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Intravenous Immunoglobulin Inhibits Staphylococcal Toxin-Induced Human Mononuclear Phagocyte Tumor Necrosis Factor Alpha Production

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Intravenous gamma immunoglobulin (IVIG) is used as therapy in superantigen-mediated disease, yet its mode of action is not clear. Pooled immunoglobulin G contains high concentrations of staphylococcal exotoxin (SE)-specific antibodies which inhibit the in vitro activation of T cells. However, SE and streptococcal exotoxins are potent stimulators of monocytes as well. Monocytes exposed to SE in vitro release large amounts of tumor necrosis factor alpha (TNF-α). The purpose of the present study was to determine if SE-specific antibodies in IVIG can inhibit the activation of monocytes by SE. We examined the in vitro effect of IVIG on the ability of staphylococcal exotoxin A (SEA) and staphylococcal exotoxin B (SEB) to stimulate release of TNF-α from human mononuclear phagocytes (MO). Pretreatment of SEA with 0.1 mg of IVIG per ml resulted in a slight decrease of SEA-induced TNF-α secretion by MO. In contrast, pretreatment of SEB with 0.1 mg of IVIG per ml resulted in significant (greater than 50%) inhibition of SEB-induced TNF-α secretion at 24, 48, 72, and 96 h ($P < 0.05$ for TNF-α levels induced by SEB alone versus SEB pretreated with IVIG at all time points). Enzyme-linked immunosorbent assay and Western immunoblotting assays of the IVIG revealed high concentrations of antibodies against SEB and lower concentrations of antibodies to SEA. These data indicate that IVIG can act in a toxin-specific manner to decrease the MO TNF-α response to superantigens. Such inhibition may be one mechanism by which IVIG exerts an immunoregulatory role in superantigen-mediated disease.

Microbial superantigens are a group of proteins which include staphylococcal exotoxin (SE) and streptococcal exotoxin, toxic shock syndrome toxin 1 (TSST-1), and exofoliative toxin. Superantigens bind to major histocompatibility complex (MHC) class II molecules on antigen-presenting cells without the internalization and proteolysis required by conventional antigen and activate T lymphocytes in a Vβ-restricted manner (8, 13, 17, 23, 30). Because a large percentage of the T-cell population share Vβ elements (13, 23), large numbers of the resting T-cell population may be stimulated, leading to excessive production of T-cell cytokines. In addition, superantigens are powerful stimulators of monocytes and B cells. TSST-1, and the related toxins SEA and SEB, induce the synthesis of interleukin 1β (IL-1β) and tumor necrosis factor alpha (TNF-α) by human monocytes (14, 18, 28, 29). Massive induction of these monokines by TSST-1 is believed to play a key role in the pathophysiology of TSS (15). Superantigens have been implicated in autoimmune diseases as well. Diseases proposed to be associated with superantigens include Kawasaki disease, (1) rheumatoid arthritis, (27) multiple sclerosis, and autoimmune thyroiditis (20).

Intravenous gamma immunoglobulin (IVIG) is used as therapy for many of these disease states (5, 16, 26, 32), and there are several possible mechanisms for its beneficial effects. IVIG preparations contain anti-cytokine antibodies (33) which could act to decrease the effects of cytokines released in response to superantigen stimulation. Takei et al. (34) proposed that the primary inhibitory mechanism of IVIG was through interference with accessory cell presentation of specific toxins to T cells, with subsequent inhibition of T-cell activation.

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We propose that an additional mechanism by which IVIG exerts its abrogating effects is through the inhibition of superantigen-induced cytokine production from monocytes. TNF-α, an important proinflammatory cytokine and a proximal mediator of the sepsis syndrome, is produced and released by monocytes in response to SE stimulation (35). The present study was undertaken to determine whether IVIG contains antibodies that can inhibit SE-induced monocyte activation and subsequent release of TNF-α. Decreased secretion of this major mediator of the inflammatory response may be an important mechanism by which IVIG exerts an immunoregulatory role in superantigen-mediated disease.

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MATERIALS AND METHODS

Reagents. Polymyxin B, SEB, SEA, and bacterial lipopolysaccharide (LPS) from *Escherichia coli* were purchased from Sigma Chemical Co. (St. Louis, Mo.). The toxins were >95% pure as determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Lyophilized toxins were reconstituted in deionized water and stored in aliquots at −20°C until used. Human gamma interferon (IFN-γ) was purchased from Genzyme Corp., Cambridge, Mass. Two commercially available preparations of IVIG were used in the experiments. The majority of the experiments were conducted with Gammagard (batch 3312 M123 AA), purchased from Baxter-Healthcare Corp., Glendale, Calif. For comparison, select experiments were conducted with Polygam S/D (batch 26206030AA), manufactured by Baxter-Healthcare Corp. and distributed by the American Red Cross Blood Services, Washington, D.C. Both products are manufactured by cold ethanol fractionation followed by ultrafiltration and ion-exchange chromatography. Polygam was further treated with an organic solvent-detergent mixture composed of tri(*n*-butyl)phosphate, octoxynol 9, and polysorbate 80.

