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J Immunol (1991) 146 (3): 826–832.
https://doi.org/10.4049/jimmunol.146.3.826

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0022-1767/91/1463-0826$02.00/0
THE JOURNAL OF IMMUNOLOGY
Vol. 146, 826–832, No. 3, February 1, 1991
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MECHANISM OF *Staphylococcus aureus* EXOTOXIN A INHIBITION OF Ig PRODUCTION BY HUMAN B CELLS[1]

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Staphylococcus enterotoxins and toxic shock syndrome toxin 1 are members of a family of exoproteins that are produced by staphylococci and bind specifically to MHC class II molecules. Upon binding to MHC class II molecules, these exoproteins are potent stimulators of T cell proliferation via interaction with specific TCR V-β segments of both $CD4^+$ and $CD8^+$ T cells. These exoproteins also directly stimulate monocytes to secrete IL-1 and TNF-α. Furthermore, these exoproteins have a profound inhibitory effect on Ig production by PBMC. We examined the effects of _Staphylococcus_ enterotoxin A (SEA) on proliferation and Ig production of highly purified human B cells. Our results demonstrated that the binding of SEA to MHC class II molecules on B cells does not alter their ability to proliferate in response to _Staphylococcus aureus_ Cowan strain I (SAC) or to produce Ig in response to SAC plus rIL-2. In contrast, the anti-DR mAb L243 inhibited both B cell proliferation and Ig production. Unable to determine a direct effect of SEA on B cell function, we investigated whether the capacity of SEA to inhibit SAC-induced Ig production by PBMC was T cell-dependent. Our results demonstrated that in the presence of T cells, under appropriate conditions, SEA can either function as a nominal Ag for stimulation of B cell proliferation and Ig production or induce T cell-mediated suppression of Ig production. SEA-induced Ig production required T cell help, which was dependent on pretreatment of the T cells with irradiation or mitomycin C; Ig production was not induced by SEA in the absence of T cells or in the presence of untreated T cells. Furthermore, SEA inhibited Ig production in SAC-stimulated cultures of autologous B cells and untreated T cells; pretreatment of the T cells with irradiation or mitomycin C abrogated SEA-induced inhibition of Ig production. Thus, T cell suppression of SAC-induced Ig production was dependent on T cell proliferation. Similar results were observed with both SEA and toxic shock syndrome toxin 1.

SE[3] (A, B, C1-3, D, and E), TSST-1, and exfoliating toxins A and B belong to a family of exoproteins produced by various strains of *Staphylococcus aureus* (1). TSST-1, exfoliating toxins, and SE, respectively, are the causative agents in toxic shock syndrome, the scalded skin syndromes, and most cases of food poisoning, as well as some cases of shock (1, 2). SE and TSST-1 are also members of a group of proteins that bind specifically to MHC class II proteins (3–5) and induce proliferation of both $CD4^+$ and $CD8^+$ T cells by interaction with specific V β segments (6–8).

Of the *Staphylococcus* exoproteins, SE are the most potent stimulators of T cell mitogenesis, resulting in increased production of IL-2 and IFN-γ which may contribute to the symptoms found with food poisoning (9–11). Increased IL-2R expression, induction of IL-1 and TNF-α and increased NK activity have also been demonstrated by human PBMC cultured with SE or TSST-1 (11–16). Recent studies have demonstrated the capacity of SE or TSST-1 to induce purified monocytes to produce IL-1 and TNF-α as a consequence of toxin binding to MHC class II molecules (17). In addition, the immunomodulatory effects of SE include profound inhibition of mitogen and Ag-induced Ig-forming cells by murine splenocytes, both in vivo and in vitro (18–20). Inhibition of Ig production by PWM-stimulated human PBMC was observed with TSST-1 (21).

B cells constituitively express MHC class II molecules on their surface, and anti-MHC class II antibodies typically inhibit B cell proliferation and differentiation (22). Therefore, we investigated whether inhibition by *Staphylococcus* exoproteins of Ig production by PBMC was the direct consequence of their binding to MHC class II molecules on B cells. Our results demonstrated differential effects of SEA and anti-DR (L243) mAb when bound to class II molecules on highly purified B cells. Furthermore, SEA and TSST-1 induced reciprocal helper or suppressor T cell effects on B cell differentiation, depending on the capacity of the T cells to proliferate.

MATERIALS AND METHODS

Reagents and antibodies. SEA and TSST-1 were purchased from Toxin Technology (Madison, WI). Formalinized SAC was a gift from Dr. R. Rossen (Baylor College of Medicine). mAb L243 (anti-HLA-DR), OKT11 (anti-CD2), OKT4 (anti-CD4), OKT8 (anti-CD8), OKM1 (anti-CD11b), anti-HNK (anti-CD57), HB57 (anti-μ), and HP-6045 (anti-γ) were obtained as hybridomas from the American Type Culture Collection and affinity purified from mouse ascites or culture supernatants. FITC-conjugated anti-HLA-DR (L243), anti-CD20,

Received for publication July 10, 1990.
Accepted for publication October 29, 1990.
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[1] This work was supported in part by United States Public Health Service grants AI24664 and AI21289.
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[3] Abbreviations used in this paper: SE, *Staphylococcus* enterotoxin; TSST-1, toxic shock syndrome toxin 1; SAC, *Staphylococcus aureus* Cowan strain I; PE, phycoerythrin; SEA, *Staphylococcus* enterotoxin A; HPO, horseradish peroxidase; SEB, Staphylococcus enterotoxin B.

