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Humoral Immunity to Aerosolized Staphylococcal Enterotoxin B (SEB), a Superantigen, in Monkeys Vaccinated with SEB Toxoid-Containing Microspheres

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Staphylococcal enterotoxin B (SEB) toxoid-containing microspheres were tested for efficacy in rhesus monkeys as a vaccine candidate for respiratory SEB toxicosis and toxic shock. Forty monkeys were randomly separated into 10 groups of four monkeys each: 9 groups were vaccinated with the microspheres via combinations of mucosal and nonmucosal routes, and 1 group served as nonvaccinated controls. Both vaccinated and nonvaccinated monkeys were then challenged with a high lethal dose of SEB aerosol. Monkeys primed with an intramuscular dose of the microspheres followed by an intratracheal booster all survived the SEB challenge. Overall, monkeys with an intratracheal booster generally had the highest antibody levels, which is consistent with their high survival rate and lower rate of illness. Protective immunity was correlated with antibody levels in both the circulation and the respiratory tract. The protection was not due to the depletion or anergy of SEB-reactive T cells, since SEB-induced proliferation in cultures of circulating lymphocytes was not significantly reduced after the microsphere vaccination. It is evident that the nonsurvivors did not die of systemic anaphylaxis or hypersensitivity because the monkeys did not die immediately after SEB challenge and there were no significant differences in histamine levels between the vaccinated and control monkeys before and after SEB challenge. The antibodies seemed to neutralize the SEB that got into the airway and the circulation.

Staphylococcal enterotoxin B (SEB) is the major staphylococcal enterotoxin associated with nonmenstrual toxic shock syndrome and accounts for the majority of the cases that are not caused by toxic shock syndrome toxin 1 (29, 36). Most of these toxic shock cases are generally due to an infection with enterotoxin-producing staphylococci, which reach the deep tissues via the openings of wounds and injuries (15, 29, 36). In recent years, however, cases of nonmenstrual toxic shock syndrome have been found in staphylococcal infections of mucosal tissues, particularly in cases of barrier contraceptive usage and of secondary infections in the respiratory tract of influenza patients (15, 22). Because antibiotic-resistant and toxin-producing staphylococci are widespread throughout the body, particularly in the nose and throat, respiratory toxicosis and toxic shock may become a serious problem during outbreaks and epidemics of respiratory infectious diseases.

The mechanism of pathogenesis of respiratory SEB toxicosis and toxic shock is still obscure. SEB is a superantigen (10, 18, 19). It binds to and forms complexes with major histocompatibility complex class II antigens on antigen-presenting cells, and the SEB-class II-antigen complexes are then presented to and activates T cells bearing certain T-cell antigen receptor Vβ elements (10, 14, 18, 19). It has been suggested that activation of both the antigen-presenting cells and T cells results in a massive production of cytokines and mediators, which then cause the disease (18, 19). In addition, other cell types may be involved. Scheuber and his co-workers (27, 28) have shown that SEB injected intradermally into cynomolgus monkeys results in an immediate-type skin hypersensitivity reaction, which is caused mostly by degranulation of mast cells. This mast cell degranulation may be caused by the direct activation by SEB of mast cells or nerve cells, which may release neuropeptides that then activate mast cells (1). Histamine H2 receptor antagonists can block SEB-induced emesis and skin reactions (27). In addition, we have recently shown that SEB can induce serotonin release from cultured mast cells (21).