Cell isolation and culture. Blood samples were collected from healthy adult volunteers by using sterile heparinized syringes (10^3 U of heparin/liter of blood). After collection, the blood was diluted 1:1 with sterile phosphate-buffered saline (PBS). The cell suspension was overlaid on Histopaque 1077 (Sigma Chemical Co.) and the mononuclear cells were separated by density gradient centrifugation. These cells were washed once with sterile PBS and then incubated for 10 min at 37°C in 0.01 M Tris-0.83% NH_4Cl (pH 7.2 to 7.4) to lyse contaminating

erythrocytes. After centrifugation, cells were washed twice more with sterile PBS prior to resuspension in RPMI 1640 (Gibco, Grand Island, N.Y.) containing 2 mM L-glutamine, 10^4 U of penicillin G per liter, 10^4 μg of streptomycin per liter, and 10% heat-inactivated fetal calf serum. Enumerated cells were greater than 95% viable by trypan blue exclusion. Monocytes were isolated from mononuclear cells by adherence to plastic. For this purpose, 10^5 mononuclear cells were allowed to adhere for 1.5 to 2 h at 37°C in 0.3 ml of medium/well in 96-well flat-bottom tissue culture trays (Costar, Cambridge, Mass.). Nonadherent cells were removed by washing five times with warm sterile PBS, and fresh culture medium was added. Monolayers were then cultured for 5 days at 37°C with 5% CO_2 prior to experimentation. The adherent cell population consisted of more than 90% monocytes as determined by staining with α-naphthyl esterase (Sigma Diagnostics, St. Louis, Mo.). Cultures of adherent cells were monitored periodically by inverted microscopy; monocytes routinely took on morphological characteristics of macrophages after 5 days in culture.

Stimulation of TNF-α. To prepare supernatant fluids, culture media from 5-day-old adherent cells were replaced with media alone or culture media containing SEA (10 μg/ml), SEB (10 μg/ml), or either toxin pretreated with IVIG. The individual toxins were pretreated with pooled immunoglobulin G (IgG) (0.1 mg/ml) for 2 h at 25°C to allow time for antigen-antibody formation. Dose-response experiments used 0.1 and 1 mg/ml of IVIG. In select experiments media were removed from the monocytes and replaced with media containing 10 μg of LPS and 100 U of IFN-γ per ml or the same concentrations of LPS and IFN-γ pretreated with IVIG. At predetermined incubation times, the plates were centrifuged at 300 × *g* for 5 min and the cell-free supernatants were harvested from each well. Supernatants from each of three wells that had been treated the same were pooled and stored at −70°C until used for TNF-α assays. All reagents had undetectable levels of endotoxin, as verified by the Limulus amoebocyte assay (Cape Cod Association, Woods Hole, Mass.) (sensitivity of 20 ng/liter).

TNF-α bioassay. TNF-α activity was determined by using an adaptation of the lytic assay of Carswell et al. (2) as described previously (4). Briefly, mouse L929 fibroblast cells were plated in 96-well plates at a concentration of 4×10^4 cells/well. After an overnight incubation, supernatants were added to the plates and serially diluted in horizontal rows. To determine the specificity of the assay, appropriate dilutions of selected supernatants or standard human recombinant TNF-α (rTNF-α) (specific activity, 5×10^7 U/mg) (Genentech, South San Francisco, Calif.) were preincubated with 5 neutralizing U of TNF-α monoclonal antibody (MAb) (specific activity, 6×10^6 neutralizing U/mg) (Genentech) for 4 h at 4°C before addition to the plates. Actinomycin D (8 mg/liter) (Sigma) was added to each well and plates were incubated overnight. Following overnight incubation, samples were removed and plates were washed with warm PBS to remove dead cells. The plates were stained with 0.05% crystal violet in 20% ethanol for 10 min. Plates were then washed with cold tap water and allowed to dry for 24 h. After drying, stain was eluted with 100 μl of 100% methanol per well. Plates were read within 10 min of methanol addition at 562 nm on a microplate reader. Optical density (OD) of L-929 cells incubated with medium alone represented 0% lysis, and cells treated with 3 M guanidine hydrochloride represented 100% lysis. One unit of TNF-α is defined as that amount of TNF-α required to produce 50% lysis. Lysis was normalized to a standard of human rTNF-α. The lower limit of detectability was 50 ng/l (2.5 U/ml).