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anti-M3 (CD14), anti-Leu11 (CD16), and PE-conjugated anti-IL-2R (CD25) were obtained from Becton Dickinson. Anti-CD3-FITC was obtained from Coulter.

Biotinylation of SEA. Biotinylation was performed by treating 0.25 mg of SEA in carbonate buffer (ph 8.0) with a threefold excess of biotinyl-E-aminocapryl *N*-hydroxysuccinimide ester (Biotin-X-NHS; Calbiochem) plus 0.128 mg of *N,N*-dimethyl foramide (Aldrich) for 2 h on ice. The reaction was stopped with the addition of 25 μl of 1 M ammonium chloride. The biotinylated SEA was then dialyzed against 0.01 M PBS with three 1-liter exchanges to remove the excess biotin.

Cell preparations. PBMC were isolated from heparinized blood of healthy volunteers or from buffy coats of units of blood donated by healthy volunteers (Gulf Coast Regional Blood Center, Houston TX) by centrifugation over Ficoll-Hypaque (Pharmacia) as described previously (23). Purified B cells were obtained by positive selection with anti-CD19-coated immunomagnetic beads (Dynal) as has been described recently (24). Briefly, PBMC were incubated with anti-CD19-coated beads for 30 min at 4°C. An iron magnet was applied for 2 min and the supernatant decanted, leaving the B cells attached to the anti-CD19-coated magnetic spheres. The B cells were gently resuspended and again the magnet was applied for 2 min and the supernatant removed. This procedure was repeated three more times and after the final wash, the cells were resuspended and placed in a 37°C, 5% CO_2, humidified atmosphere to allow for disassociation of the B cells from the magnetic beads. After 16 to 18 h, the cells were vigorously resuspended, the magnet applied, and after 2 min, the supernatant containing the B cells was collected. The remaining beads were vigorously washed an additional four times. To eliminate the rare residual non-B cells, the B cells were incubated with anti-CD2, anti-CD4, anti-CD8, anti-CD11b, and anti-CD57 mAb and baby rabbit complement (Pelfreeze) for 45 min at 37°C. Viability of the highly purified B cells, as measured by Trypan blue dye exclusion, was 86 to 91%. T cells were purified from the PBMC depleted of B cells by anti-CD19 beads. Monocytes were partially depleted by plastic adherence for 1 h at 37°C. The nonadherent cells were incubated at 4°C for 2 h with 1-aminoethylisothiouronium bromide-treated SRBC, then centrifuged over Ficoll-Hypaque. The SRBC were lysed with hypotonic ammonium chloride, and the E-rosette$^+$ cells were again incubated 2 h at 4°C with SRBC, then centrifuged over Ficoll-Hypaque. The SRBC were lysed and E-rosette$^+$ cells were incubated with L243, anti-CD11b, and anti-CD57 mAb, and baby rabbit complement to remove residual non-T cells. Purity of the B and T cells was assessed by flow cytometry and functional studies. B cells were found to be >98% CD20$^+$ and no CD3$^+$, CD14$^+$, or CD16$^+$ cells could be identified by flow cytometry. The B cells proliferated to the B cell mitogen, SAC, but not to Con A or plastic bound anti-CD3 mAb plus PMA (Sigma). The T cell population was found to be >97% CD3$^+$ with <1% CD20$^+$, <1% CD14$^+$, and <1% CD16$^+$ cells by flow cytometry. The T cells did not proliferate to SAC, but did proliferate to plastic bound anti-CD3 plus PMA. Flow cytometry was performed on an EPICS C (Coulter) or an EPICS Profile II (Coulter).

Cell culture. All cultures were carried out in triplicate in 96-well, round-bottomed microtiter plates (Linbro). Cells were incubated in 200 μl of culture media containing RPMI 1640, with 10% FCS, 2 mM L-glutamine, and 10 μg/ml gentamicin at 37°C in a 5% CO_2 humidified atmosphere. SAC was added to cultures at a concentration of 0.01% (v/v) for optimum B cell proliferation and Ig production, unless otherwise noted. TSST-1 was added at 1 μg/ml. Concentrations of L243 and SEA varied in different experiments, and exact doses are noted for each experiment.