If SEB intoxication is caused by activated macrophages, T cells, and other cell types and their mediators, the toxicosis could be a cascade and/or a combination of sequential pathophysiological changes among functionally interrelated tissues. A blockade of the initial step in which SEB reacts with the target cells should result in effective immunity. It is well known that SEB toxoid is a relatively good immunogen in inducing high-titered antibodies in monkeys and rabbits (3, 4, 31, 35), and recently, SEB toxoid-containing microspheres made of poly(DL-lactide-co-glycolide), a nontoxic biodegradable adjuvant, have been shown to be capable of inducing long-lasting, high-titered antibodies in the serum and gut of mice (13). Because immunity in the lung is mainly manifested by antibodies in the respiratory tract and the circulation (reviewed by Bienenstock [5]), the SEB toxoid-containing microspheres appear to be a good vaccine candidate for preventing respiratory SEB toxicosis and toxic shock. Previously, we had conducted a pilot experiment to test the protective immunity to aerosolized SEB in rhesus monkeys by oral immunization with the toxoid-containing microspheres (33). In the present study, we have further tested the efficacy of the toxoid-containing microspheres, also in rhesus monkeys, against aerosolized SEB by vaccination via combinations of mucosal and nonmucosal routes. We have shown here that an intramuscular (i.m.) priming dose followed by an intratracheal (i.t.) booster of the mi-

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crospheres elicits immunity to a relatively high lethal dose of SEB aerosol. Depletion or anergy of SEB-reactive T cells did not occur after vaccination. The immunity was mainly due to anti-SEB antibodies in the circulation and in the respiratory tract rather than to the depletion and/or anergy of the T cells. The microsphere vaccination and SEB challenge did not induce allergic or hypersensitivity reactions.

MATERIALS AND METHODS

SEB. SEB prepared according to the method of Schantz et al. (26) was obtained from the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Ft. Detrick, Frederick, Md. SEB toxoid was prepared at pH 7.5 and precipitated with alum by John Eldridge in the Department of Microbiology, University of Alabama, Birmingham, according to the procedure of Warren and coworkers (35). The SEB toxoid was incorporated into poly(DL-lactide-co-glycolide) microspheres, which were prepared by the Southern Research Institute, Birmingham, Ala., under a research contract.

Animal care and use. The experiments conducted in monkeys strictly adhered to the 1985 Amendments to the Animal Welfare Act (7 U.S.C. 2131, et seq.), Army regulation AR 70-18, and Public Law 99-198 and to the *Guide for the Care and Use of Laboratory Animals* (23a) as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, and adopted by the Laboratory Animal Care and Use Committees of each of our research institutes.

Immunization. Forty young rhesus monkeys (*Macaca mulatta*), male and female, weighing 2 to 3 kg, were randomly separated into 10 groups of four. Nine groups were immunized by the administration 7 weeks apart of two doses of microspheres containing 100 μg of SEB toxoid via combinations of mucosal and nonmucosal routes. One group of nonimmunized monkeys served as controls. Although monkeys administered plain microspheres via various route combinations similar to those of the vaccinated ones would have been more appropriate controls, such controls were not used in this study. However, once an effective immunization regimen is determined, vehicle controls, in which monkeys are injected with empty microspheres, will be employed in further testing of the microsphere vaccine. The decision not to use vehicle controls in the experiments described below had the advantage of reducing the use of monkeys, since vehicle controls would have had to be used for each immunization regimen. In addition, the nonimmunized controls already performed could also be used as controls for monkeys given other SEB vaccine formulations.

Oral or i.t. priming and booster immunizations were performed in Telazol-anesthetized monkeys by stomach or i.t. intubation, respectively. All priming and booster immunizations were performed in John Eldridge's laboratory, University of Alabama, under a research contract. The monkeys were then shipped to USAMRIID 7 weeks after the booster. Three to 4 weeks after arriving at USAMRIID (10 to 11 weeks after the boosters), the monkeys were challenged with a lethal dose of aerosolized SEB. The immunization regimens and challenge dates were chosen for the purpose of testing the efficacy of the SEB toxoid-containing microspheres as a vaccine candidate.