Measurement of antibodies to staphylococcal toxins. The enzyme-linked immunosorbent assay (ELISA) was performed as described by Walker et al. (36). Assay wells were coated with 5 μg of (0.5 μg per well) toxin per ml. Pooled IgG (adjusted to 10 mg/ml to approximate the normal serum IgG level) was diluted 1:1,000 or greater. Bound IgG was detected by addition of an affinity-purified goat anti-human IgG conjugated with alkaline phosphatase (Bio-Rad Laboratories, Richmond, Calif.). Each dilution of pooled IgG was assayed in duplicate on nonantigen-coated (background) wells and antigen-coated wells. Background values, which averaged <0.01 OD, were subtracted from the antigen-coated well values. The concentration of antibody in the IgG preparation was defined as the reciprocal dilution of the sample giving an OD of 0.2 extrapolated from a standard curve. The relationship between absorbance and the dilution of IgG was linear on a log-log plot between an OD of 0.05 and 1.50, with a similar slope of binding for each toxin (data not shown).

Western blot (immunoblot) analysis. Equal amounts of SEA and SEB (0.3 μg) were electrophoresed on 10% acrylamide minigels by the discontinuous denaturing method of Laemmli (22). Before electrophoresis, samples were mixed with an equal volume of solubilizing solution (0.1 M Tris-HCl, pH 6.8) containing 2.5% SDS, 20% glycerol, and 0.01% bromphenol blue and boiled for 10 min. Polyacrylamide gel electrophoresis in Tris-glycine buffer (pH 8.6) containing 0.01% SDS was carried out at a constant current of 20 mA. The proteins were electrophoretically transferred to nitrocellulose paper (Millipore, Bedford, Mass.) at 150 mA for 2 h. After blocking, the sheet was incubated overnight at 4°C with 20 μg of Gammagard IVIG per ml (same lot of Gammagard IVIG as that used for cellular incubations). After washing in PBS, the membrane was incubated with alkaline phosphatase-conjugated goat anti-human IgG (Bio-Rad) diluted 1:1,000 with gelatin diluent. Blots were developed with alkaline phosphatase-conjugate substrate kit (Bio-Rad) for 10 min.

Data calculations. Data are presented as the mean ± standard error of the mean (SEM) for triplicate determinations for four individuals. The triplicate values for each individual varied 2 to 12%. The two-sample *t* test for unpaired data was used for statistical analysis. Differences were considered significant at the level that two-tailed *P* was <0.05.

RESULTS

Evaluation of TNF-α response in adherent MO exposed to SEA or SEB. After 5 days in culture, human mononuclear phagocytes (MO) were replated with culture medium alone or with medium containing SEA or SEB. Supernatants collected at specific intervals (24, 48, 72, and 96 h) were assayed for TNF-α production by L929 assay. Little to no TNF-α activity was detected in supernatants harvested from MO incubated in media alone (<80 U/ml) at all time points (data not shown). In contrast, supernatants harvested from MO incubated with either SEA or SEB contained significant amounts of TNF-α (Fig. 1). Exposure of MO to SEB resulted in the rapid accumulation of high levels of TNF-α, with levels of 10.2×10^3 U/ml being found after only 24 h of incubation. In comparison, relatively low levels of TNF-α were seen in MO incubated with SEA for 24 h (1.6×10^3 U/ml). By 72 h of incubation, extremely high levels of TNF-α were detected from MO incubated with either toxin. Thus, both SEA and SEB stimulate prolonged production of high levels of TNF-α by MO.

Effect of IVIG on MO TNF-α response induced by SEA or SEB. To investigate the ability of commercially available IVIG to inhibit SE-induced release of TNF-α, SEA and SEB were each pretreated with IVIG (Gammagard; Baxter-Healthcare) for 2 h prior to their addition to MO cultures. Supernatants harvested in parallel to those described above were assayed for TNF-α (Fig. 2). Although preincubation of SEA with IVIG led to a slight decrease in the levels of TNF-α induced by SEA (Fig. 2A), the decrease was not significant at any time point. The kinetics of TNF-α secretion were the same as those with SEA alone, with extremely high levels again being seen at 72 h of incubation (15.0×10^3 U/ml). In contrast to results obtained with SEA, pretreatment of SEB with IVIG led to greater than 50% inhibition of the SEB-induced TNF-α response at 24, 48, 72, and 96 h of incubation (Fig. 2B). At all time points, TNF-α levels from MO stimulated with SEB pretreated with IVIG were significantly less than those from MO stimulated with SEB alone (Fig. 2B).

