sites were virtually identical for SEB and anti–HLA-DR MoAb, 2) SEB mobilized intracellular free calcium in HLA-DR–positive but not in HLA-DR–negative keratinocytes, and 3) anti–MHC class II MoAb partially prevented binding of SEB to HLA-DR–positive keratinocytes, and completely blocked calcium mobilization by SEB. Because anti–HLA-DR MoAb (L243) and anti–MHC class II MoAb (CR3/43) bind to β-chain of HLA-DR, and because the amino acid sequence of the α-chain is critical for SEB binding to HLA-DR [22], partial inhibition of SEB binding by these MoAbs may have been due to steric hindrance but probably not to topographic overlapping of the binding sites. In addition, it is plausible that SEB binds to IFN-γ–treated transformed keratinocytes that had been pretreated with L243, which blocks HLA-DR molecules only, because of the binding ability of SEB for HLA-DQ molecules other than DR [20] and the induction of both molecules on keratinocytes by IFN-γ [21]. In contrast, MoAb obtained from clone CR3/43 reacts with the β-chain of all products of the gene subregions DP, DQ, and DR. Thus, CR3/43 blocks SEB binding to DJM-1 cells more efficiently than does L243.

Cross-linking of SEB by other reagents such as anti-SEB antibody was not necessary to mobilize calcium. The present data are inconsistent with the previous finding that calcium mobilization occurs only after cross-linking of SEB on the cell surface [23–25]. However, even without cross-linking, SEB activates protein tyrosine kinase and phosphoinositide-specific phospholipase C [26–28], which cleaves phosphoinositide into diacylglycerol [29] and inositol triphosphate, which in turn releases free calcium from the endoplasmic reticulum [30]. These observations suggest that monovalent binding of SEB to HLA-DR molecules induces calcium mobilization and signal transduction.

Alteration of adhesion molecules expression by SEB binding to the MHC class II molecule has also been previously shown in B cells. For example, SEB induces B cell–B cell adhesion [31] that is caused by qualitative changes of lymphocyte function-associated antigen-1 molecule expression without upregulation of ICAM-1 molecules. Thus, expression of adhesion molecules is differentially regulated in keratinocytes and B cells *via* the binding of SEB to MHC class II molecules.

At least three mechanisms could explain SEB-mediated upregulation of ICAM-1 expression in the IFN-γ–treated DJM-1 cells: 1) SEB binds to HLA-DR molecules and transduces signals that directly upregulate synthesis of ICAM-1 molecules, 2) SEB protects ICAM-1 molecules on the cell surface from degradation or shedding, and 3) SEB stimulates keratinocytes to secrete soluble factors, such as TNF-α, that upregulate ICAM-1 expression in an autocrine fashion. The first mechanism is most plausible because ICAM-1 mRNA was detected 4 h after the addition of SEB, during which time TNF-α was not detected by enzyme-linked immunosorbent assay in culture medium (unpublished observation). In addition, conditioned medium that might contain biologically active molecules did not augment ICAM-1 expression on IFN-γ–treated keratinocytes. This indicated that under our experimental conditions, soluble molecules were not responsible for upregulation of ICAM-1 expression on IFN-γ–treated keratinocytes. The second mechanism is also possible in the present study and not mutually exclusive with the first one. Levels of soluble ICAM-1 in culture medium could be measured to test this.

One of the critical immunologic and inflammatory events within the epidermis is binding of infiltrating leukocytes, such as T cells, monocytes, and possibly eosinophils, to epidermal keratinocytes