Aerosol challenge. The SEB aerosol challenge was done according to the procedure described previously (33). One week before SEB challenge, the monkeys were anesthetized with Telazol. Samples of blood and bronchoalveolar lavage (BAL) were collected. To prevent proteolytic digestion of antibodies, the BAL fluid was immediately mixed with an equal volume of a cocktail of proteolytic enzyme inhibitors (1 mM phenylmethylsulfonyl fluoride, 100 mM EDTA, 100 mM iodoacetamide). The BAL and plasma samples were then used for determinations of prechallenge anti-SEB antibody levels. At the time of challenge, monkeys were weighed and anesthetized with Telazol, placed in a head-only exposure chamber modified from a Henderson aerosol apparatus, and individually exposed for 10 min to approximately 7 or 15 50% lethal doses (LD_{50}) of SEB aerosol. The LD_{50} was determined on the basis of historical data accumulated since 1960 at USAMRIID. During the exposure of each monkey, a sample of SEB was taken directly from the sampling port, using an all-glass impinger (AGI-30), and quantified to ascertain the dose. After SEB challenge, monkeys were removed from the chamber, and blood samples were collected 30 and 90 min later. The plasma from this blood served as postchallenge samples. Monkeys were then allowed to rest and recover in their own cages and were observed closely around the clock for clinical symptoms for the next 10 days. According to published reports (2, 17, 33) and the experience of our veterinarians (Robert Hunt, Anthony Johnson, and David Ruble), a relative index of illness was determined on a scale of 0 to 10 on the basis of severity of clinical symptoms as follows: 0, no symptoms; 2, anorexia; 4, vomiting and/or diarrhea and/or loose stool; 6, depression and recumbency; 8, shock; 10, death. Monkeys in shock were fatally ill; they were humanely euthanized.

Lymphocyte proliferation. Lymphocytes from the blood were separated by Ficoll-Paque gradient centrifugation (6). They were cultured in 96-well culture plates in RPMI 1640 medium supplemented with 10% fetal bovine serum, 10 μg of gentamicin per ml, and 5×10^{-5} M 2-mercaptoethanol. SEB, concanavalin A (ConA), and phenol-water-extracted lipopolysaccharide (LPS) from *Salmonella typhimurium* at the maximal mitogenic concentrations (SEB, 2 μg/ml; ConA, 1 μg/ml; LPS, 100 μg/ml) were added when the cultures were established. ConA and LPS were purchased from Sigma Chemical Co., St. Louis, Mo. Four to six cultures were stimulated with or without a mitogen. The lymphocytes were cultured in a humidified incubator at 37°C under 5% CO_2. Cells were harvested onto glass fiber filters in a cell harvester at day 4 after initiation of culture. Eighteen to 24 h before harvest, [^{3}H]thymidine (0.02 μCi/ml) or [^{125}I]iododeoxyuridine (0.02 μCi/ml) was added to label the DNA of the actively dividing cells. Cells harvested on the glass fiber filters were processed for radioactive counting in a beta or gamma counter. Proliferation indexes, defined as the counts per minute of mitogen-stimulated cultures divided by those of cultures without mitogen, were then calculated.

ELISA. Antibodies from plasma and BAL were quantified by enzyme-linked immunosorbent assay (ELISA) as described previously (33). Briefly, each well of a 96-well Immulon II plate (Dynatech Laboratories, Inc., Chantilly, Va.) was coated overnight at room temperature with 75 μl of 3 μg of SEB per ml in 0.1 M bicarbonate buffer (pH 9.0). Each well was then blocked with 150 μl of 1% bovine serum albumin in phosphate-buffered saline (PBS). Monkey plasma or BAL samples in serial fourfold dilutions (100 μl) were then added. Rabbit anti-monkey serum (Nordic Immunological Laboratories, Capistrano Beach, Calif.) specific for the immunoglobulin A (IgA), IgG, or IgM isotype in a pretitrated dilution (100 μl) was used to determine and quantify the isotype of immunoglobulin. The rabbit antisera were diluted in bovine serum albumin-PBS containing 20 μg of SEB per ml and were allowed to react for at least 1 h to inhibit the residual anti-SEB antibodies that could not be removed by SEB affinity column chromatography. Peroxidase-conjugated goat anti-rabbit immunoglobulin (Zymed, South San Francisco, Calif.) at a pretitrated maximal dilution (100 μl) was then added. Color was then developed by adding a solution (100 μl) of hydrogen peroxide mixed with ABTS (2,2′-azino-di-[3-ethylbenzthiazoline sulfonate]) (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.). Optical density was then read at a wavelength of 405 nm, with subtraction of background read at 630 nm in a Bio-Tek ELISA Kinetics Reader (Bio-Tek Instruments, Inc., Winooski, Vt.). A monkey from our colony produced high titers of anti-SEB antibodies (midpoint titers, 9,000 for IgG, 4,200 for IgA, and 410 for IgM); this monkey plasma was used as a reference standard and was titrated in the same plates in which unknown samples were to be quantified. Antibody units per 100 μl of plasma were calculated from the reference standard. The half-maximal absorbance of the reference serum was used to define the endpoint of the titration curves of the unknown sera. The reciprocal of the highest dilution of the unknown sera that gave an absorbance equal to this half-maximal absorbance of the reference standard was defined as the antibody level (units) of the unknown samples.