A second experiment was conducted with a different brand and lot of IVIG, Polygam S/D (batch 26206030AA). This product is also made by Baxter Healthcare, but it undergoes treatment with an organic solvent-detergent mixture to ensure viral inactivation and clearance. Supernatants from MO incubated for 48 h with either SEA or SEB or either toxin pretreated with Polygam IVIG (0.1 mg/ml) were assayed for TNF-α by L929 assay. As with Gammagard IVIG, preincubation of SEB with Polygam IVIG resulted in a significant decrease in SEB-induced TNF-α release (levels decreased from $9.1 \pm 3.2 \times 10^3$ to $4.8 \pm 1.1 \times 10^3$ U/ml [means ± SEM of triplicate determinations for four individuals]). This represents a 48% reduction. Pretreatment of SEA with Polygam IVIG also resulted in a significant decrease in SEA-induced TNF-α secretion (levels decreased from $7.7 \pm 1.9 \times 10^3$ to $3.8 \pm 0.8 \times 10^3$ U/ml, a 51% reduction).

In an effort to prove the specificity of the mechanism of inhibition, MO were incubated with large amounts of LPS and IFN-γ or with LPS and IFN-γ preincubated with IVIG. Pretreatment of these stimulants with IVIG did not result in any inhibition of TNF-α production ($5.6 \pm 2.8 \times 10^3$ U/ml for LPS- and IFN-γ-stimulated MO versus $6.1 \pm 3.2 \times 10^3$ U/ml for MO stimulated with LPS and IFN-γ that had been preincubated with IVIG).

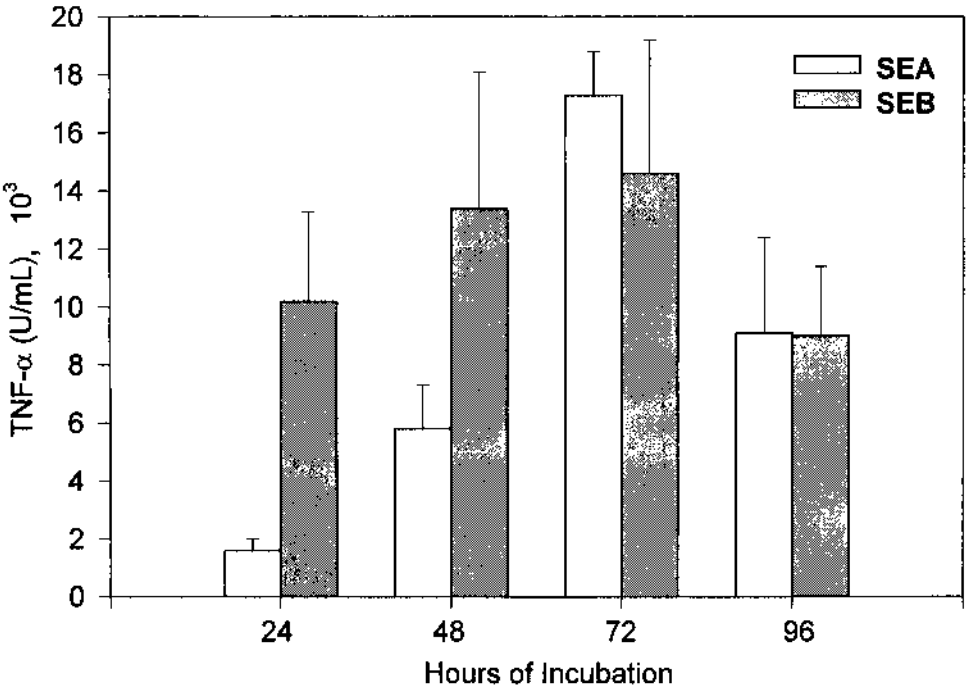

FIG. 1. TNF-α produced in response to SEA and SEB. MO were incubated with 10 μg of the respective toxins per ml for 24 to 96 h. Cell-free supernatants were harvested and tested for the presence of TNF-α as measured by cytotoxicity toward the TNF-α-sensitive murine fibroblast cell line L929. Results are means ± SEM for triplicate determinations for four individuals. Supernatants from untreated control cells contained no TNF-α activity at any time point examined (data not shown). The exotoxins had no direct effect on the L929 cells. Anti-TNF-α neutralized cytotoxicity ≥95%.