Proliferation studies. Cells (10^5 B cells or 10^5 B and 10^5 T cells) were cultured for 54 h and then 1 μCi of [^{3}H]thymidine (Amersham) was added to each of the wells. The cells were harvested 18 h later onto glass filter paper, and the [^{3}H]thymidine incorporation was determined by β-spectrophotometry. In some of the experiments, T cells were treated with mitomycin C (50 μg/ml; Sigma) for 45 min and then washed extensively before co-culture with the B cells. In other experiments, the T cells or B cells were γ-irradiated with 3000 R before culture.

Ig production. PBMC (2 × 10^5), 10^5 B cells, or 10^5 B cells and 10^5 T cells were cultured for 10 days. In some studies, T cells were mitomycin C-treated or irradiated. In cultures with PBMC or B and T cells, SAC was added as the stimulus for Ig production; for coculture experiments, preliminary studies demonstrated optimum Ig production at a one-to-one ratio of B cells and T cells. In those cultures containing only purified B cells, 100 U/ml rIL-2 (Amgen) was added in addition to SAC to stimulate Ig production. Supernatants were harvested on day 10 for determination of Ig concentrations by isotype-specific ELISA.

Cell surface receptor analysis. Binding studies with biotinylated SEA and anti-HLA-DR-FITC were carried out at 4°C on freshly isolated B cells at saturating doses of both compounds. The capacity for SEA or L243 mAb to modulate IL-2R expression was determined on B cells cultured for 48 h with saturating concentrations of SEA or L243 mAb.

Ig isotype-specific ELISA. Microtiter plates (Titertek) were coated with 10 μg/ml of affinity-purified anti-μ or anti-γ mAb, or affinity-purified goat anti-human IgA (Calbiochem) in 0.05 M Tris and incubated overnight at 4°C. The plates were washed once with PBS and Tween and blocked with 10% FCS in PBS and Tween for 1 h at room temperature. Plates were washed once and then incubated with the serial dilutions of culture supernatants, or serial dilutions of known concentrations of affinity-purified IgG, IgM, or IgA, for the standard curve, for 2 h at 37°C. The plates were washed five times and HPO-conjugated, affinity-purified goat anti-human IgM and IgA antibodies (Calbiochem) were added at a 1:10,000 dilution. Murine anti-human IgG F(ab′)$_2$ (heavy and light chains)-HPO (Jackson Laboratories) was added at a 1:10,000 dilution, and all plates were incubated overnight at 4°C. Substrate for the HPO was made by adding 100 μl of a 10 mg/ml solution of tetramethylbenzidine (Sigma) in DMSO to 10 ml of 0.5 M sodium acetate (pH 6.0) and 3.3 μl of concentrated hydrogen peroxide. The plates were washed extensively, the substrate was added, and the reaction was stopped at 10 min with the addition of 4 N sulfuric acid. The plates were read at 450 nm wavelength (Titertek, Multiskan) and the amount of Ig determined by comparison with the standard curve with the use of the Titersoft program.

RESULTS

Effects of SEA or anti-DR mAb (L243) on Ig production by SAC-stimulated PBMC. The ability of SEA to inhibit in vitro production of Ig was demonstrated in SAC-stimulated 10-day cultures of unfractionated human PBMC. IgG, IgM, and IgA concentrations in culture supernatants were determined by isotype-specific ELISA. Increasing doses of SEA added at the initiation of the cultures inhibited Ig production in a dose-dependent manner (Fig. 1). At 1 pg/ml of SEA, the inhibition was almost maximal. IgG and IgA production were completely inhibited, whereas IgM production was decreased by 87%. Since SEA binds specifically to MHC class II molecules, we studied the potential for the anti-DR-mAb L243 to also inhibit SAC-induced Ig production by PBMC. As with SEA, L243 mAb inhibited IgG, IgM, and IgA production, with maximal inhibition at 5 μg/ml of L243 mAb (data not shown). Ig production was maximally inhibited when SEA was added within the first 24 h of culture (Fig. 2). Similar inhibition kinetics were observed with L243 mAb (data not shown).

Comparison of effects of SEA and anti-HLA-DR mAb (L243) on proliferation of purified B cells. We next investigated whether inhibition by SEA and L243 mAb of

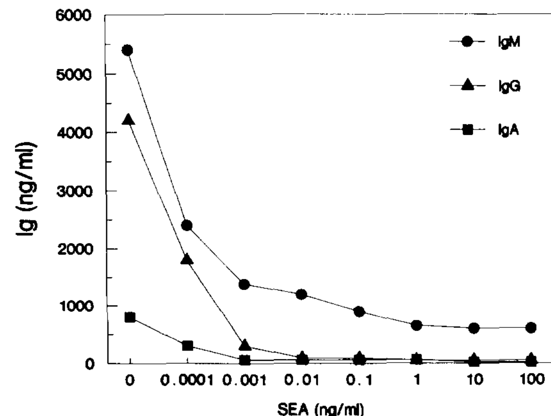

Figure 1. SEA inhibition of Ig production by PBMC. PBMC (2 × 10^5 per well) were cultured with 0.01% SAC and increasing concentrations of SEA. Supernatants were collected on day 10 and analyzed by isotype-specific ELISA. Cultures were performed in triplicate. The SEM for each data point was less than 10% of the mean.