Histamine quantitation. Histamine levels in the plasma and BAL were quantified by the methyltransferase method (7, 32) in the laboratory of Anne Kagey-Sobotka, Johns Hopkins Asthma & Allergy Center, Baltimore, Md., under a research service contract.

Postmortem pathology. Euthanized monkeys were necropsied. Tissues were processed for histopathologic studies. Tissue sections (4 to 10 μm thick) were stained with hematoxylin and eosin, and pathologic diagnoses were made by light microscopy.

Statistical analyses. Means, standard errors of the mean, the Kruskal-Wallis test for differences in antibody isotype levels in survivors and nonsurvivors, and Pearson correlation matrices were calculated by using the Statpal program (Statpal Associates, Montpelier, Vt.) (9), which was installed in a Zeos 486 personal computer. The relationship between antibody levels, route of administration of antigen, and survival was analyzed by binary logistic regression, using the BMDP statistical software package (BMDP Statistical Software, Los Angeles, Calif.).

RESULTS

Immunization routes. Forty rhesus monkeys were separated into 10 groups of four monkeys each, primed and boosted with two doses of the microsphere-encapsulated toxoid 7 weeks apart via combinations of mucosal and nonmucosal routes, and challenged with a lethal dose of aerosolized SEB 10 to 11 weeks after the booster immunization to study protective immunity. The results are summarized in Table 1. Monkeys primed i.m. followed by an i.t. booster (i.m.-i.t. immunization) all survived the SEB challenge whether the SEB aerosol was at a high (15 LD_{50}) or a low (7 LD_{50}) dose. The survival rate was also relatively high in monkeys primed via the i.t. or oral route and boosted i.t. with microspheres (i.t.-i.t. and oral-i.t. immunizations). In both the i.t.-i.t. and oral-i.t. groups, one of three monkeys survived the challenge with a high dose of SEB, and the single monkey challenged with a low dose of SEB also survived, though most monkeys were sick. When the monkeys were grouped on the basis of booster immunizations (X-i.t., X-i.m., or X-oral immunization in Table 1, where X represents

TABLE 1. Survival and illness rates in rhesus monkeys vaccinated with SEB-toxoid microspheres and challenged with SEB

Immunization route	No. of monkeys sick or that survived/no. tested					
	15 LD_{50}		7 LD_{50}		Accumulated	
	Sick	Survival	Sick	Survival	Sick	Survival
i.m.-i.t.	1/3	3/3	1/1	1/1	2/4	4/4
i.t.-i.t.	2/3	1/3	0/1	1/1	2/4	2/4
Oral-i.t.	3/3	1/3	1/1	1/1	4/4	2/4
X-i.t.[a]	6/9	5/9	2/3	3/3	8/12	8/12
i.m.-i.m.	3/3	0/3	1/1	1/1	4/4	1/4
i.t.-i.m.	3/3	0/3	1/1	1/1	4/4	1/4
Oral-i.m.	3/3	1/3	1/1	0/1	4/4	1/4
X-i.m.[a]	9/9	1/9	3/3	2/3	12/12	3/12
i.m.-oral	3/3	0/3	1/1	0/1	4/4	0/4
i.t.-oral	3/3	0/3	1/1	0/1	4/4	0/4
Oral-oral	3/3	0/3	1/1	1/1	4/4	1/4
X-oral[a]	9/9	0/9	3/3	1/3	12/12	1/12
Control	3/3	0/3	1/1	0/1	4/4	0/4