Concentrations of antibodies to SE in IVIG. We hypothesized that the amount of toxin-specific antibodies present in a particular lot of IVIG determined the efficiency with which IVIG inhibited responses induced by a specific toxin. We determined the relative concentrations of antibodies against SEA and SEB present in the lots of IVIG used for the above experiments. When the concentrations of antibodies were measured by ELISA, a higher concentration of antibody to SEB (positive to a dilution of 1:15,100) than to SEA (1:3,300) was found in the Gammagard IVIG. In the lot of Polygam IVIG,

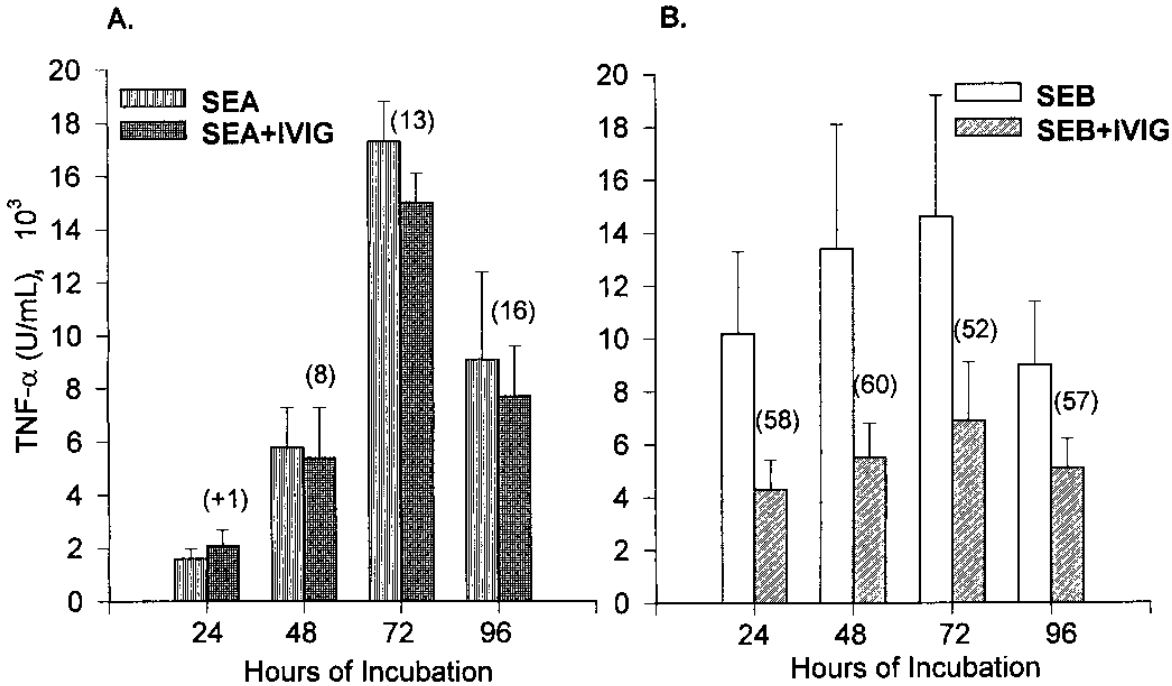

FIG. 2. Inhibition by IVIG of SE-induced TNF-α production by MO. Cultured MO were incubated with media containing SEA (10 μg/ml) (A), SEB (10 μg/ml) (B), or toxin that had been pretreated with IVIG (0.1 mg/ml). Cell-free supernatants were harvested at 24 to 96 h and tested for the presence of TNF-α by L929 assay. Results are means ± SEM of triplicate determinations for four individuals. Percent inhibition of TNF-α by the IVIG is noted above the bars in parentheses. The IVIG had no effect on the viability of the L929 cells.

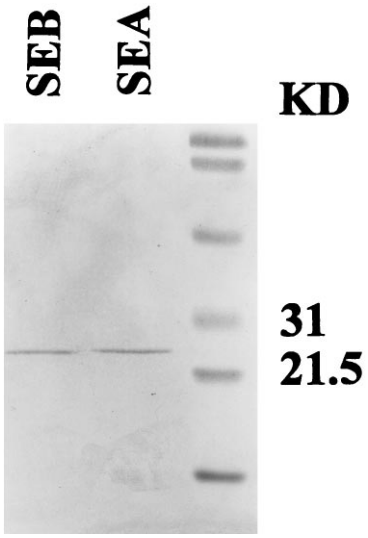

FIG. 3. Polyacrylamide gel electrophoresis of equal amounts of SEA and SEB (0.3 μg) followed by staining revealed bands of equal intensity in the 20- to 30-kDa (KD) range. Molecular mass markers are noted on the right.

the concentrations of antibodies to the two toxins were similar (ELISA positive to a dilution of 1:14,400 for SEB and to a dilution of 1:12,200 for SEA). Western blot assay confirmed the presence of a higher concentration of antibody specific for SEB in the Gammagard IVIG (Fig. 3 and 4). When the toxins were loaded in equal amounts and run on a gel, bands of equal intensity were obtained in the 20- to 30-kDa range (Fig. 3). The proteins were electrophoretically transferred to nitrocellulose and probed with IVIG. A darker band to SEB than to SEA was revealed, reflecting the higher concentration of antibody to SEB in the Gammagard lot of IVIG (Fig. 4).