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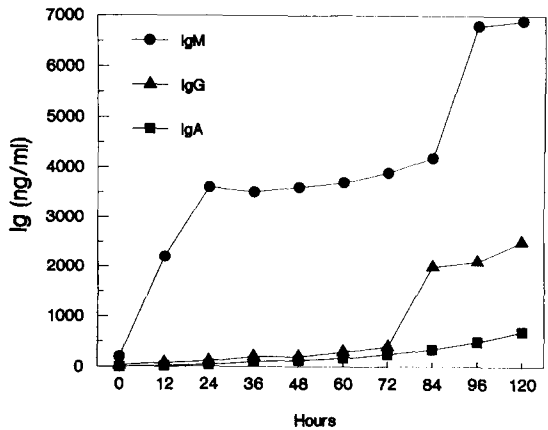

Figure 2. Kinetics of SEA inhibition of Ig by PBMC. SEA (2500 ng/ml) was added to SAC-stimulated PBMC at 12-h intervals. Maximal inhibition occurred when SEA was added within the first 24 h. Cultures were performed in triplicate. The SEM for each data point was less than 10% of the mean.

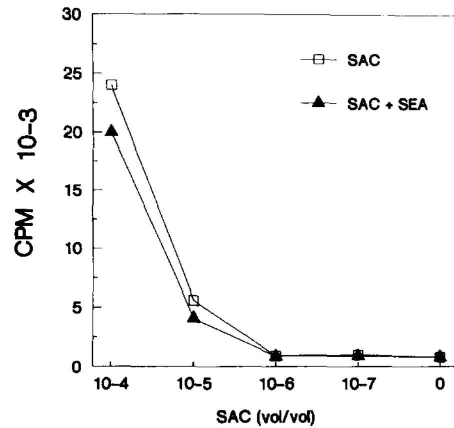

Figure 4. Effects of SEA on submaximal SAC stimulation of B cells. Submaximal concentrations of SAC were added to 10^5 B cells with 2500 ng SEA/ml. [^{3}H]Thymidine incorporation was measured at 72 h. Cells were cultured in triplicate. The SEM for each data point was less than 10% of the mean.

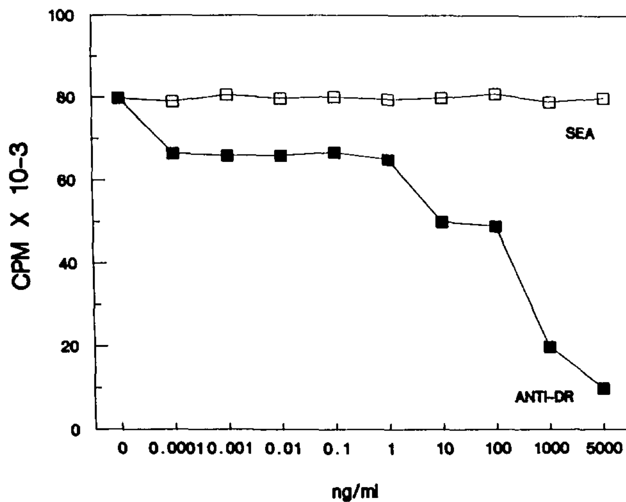

Figure 3. SEA vs anti-DR mAb (L243) effects on SAC-induced proliferation. Increasing concentrations of either SEA or anti-HLA DR mAb (L243) were added to 10^5 purified B cells stimulated with 0.01% SAC. Cells were pulsed with [^{3}H]thymidine for the last 16 h of culture and harvested at 72 h. Cells were cultured in triplicate. The SEM for each data point was less than 10% of the mean.

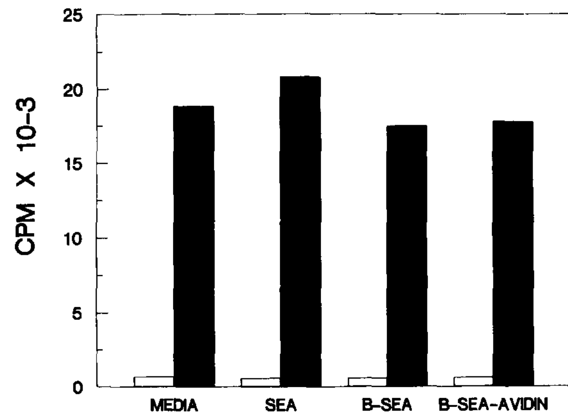

Figure 5. Role of cross-linking. SEA (2500 ng/ml), a saturating concentration of biotinylated SEA (*B-SEA*), or biotinylated SEA conjugated to avidin were added to 10^5 untreated (*open bars*) or SAC (0.01%)-stimulated B cells (*solid bars*). [^{3}H]Thymidine uptake was measured at 72 h. Cells were cultured in triplicate. The SEM for each data point was less than 10% of the mean.