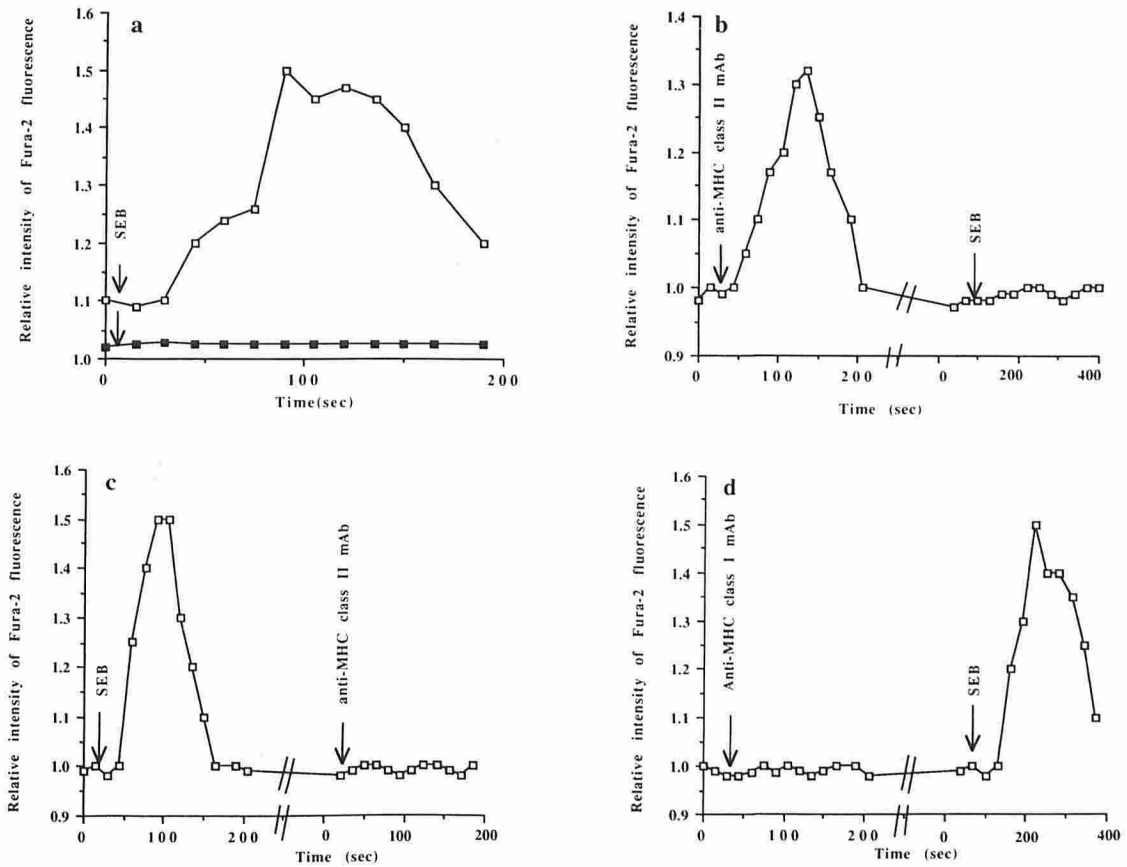

Figure 6. SEB and anti-MHC class II MoAb mobilize cytoplasmic free calcium. Fura-2–loaded DJM-1 cells were either untreated (*closed squares*) or pre-treated with 50 U/ml of IFN-γ for 48 h (*open squares*) and then incubated with 600 ng/ml of SEB (*a*). IFN-γ–pretreated cells were stimulated with 10 μg/ml of anti–MHC class II MoAb (*b*), 600 ng/ml of SEB (*c*), or 10 μg/ml of anti-MHC class I MoAb (*d*) and exposed to SEB (*b,d*) or anti–MHC class II MoAb (*c*) 5 min later.

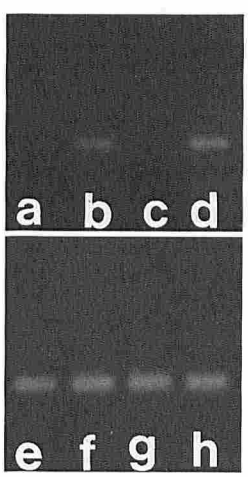

Figure 7. SEB induces ICAM-1 mRNA in IFN-γ–treated keratinocytes. Normal human keratinocytes were either untreated (*a,e*) or treated (*b,c,f,g*) for 48 h with 100 U/ml of IFN-γ. After a 48-h incubation without stimulant, medium was replaced with fresh KC-SFM containing either no stimulants (*a,c,e,g*) or 1 μg/ml of SEB (*b,f*) for an additional 4 h. Normal human keratinocytes without pretreatment were incubated with 100 U/ml of IFN-γ for 4 h as a control (*d,h*). Total RNA was isolated and 1 μg was subjected to reverse transcriptase–polymerase chain reaction using primers for ICAM-1 (*a–d*) or β-actin (*e–h*).

through the interaction of ICAM-1 and lymphocyte function-associated antigen-1 [32,33]. *In vitro* studies have shown that such interaction is a prerequisite for MHC class II–bearing keratinocytes to serve as accessory cells in the T-cell response to bacterial superantigens [4]. The present findings indicate that SEB facilitates the accessory cell function of keratinocytes by inducing and upregulating ICAM-1 expression. Superantigens from superficially colonized *Staphylococcus aureus* may penetrate viable epidermal layers through the disrupted skin barrier, augmenting and prolonging inflammation in various cutaneous diseases by upregulating ICAM-1 expression of keratinocytes. Furthermore, chronic exposure to exogenous superantigens induces profound deletion of reactive mature T cells [34], thus downregulating cutaneous inflammation.

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