Dose response. Pretreatment of either toxin with higher concentrations of Gammagard IVIG resulted in better inhibition of toxin-induced TNF-α production (Fig. 5). Significant inhibition of SEA-induced TNF-α required concentrations of Gammagard IVIG 10-fold greater than those required to inhibit SEB-induced TNF-α activity.

Time dependence. To determine the time dependence of the IVIG, MO were incubated in IVIG for 18 h followed by washing and subsequent addition of SEB. Pretreating the MO with IVIG did not inhibit subsequent SEB-induced TNF-α production, as shown in Table 1. Thus, the IVIG was not inhibitory by a direct effect on the MO themselves.

Studies were performed to establish the effect of delayed addition of IVIG to SEB-stimulated MO (Table 1). When MO were exposed to SEB for 18 h and then IVIG was added, the TNF-α response seen after a subsequent 24 h of incubation was decreased only 23% from control. But, TNF-α levels found after 48 h of incubation were 55% less than those from MO to which no IVIG was added. Thus, delayed addition of IVIG to SEB-stimulated MO resulted in significant inhibition of the SEB-induced TNF-α response if time was allowed for IVIG to interact with the toxin.

DISCUSSION

This study confirmed the ability of SE to induce the prolonged release of high levels of TNF-α from human MO (6). We detected high levels of TNF-α in supernatants from MO incubated in vitro for as long as 96 h with either SEA or SEB. Fast et al. detected TNF-α activity in culture supernatants from MO incubated with various exotoxins through day 6 and showed that the persistence of TNF-α was the result of continued production of the cytokine, rather than the result of accumulation of the product (6). This contrasts with the time course of endotoxin-induced TNF-α production from human MO in vitro, where protein levels are highest at 24 h and begin to decline by 48 h (6). Prolonged stimulation of MO to produce TNF-α by SE in vivo may contribute to the pathophysiology of superantigen-mediated disease.

Animal studies have shown that depletion of MO in vivo has minimal effect on the superantigen-induced T-cell response in the spleen (19). Our study does not suggest that the T-cell response to superantigens in and of itself is unimportant, nor that MO are essential for the T-cell response to superantigens, nor that they are necessary for superantigen-mediated disease to occur. Our data simply indicate that SE-induced MO TNF-α production may contribute to the pathophysiology of superantigen-mediated disease states, and through an inhibition of toxin-MO interaction, IVIG may decrease the release of TNF-α. TNF-α is an important proinflammatory mediator and an initiator of the cytokine cascade promoting the systemic inflammatory response syndrome. Thus, IVIG inhibition of further increases in TNF-α that might occur through continued SE-MO interaction may be one mechanism by which IVIG exerts therapeutic benefit in patients with superantigen-mediated disorders. It is possible that in mice in which the monocyte population is depleted, other antigen-presenting cells (dendritic cells or B cells) compensate for the lack of MO in vivo.

Studies have suggested that the induction of cytokines from MO by SE requires the participation of T cells (9, 10, 19). Our experiments were conducted with adherent mononuclear cells, of which more than 90% stained positive for α-naphthyl esterase. In addition, cells were maintained in culture for 5 days and were observed to take on the morphology of macrophages prior to the addition of toxins. Thus, our data suggest that the production of TNF-α by SE can be T-cell independent. This is supported by experiments of Trede et al. (35) that demonstrated the induction of TNF-α and IL-1β mRNA in human monocytes and in the monocytic cell line THP-1 by stimulation

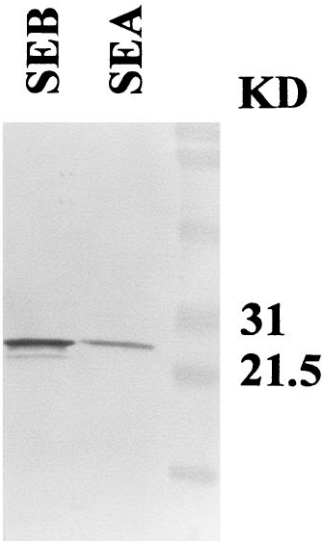

FIG. 4. Binding of IVIG to the SE as assayed by Western blot. The protein bands depicted in Fig. 3 were electrophoretically transferred to nitrocellulose and exposed to 20 μg of IVIG per ml. The bound IgG was developed with IgG-specific antiserum. Molecular mass markers are noted on the right.