Ig production by PBMC was the consequence of a direct effect on B cells. Highly purified peripheral blood B cells were initially studied for the ability of SEA or L243 mAb to inhibit SAC-induced proliferation. L243 mAb demonstrated a dose-dependent inhibition of SAC-stimulated proliferation (Fig. 3). In contrast, the addition of SEA to SAC-stimulated purified B cells did not inhibit or enhance the proliferative response (Fig. 3). L243 mAb also inhibited the SAC-induced increase in B cell IL-2R expression as determined by flow cytometry with anti-CD25 mAb, whereas SEA did not alter IL-2R expression induced by SAC (data not shown). Since the 0.01% dose of SAC used in the above experiments gives a maximal proliferative signal for B cells at 72 h, we further examined the potential for SEA to enhance B cell proliferation in response to submaximal doses of SAC. There was no direct stimulation of B cell proliferation by SEA alone, nor was there enhancement of proliferation with a submaximal SAC stimulus (Fig. 4). We next examined the possibility that the difference in inhibitory effect between SEA and L243 mAb was due to the ability of the anti-DR antibodies to cross-link molecules on the surface of the B cells, whereas the SEA might not. To achieve SEA cross-linking, SEA was biotinylated and used, at a concentration in severalfold excess of that which gave maximum B cell binding, in conjunction with avidin. The biotinylated SEA was not altered functionally by the biotinylation process as was demonstrated by its ability to bind to B cells but not T cells as assessed by flow cytometry with streptavidin-PE and by its ability to stimulate proliferation of T cells cultured with irradiated (3000 R) B cells (data not shown). No significant difference in SAC-induced B cell proliferation was observed when the biotinylated SEA was cross-linked with avidin (Fig. 5), suggesting that the functional differences observed between SEA and L243 mAb were not due to differences in their potential to cross-link HLA-DR molecules.

Effects of SEA and anti-DR antibody on Ig production by purified B cells. Ig production by purified B cells was induced by SAC plus 100 U/ml of rIL-2. When SEA was added to the cultures, at concentrations up to 10^6 times greater than that required to inhibit Ig production by PBMC, no inhibition of Ig production was observed. In contrast, L243 mAb at 5 μg/ml inhibited the production of IgG, IgM, and IgA by 82 to 85% (Fig. 6).

Lack of competitive effects of SEA and anti-DR anti-

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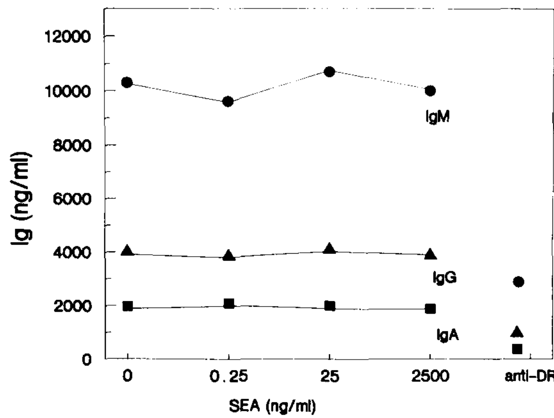

Figure 6. SEA and anti-DR mAb (L243) effects on Ig production by purified B cells. B cells (10^5) were cultured with 0.01% SAC, 100 U rIL-2/ml and increasing doses of SEA or 2500 ng anti-DR/ml. Supernatants were harvested on day 10 and analyzed by isotype-specific ELISA. Cells were cultured in triplicate. The SEM for each data point was less than 10% of the mean.

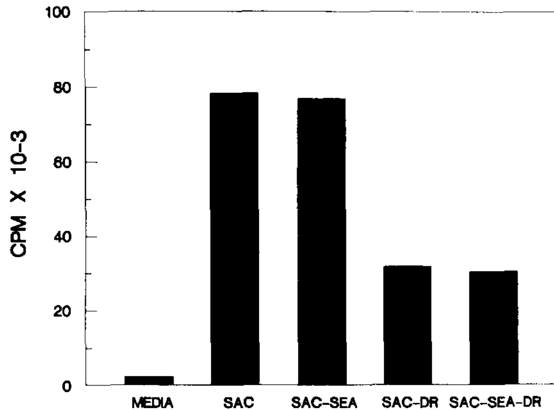

Figure 7. Lack of functional competition between SEA and anti-DR mAb (L243). SEA (5 μg/ml) and anti-DR mAb (5 μg/ml) were either added alone or in combination to SAC (0.01%) stimulated B cells. [^{3}H]Thymidine incorporation was measured at 72 h. Cells were cultured in triplicate. The SEM for each data point was less than 10% of the mean.

body binding on purified B cells. We next investigated the potential for SEA to prevent the inhibitory effects of L243 mAb on B cells. In functional competition studies, supersaturating concentrations of SEA (5 μg/ml) were unable to alter the L243 mAb-induced inhibition of SAC-stimulated B cells (Fig. 7). Pre-incubation of the cells with SEA for 1 h before the addition of the L243 mAb also did not abrogate the inhibitory effect of the anti-DR mAb (data not shown). Additional evidence that SEA and L243 mAb do not compete for binding epitopes was demonstrated by flow cytometry. Saturating concentrations of biotinylated SEA + strepavidin-PE and L243 mAb-FITC did not competitively inhibit binding of either molecule to the surface of purified peripheral B cells (Fig. 8, *a* to *c*). Preincubation for 1 h, at 4°C or 37°C, with either molecule did not alter the binding pattern.