[a] Grouping of monkeys based on different priming but the same booster immunization route of i.t., i.m., or oral, represented by X-i.t., X-i.m., and X-oral, respectively. The numbers are accumulated numbers within the same group. X represents priming via the i.m., i.t., or oral route.

any primary immunization), monkeys with an i.t. booster showed the highest survival rate and the lowest illness rate compared with those with i.m. and oral boosters. The importance of the i.t. booster was further confirmed by binary logistic regression and the odds ratio test in which a monkey with an i.t. booster was seven times more likely to survive than one that was boosted via a different route. Thus, the i.t. booster immunization was the best route of eliciting immunity to SEB aerosol.

Antibody levels. To understand the source of protective immunity in the monkeys, anti-SEB antibodies in plasma and BAL samples taken before SEB challenge were quantified. The results grouped by booster immunization are shown in Fig. 1 and 2. Monkeys boosted via the i.t. route (X-i.t. monkeys) showed the highest levels of circulating IgG and IgA antibodies (Fig. 1). However, statistically, these high levels of IgG and IgA antibodies were not significantly different from those of monkeys with an i.m. booster (X-i.m. monkeys). When IgA antibodies in BAL were quantified (Fig. 2), monkeys with an i.t. booster showed the highest antibody levels. These high antibody levels in the lung and circulation of the X-i.t. monkeys were consistent with their high survival rate and low illness rate (Table 1).

Antibody levels in surviving monkeys and those that died. To further study the role of antibodies in protective immunity, antibodies in both the survivors and nonsurvivors were quantified and compared. Changes of antibody levels in the plasma before and after SEB challenge were also examined. When the antibodies were quantified shortly after SEB challenge (30 to 90 min postchallenge), there were dramatic reductions in the levels of all antibody isotypes (Fig. 3). Prominent reductions in circulating IgA and IgG antibodies were seen in the survivors (Fig. 3). When prechallenge levels of circulating antibodies in the survivors and nonsurvivors were compared (Fig. 3), the survivors had significantly higher average levels of IgG and IgA antibodies than the nonsurvivors whether they were challenged with high or low doses of SEB. IgM levels were not significantly different for survivors and nonsurvivors. These results suggest