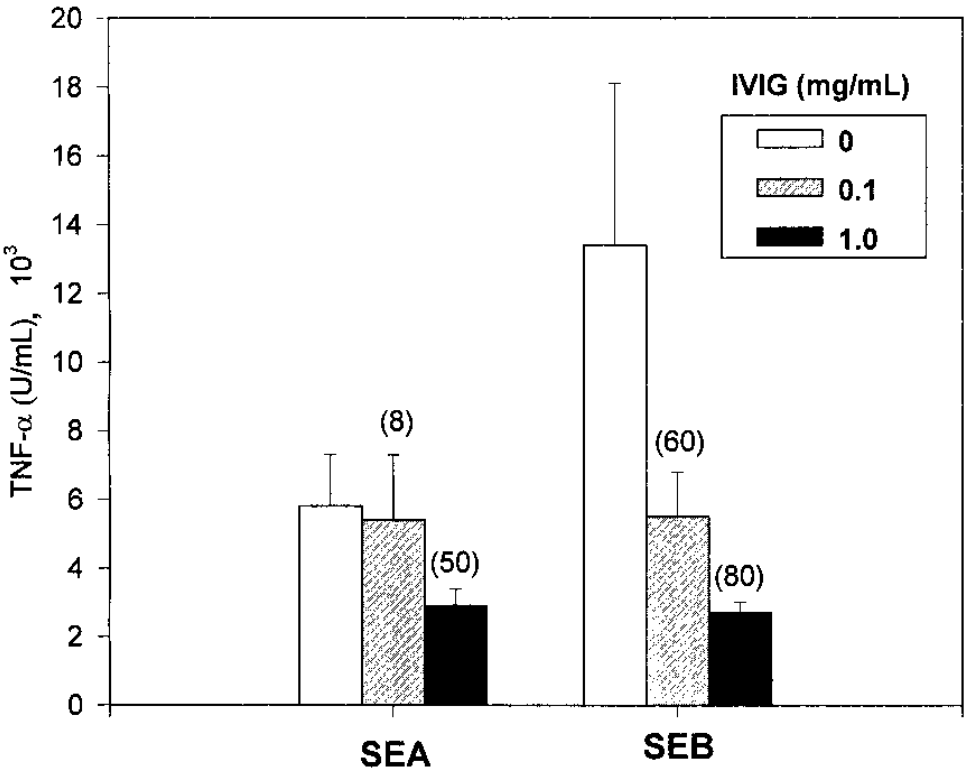

FIG. 5. Concentration dependence of IVIG inhibition of SE-induced TNF-α production by MO. Cultured MO were incubated with media containing SEA (10 μg/ml), SEB (10 μg/ml), or either toxin pretreated with two concentrations of Gammagard IVIG: 0.1 or 1.0 mg/ml. Cell-free supernatants were harvested at 48 h and tested for the presence of TNF-α by L929 assay. Percent inhibition of TNF-α by the IVIG is noted above the bars in parentheses. Results are means ± SEM of triplicate determinations for four individuals. The toxins and the IVIG had no effect on the L929 cells (data not shown).

with SEB and TSST-1. Furthermore, studies of the signal transduction mechanism revealed that protein tyrosine kinase activation is involved in TSST-1 induction of IL-1β mRNA in THP-1 cells (31) and that SEA induces an increase in intracellular calcium levels in human peripheral blood monocytes (3). In other work with human MO isolated by countercurrent centrifugal elutriation, Matsuyama et al. demonstrated that both protein tyrosine kinase and protein kinase C activation are involved in SEB-induction of IL-1β and TNF-α gene expression (24). A possible explanation for the requirement for T cells in some experiments is the amount of toxin used for stimulation. At low toxin concentrations monocytes might need a second signal for successful production of cytokines. This signal could be provided by T cells in the form of cytokines, such as IFN-γ, released from SE-activated T cells (21).