Role of SEA in T cell-dependent model of Ig production. In light of the recent demonstration that TSST-1 could function as a nominal Ag to induce B cells to proliferate and differentiate into Ig-producing cells in a cell surface contact-dependent manner with nonproliferating (irradiated) T cells (25), we investigated a similar potential for SEA. We confirmed the previous report with respect to TSST-1 (data not shown), and similarly found that irradiated or mitomycin C-treated T cells cultured

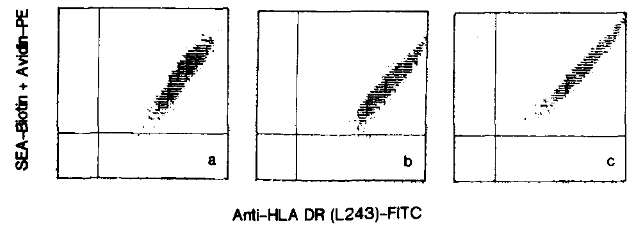

Figure 8. Competitive binding of SEA and anti-DR mAb (L243). Saturating concentrations (5 μg/ml) of biotinylated SEA-streptavidin PE and anti-DR FITC were added to purified B cells for 1 h (*a*). Biotinylated SEA-streptavidin PE was added 1 h before anti-DR FITC (*b*). Anti-DR FITC was added 1 h before addition of biotinylated SEA-streptavidin (*c*).

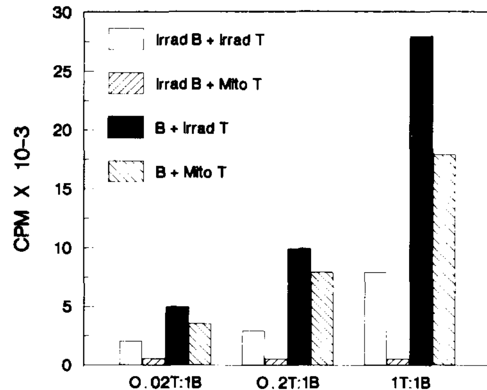

Figure 9. SEA effects on B cell proliferation in the presence of inactivated T cells. DNA synthesis was measured at 72 h in cultures containing 1000 ng SEA/ml and 10^5 untreated or irradiated (3000 R) B cells with varying ratios of irradiated (3000 R) or mitomycin C-treated T cells. Cells were cultured in triplicate. The SEM for each data point was less than 10% of the mean.

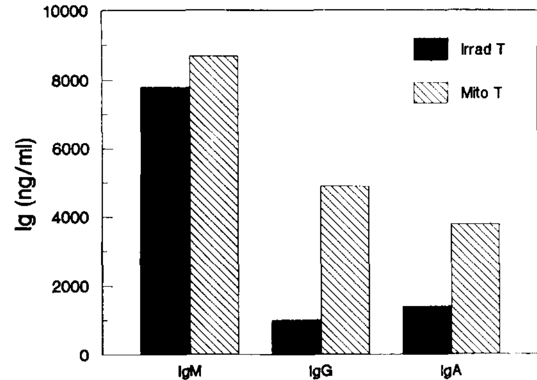

Figure 10. SEA effects on Ig production in the presence of inactivated T cells. Ig production was measured after 10 days from supernatants of 10^5 B cells cultured with 1000 ng SEA/ml and 10^5 irradiated or mitomycin C-treated T cells. Cells were cultured in triplicate. The SEM for each data point was less than 10% of the mean.

with purified B cells in media containing SEA resulted in substantial B cell proliferation (Fig. 9) and Ig production (Fig. 10). It is of interest that irradiation (3000 R) did not completely abolish the ability of the T cells to respond to either TSST-1 or SEA, as can be seen when the irradiated T cells are cultured with irradiated B cells (Fig. 9). Treatment with mitomycin C totally inhibits T cell proliferative responses to TSST-1 or SEA, but does not affect the T cell-mediated induction of B cell proliferation and differentiation in response to TSST-1 or SEA. In the comparison of the effects of the SEA vs TSST-1 on Ig production, no significant differences were noted between SEA and TSST-1 (data not shown). With both SEA and TSST-1, IgG and IgA production were lower with irradiated T cells

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than mitomycin C-treated T cells.