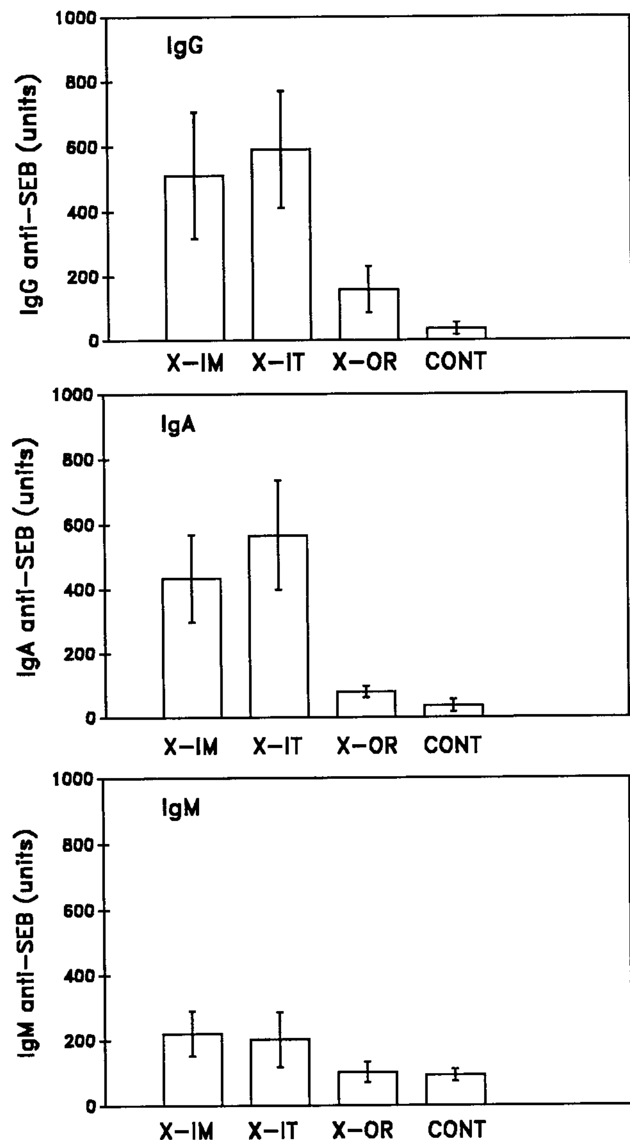

FIG. 1. Circulating antibody levels before SEB aerosol challenge in monkeys vaccinated with SEB toxoid-containing microspheres. Rhesus monkeys were primed and boosted via mucosal and nonmucosal routes with two doses of the microspheres before SEB aerosol challenge. X represents priming via the i.m. (IM), i.t. (IT), or oral (OR) route. IM, IT, and OR represent booster immunizations. CONT represents non-immunized controls. X-IM, X-IT, and X-OR groups each consist of 12 monkeys; the CONT group consists of 4 monkeys.

that antibodies in the circulation play an important role in protective immunity.

Correlation among circulating and lung antibodies. Correlation analyses were done for the circulating and lung antibodies in all survivors and nonsurvivors. No significant correlation was seen between the levels of circulating IgA antibodies and BAL IgA antibodies. The correlation between the levels of circulating IgG antibodies and BAL IgA antibodies was not statistically significant either (data not shown). Thus, the circulating antibodies and BAL antibodies may be produced and may be functioning in separate tissues.

To further study the relationship between IgG and IgA antibodies in the circulation, a correlation analysis was done and a scatter plot was made. The results showed that there is a significant correlation between the IgG and IgA antibodies in

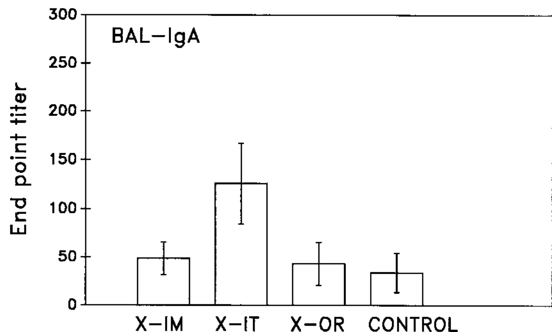

FIG. 2. BAL IgA antibody levels before SEB aerosol challenge in monkeys vaccinated with SEB toxoid-containing microspheres. Monkeys were grouped on the basis of booster immunizations. Groups are the same as given in the legend to Fig. 1.

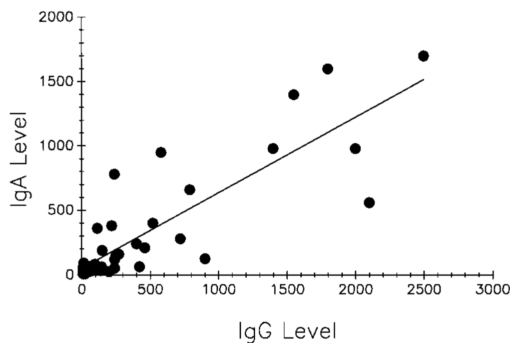

FIG. 4. Scatter plot of circulating IgG and IgA antibody levels in monkeys vaccinated with SEB toxoid-containing microspheres. There is a significant correlation between IgG and IgA antibody levels ($r = 0.85$; $P < 0.001$).

the circulation (Fig. 4) ($r = 0.85$; $P < 0.001$). Thus, the IgG and IgA antibodies in the circulation tend to associate with each other in function and production.