TABLE 1. Time dependence of inhibition

Experimental conditions	10^3 TNF-α (U/ml) at[a]:	
	24 h	48 h
MO alone	0	0
MO alone for 18 h, SEB added	10.2 ± 3.1	13.6 ± 4.7
MO + IVIG for 18 h, wash, SEB added	9.9 ± 2.8 (0)	14.3 ± 2.3 (0)
MO + SEB for 18 h, no wash	11.3 ± 1.2	13.9 ± 1.1
MO + SEB for 18 h, no wash, IVIG added	8.7 ± 2.2 (23)	6.2 ± 0.8 (55)

[a] Expressed as means ± SEM of triplicate determinations for four individuals. Anti-TNF-α neutralized L929 cytotoxicity >95%. Data in parentheses are percent inhibition.

The concentration of toxin used in our experiments was 10^3 times higher than that used in investigations indicating a requirement for T cells (9, 10). Of course, 10% of the cells used in our experiments were not positive for α-naphthyl esterase and may have been T cells. Thus, we cannot rule out a possible direct or indirect contribution of T cells to the production of TNF-α. It is entirely possible that such a small population of T cells might provide enough of a second signal through IFN-γ release or cross-linking of class II molecules through T cell-Vβ-SE binding to activate the MO for TNF-α production in the presence of SE. T cells produce TNF-α and TNF-β in response to stimulation with SE (10). More than 95% of the TNF activity detected by the L929 assay was made up of TNF-α, as shown by neutralization of TNF activity by preincubation of cell supernatants in antibody specific for TNF-α. Considering that TNF-α is primarily produced by MO, the majority of the TNF-α detected in our experiments was likely produced by MO.

IVIG has proven clinical efficacy in patients with Kawasaki disease (26), and case reports suggest it may be of benefit in patients with superantigen-mediated TSS (25). Its therapeutic mode of action in these disease states is not clear. In the present in vitro study, we demonstrated the ability of IVIG to inhibit superantigen-induced release of TNF-α from MO. Experiments showed that preincubation of toxins with a predetermined concentration of Gammagard IVIG resulted in significant inhibition of the TNF-α response induced by SEB but minimal inhibition of the response induced by SEA. We found a higher titer of antibodies specific for SEB in this IVIG preparation than for SEA; inhibition of SEA-induced TNF-α production required the use of higher concentrations of this lot of

IVIG. A second experiment showed that incubation of either SEB or SEA in a lot of Polygam IVIG that contained high titers of antibody to both toxins resulted in inhibition of both SEB- and SEA-induced TNF-α production. Thus, inhibition is likely mediated by toxin-specific antibodies present in the IVIG, and high titers of specific antibody seem to be necessary for significant inhibition to occur. Studies have shown that substantial variation occurs in the levels of antibody against various bacterial antigens in different preparations of IVIG and among lots of the same preparation (7, 12). Indeed, the preparations of IVIG used in our experiments had significantly different titers of antibody to SEA. This may reflect the prevalence and level of specific antibodies in a donor population (12). Our data suggest that the amount of toxin-specific antibodies present in a particular lot of IVIG may determine its effectiveness in a specific toxin-mediated disease. These data also suggest that a minimal dose of IVIG may be required for it to be of therapeutic benefit.

The specificity of inhibition was indicated by the fact that very little inhibition of SEA-induced TNF-α production occurred when the IVIG used for preincubation contained a low titer of antibody specific for SEA. The fact that MO stimulated by LPS combined with IFN-γ produced high levels of TNF-α despite preincubation of these stimulants in IVIG also showed that IVIG is not nonspecifically inhibitory. These data prove that the IVIG does not contain TNF-α binding proteins or anti-TNF-α antibodies sufficient to interfere with the assay for TNF-α. Rather, the mechanism of inhibition is through toxin-specific antibody binding.

Late addition of IVIG to MO already exposed to SEB for 18 h continued to result in inhibition of subsequent toxin-induced release of TNF-α. This may be due to the prolonged time over which SE stimulate MO to release TNF-α. If the same were to hold true in vivo, these data suggest that the administration of IVIG to patients with SE-mediated disease may be helpful even if administration is relatively delayed.

Pretreatment of the MO with IVIG had no effect on the TNF-α response induced by subsequent exposure to toxin. These data suggest that IVIG had no inhibitory effect on the MO themselves but interfered with the interaction of the toxin with the MO, thus preventing toxin-induced MO release of TNF-α. In conclusion, the means by which IVIG is effective for a number of diverse disease conditions, including Kawasaki disease, rheumatoid arthritis and TSS, remain uncertain (16, 26, 32, 33). We propose that one mechanism by which IVIG exerts its immunomodulating effects is through toxin-specific antibody inhibition of toxin-induced release of TNF-α from MO. The ability of IVIG to down-regulate the production of this important proinflammatory cytokine makes it an attractive treatment consideration for severe SE-mediated disease as well as for other proposed superantigen-mediated disease states.

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