SEA inhibition of Ig production requires T cell proliferation. Since SEA inhibited SAC-induced Ig production by PBMC but induced Ig production by B cells in the presence of inactivated T cells, we investigated whether the inhibition of SAC-induced Ig production by SEA required T cell proliferation. Purified B cells and purified T cells were cocultured at a 1:1 ratio in media with or without SAC. Cultures without SAC produced no Ig, whereas cultures containing SAC readily produced IgG (2.4 μg/ml), IgM (6.6 μg/ml), and IgA (0.9 μg/ml). However, when SEA was also added to the cultures, production of Ig was inhibited (Fig. 11). Thus, inhibition of Ig production seen with the addition of SEA to SAC-stimulated PBMC (Fig. 1) can, at least in part, be attributed to the T cell compartment. The abrogation of inhibition by irradiation or mitomycin C treatment of the T cells before culture (Fig. 11) indicates that T cell proliferation is required for the induction of inhibition or the production of an inhibitory factor for B cell differentiation. Similar results were observed when TSST-1 was used instead of SEA (data not shown).

DISCUSSION

This report provides insight into the physiologic mechanism by which *Staphylococcus* exoproteins inhibit Ig production by human PBMC. Since SEA binds specifically to MHC class II molecules, its direct effect on proliferation and differentiation of highly purified B cells was compared with that of anti-HLA DR mAb (L243), which suppresses B cell proliferation and differentiation. In contrast to L243, SEA did not inhibit SAC-induced proliferation or SAC and rIL-2-induced differentiation. Also, SEA did not directly induce B cell proliferation, even in the presence of a phorbol ester; nor did SEA enhance SAC-induced B cell proliferation. Thus, no direct inhibitory or enhancing effects of SEA on B cell proliferation or differentiation were demonstrated. However, in the presence of highly purified autologous T cells, SEA almost totally inhibited Ig production by SAC-stimulated B cells. Such inhibition was abrogated by irradiation or mitomycin C treatment of the T cells before their addition to culture. Thus, at least one mechanism by which SEA inhibited Ig production by PBMC was a proliferating T cell-dependent mechanism. Likewise, the exoprotein

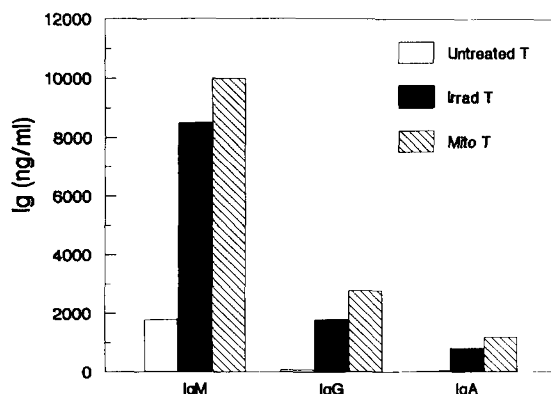

Figure 11. Inhibition of Ig production by SAC-stimulated B cells and T cells. Ig was measured after 10 days from supernatants of 10^5 B cells cultured with SAC (0.01%), 1000 ng SEA/ml, and 10^5 untreated, irradiated (3000 R) or mitomycin C-treated T cells. Cells were cultured in triplicate. The SEM for each data point was less than 10% of the mean.

TSST-1 inhibited SAC-induced Ig production by a proliferating T cell-dependent mechanism.

Consistent with our observations of the effects of SEA and TSST-1 on human lymphocytes, murine studies have demonstrated that SEB inhibits antibody responses both in vivo and in vitro (26, 27). Such inhibition of Ig production was dependent on a regulatory network in which $CD4^+$ suppressor/inducer cells activated $CD8^+$ suppressor/effector cells (28). The activity of the SEB-induced suppressor was mediated by an I-J-restrictive 26-kDa factor that inhibits antibody responses in an Ag-nonspecific manner. Ag nonspecificity has been hypothesized to be the consequence of SEB polyclonal activation of multiple Ag-specific T suppressor cell populations (29, 30). Whether an analogous network exists for human T cells is under investigation.