SEB-reactive T cells. SEB is a superantigen (10, 18, 19). It activates T cells bearing certain Vβ chains of the T-cell receptors to proliferate and results in a depletion and anergy of these T cells (10, 19, 20, 23, 24). SEB toxicosis and lethal toxic shock seem to be due to an overproduction of cytokines produced directly or indirectly by these SEB-activated T cells (18, 19). It is possible that the monkeys might have had their SEB-reactive T cells depleted or anergized after the microsphere immunization and thereby might have become tolerant to SEB aerosol intoxication, provided that the SEB toxoid in the microspheres retained superantigenicity. If SEB-reactive T cells had been depleted or anergized by the vaccination, then the protection conferred by vaccination might be attributed, completely or in part, to the lack and/or anergy of SEB-responsive T cells in the immunized monkeys. To test this possibility, circulating lymphocytes from all monkeys prior to SEB challenge were cultured with SEB as well as with ConA or LPS to study their proliferation capability. If the SEB-reactive T cells had been depleted or anergized after immunization with microspheres, a reduction in SEB-stimulated proliferation should have been seen in the lymphocyte cultures. The results showed that both the survivors and nonsurvivors had essentially the same proliferation indexes in their lymphocyte cultures stimulated with SEB, ConA, or LPS when prechallenge lymphocytes from immunized and control monkeys were compared (data

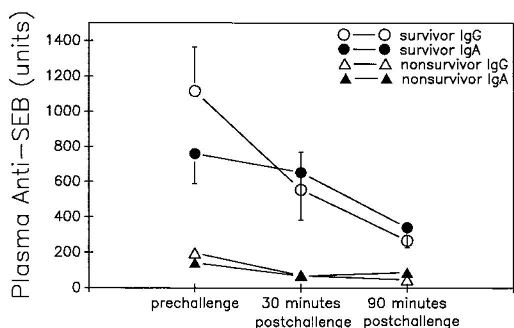

FIG. 3. Reduction of IgG and IgA antibodies shortly after SEB aerosol challenge in the survivors and nonsurvivors of the monkeys vaccinated with SEB toxoid-containing microspheres and challenged with SEB aerosol. By Kruskal-Wallis tests, prechallenge anti-SEB levels of IgG and IgA, but not IgM (not shown), were significantly different between the survivors and the nonsurvivors.

not shown). Thus, depletion and/or anergy of SEB-reactive T cells did not occur in the microsphere-immunized monkeys, and the immunity to SEB aerosol in the monkeys was not due to the depletion or anergy of SEB-reactive T cells.

Histamine release and anaphylaxis. SEB is capable of inducing degranulation of mast cells in the skin when it is injected intradermally into monkeys (28). Also, it is conceivable that aerosolized SEB may form immune complexes with anti-SEB antibodies and cause systemic anaphylaxis in immunized monkeys. To test these possibilities, histamine contents in both the plasma and BAL before and shortly after SEB challenge were quantified. The result showed that there were no significant differences in the histamine levels between immunized and control monkeys before and after SEB challenge, nor were there significant increases of histamine levels after SEB challenge in the control or immunized monkeys (data not shown). Furthermore, monkeys generally died at 40 to 65 h after SEB challenge rather than immediately after challenge, as would have been expected for an anaphylactic reaction. Additionally, postmortem histopathologic examinations did not reveal clear and prominent type III hypersensitivity reactions. Thus, the nonsurvivors did not die of allergic reactions or of anaphylaxis to SEB challenge, and the vaccination did not significantly induce allergic sensitization in the immunized monkeys.

DISCUSSION

The present study shows that SEB toxoid-containing microspheres are capable of eliciting protective immunity to a lethal aerosol challenge of SEB in rhesus monkeys when the booster vaccination is conducted via the i.t. route. This was most impressively seen when the monkeys were primed i.m. and boosted i.t. The protective immunity was highly correlated with the antibody levels in both the lung and the circulation. There were no significant decreases of SEB-induced proliferation after immunization, suggesting that the protective immunity in the monkeys was not due to a lack or tolerance of SEB-reactive T cells as a result of apoptosis or anergy. The death of nonsurvivors was consistent with the lack of antibodies rather than with possible anaphylaxis caused by allergic reactions or hypersensitivity reactions due to antigen-antibody (SEB–anti-SEB) complexes.