In the context of investigating the mechanisms of *Staphylococcus* exotoxin inhibition of Ig production, we confirmed the observation that TSST-1 could behave as a nominal Ag by binding to MHC class II molecules on B cells and thereby induce irradiated T cells to provide cell contact-dependent help for induction of B cell proliferation and Ig production, in the absence of other exogenous stimuli (25). We demonstrated a similar effect with SEA. Thus, a dichotomy exists regarding exotoxin effects on B cell differentiation in the presence of inactivated vs untreated T cells. Although the induction of Ig production by these exotoxins in the presence of inactivated T cells provides a novel model for examining T cell-B cell interactions, the physiologic effect of the exotoxins is a proliferating T cell-dependent inhibition of B cell differentiation. There are several possible explanations for the differential effects of the exotoxins in the presence of inactivated vs untreated T cells. One alternative is that inactivated T cells produce qualitatively or quantitatively different cytokines. Alternatively, proliferating T cells might consume cytokines, which are important for mitogen-induced B cell differentiation. Such a trivial explanation is unlikely since, in our model, only IL-2 is necessary to support SAC-induced Ig production, and the addition of excess rIL-2 to cultures containing both SAC and SEA was not able to overcome the inhibition of Ig production. Likewise, the capacity of *Staphylococcus* exotoxins to inhibit B cell differentiation in vivo in mice argues against consumptive depletion of cytokines as the basis for the inhibition observed. Another possibility is that the exotoxins induce a proliferation-dependent T cell regulatory network with human lymphocytes similar to that described in a murine system. In unfractionated PBMC, direct stimulation of monocytes resulting in the production of IL-1 and TNF-α and T cell-derived IL-2 stimulation of NK cell activity could also play a role in the immunoregulation of Ig production by these exotoxins. However, our results indicate that T cells constitute a major cellular component involved in *Staphylococcus* exotoxin-induced inhibition of B cell differentiation.

The effects of the *Staphylococcus* exoproteins on the immune system are dependent on binding of the toxins to MHC class II molecules. However, our data clearly demonstrate that exotoxin binding to the MHC class II molecules does not affect B cell proliferation and differentiation. Such a lack of response was somewhat surprising in light of the effects that anti-MHC class II antibodies can have on B cell function. For example, anti-Ia

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antibodies have been shown to inhibit lipopolysaccharide-stimulated proliferation of murine B cells (31). Conversely, murine B cells activated with anti-μ antibodies and IL-4 proliferate in response to immobilized anti-Ia antibodies and differentiate into Ig secreting cells in the presence of additional cytokines (32). Such studies have demonstrated that signal transduction through the MHC class II molecules varies with the nature of the signal (soluble vs immobilized), the timing of the signal, and the epitope. To date, studies with human B cells have demonstrated that antibodies to MHC class II molecules inhibit B cell activation and proliferation. The well characterized anti-HLA DR mAb, L243, has been found to inhibit mitogen-induced proliferation of purified B cells and spontaneous proliferation by EBV transformed B cell lines (22, 33). L243 has also been shown to inhibit spontaneous Ig production by pure B cells from SLE patients (34). We confirmed the inhibitory effect of L243 on the proliferation of SAC-stimulated highly purified B cells and extended this observation to include the inhibition of SAC and rIL-2-induced Ig production by pure B cells. In contrast, SEA did not inhibit B cell proliferation or differentiation even at doses 100 times that used to activate T cells. These observations were not limited to the SAC-stimulated system, as we have seen similar results with EBV-transformed cell lines and tonsillar B cells, even with the addition of phorbol ester (A.B. Moseley and D.P. Huston; unpublished data). Although IL-2R expression plays a significant role in B cell activation and differentiation (35), the exact relationship between MHC class II signaling and IL-2R expression is unclear. L243 has been shown to down-regulate the expression of IL-1R on lupus B cells (34) and the expression of CD20 and CD23 on EBV-transformed cell lines (33). We demonstrated a SAC-induced increase of B cell IL-2R expression, confirming a recent report (36), and have also shown that the addition of L243 to SAC-activated B cells inhibited IL-2R expression. In contrast, SEA did not inhibit or enhance the SAC-induced IL-2R expression, suggesting differential signaling by SEA and L243 through MHC class II molecules. Recently, competitive binding studies have suggested that SEB and TSST-1 bind to distinct epitopes on HLA-DR expressed by L cell transfectants (37). However, binding of both SEB and TSST-1 could be inhibited by L243, suggesting that more than one epitope served as binding sites. We found that L243 and SEA did not competitively inhibit each other from binding to B cells or EBV-transformed B cell lines. Thus, SEA and L243 bind different epitopes on MHC class II molecules, suggesting that SEA binds to a site or sites distinct from those of SEB and TSST-1 since their binding is blocked by L243. Although binding to different epitopes on the MHC class II molecule could result in differences in signal transduction between SEA and L243, the similarity of functional effects of SEA and TSST-1 suggest that these differences are less the consequence of functional heterogeneity of MHC class II epitopes, but rather reflect unique properties of staphylococcal exotoxins.

Staphylococcal exoproteins are the most potent known stimulators of T cell mitogenesis. As members of the unique family of bacterial-derived proteins that bind specifically to MHC class II molecules with consequent direct stimulation of cytokine production by monocytes and cytokine production by T cells upon interacting with TCR, they have the potential to be engineered as therapeutic biologic response modifiers. In their native form, this potential is manifest as a variety of pathologic clinical syndromes. Given the effect of the *Staphylococcus* exoproteins on B cell differentiation, their potential in the pathogenesis of hypogammaglobulinemia syndromes and as modifiers of hypergammaglobulinemia disorders remains to be explored.

Acknowledgments. We appreciate the useful discussions with Dr. M. Huston, the assistance of Dr. D. Lewis with flow cytometry, and the expert secretarial assistance of S. Petrash.

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