It is generally agreed that humoral immunity in the lung is manifested by mucosal antibodies in the upper airway and by circulating antibodies in the alveolar and lower airways (see review by Bienenstock [5]). This difference in the nature of antibody-mediated immunity is due to the differences in the anatomic structure of the mucous membrane of the respiratory

tract. Although we do not know in the present study how the SEB aerosol affects mucous tissues and from where in the airway the SEB enters the blood circulation, protective immunity was consistent with high levels of antibodies in both the lung and the circulation. This is supported by the findings that (i) antibody levels in both the lung and the circulation correlate with protective immunity; (ii) monkeys with an i.t. booster generally had the highest survival rate and lowest illness rate, which is consistent with the fact that they had the highest levels of antibodies in the circulation and in the lung; (iii) the survivors had significantly higher mean levels of antibodies than the nonsurvivors in the circulation; and (iv) shortly after SEB challenge, there was a dramatic reduction of circulating antibodies, which may have been due to their neutralization by SEB that passed through the mucous membranes into the circulation.

SEB is a superantigen (10, 18, 19, 23). It binds to and forms complexes with major histocompatibility complex class II antigens on antigen-presenting cells (10, 14, 18, 19). The SEB-class II antigen complexes are then presented to and activate T cells bearing certain Vβ chains of the T-cell receptor (10, 14, 18, 19). Activation of both the antigen-presenting cells and the T cells results in the production of large amounts of cytokines, which then appear to cause the toxicosis and toxic shock (18, 19, 23). Additionally, the T cells undergo apoptosis after SEB activation, and in the mouse a depletion of these activated T cells results in anergy or a tolerant state to a second challenge of SEB (20, 24). If the SEB toxoid-containing microspheres had retained some of the superantigenicity of native SEB, depletion and/or anergy of T cells could have occurred in the monkeys after vaccination and could have rendered the monkeys resistant to SEB aerosol challenge. To test this possibility, we cultured circulating lymphocytes from the monkeys (vaccinated and controls) and found that there is no reduction of lymphocyte proliferation in response to in vitro stimulation with SEB, ConA, or LPS. These results suggest that there was no depletion or anergy of T cells in the monkeys after the vaccinations, which is consistent with the idea that antibodies were responsible for conferring resistance to the aerosol challenge with SEB.

It has been shown that monkeys intradermally injected with SEB develop an immediate-type hypersensitivity reaction in the injected site (27, 28). This skin reaction is manifested by mast cell infiltration and mast cell degranulation (28). In lymphocyte cultures, SEB is a potent inducer of interleukin-4, a lymphokine that enhances IgE and IgG1 production (11, 30, 34). Furthermore, in the microsphere-immunized monkeys there are substantial amounts of circulating anti-SEB antibodies. One may suspect that these antibodies form immune complexes with aerosolized SEB, trigger the complement pathway, and result in systemic anaphylaxis. Thus, one may suspect that the nonsurvivors died of anaphylaxis, which could have been caused by either the mediators, the IgE anti-SEB antibodies, and/or SEB–anti-SEB immune complexes activating the complement pathway. However, our results indicate that the death of the nonsurvivors was due to insufficiency of antibodies. This is supported by the findings that (i) nonsurvivors generally had low antibody levels in the lung and circulation; (ii) there was no significant difference of histamine levels in the BAL and plasma between vaccinated and control monkeys before SEB challenge; (iii) there were no significant differences in histamine levels in the plasma of vaccinated and control monkeys after SEB challenge; (iv) the nonsurvivors did not die immediately following SEB challenge but rather mostly in the time frame of 40 to 65 h after SEB challenge, a time inconsistent with systemic anaphylaxis; and (v) postmortem histopathologic examination did not reveal clear type III hypersensitivity reactions in tissues.

Intestinal immunizations or combinations of parenteral and intestinal immunizations generally fail to elicit high antibody titers in the intestine unless a special antigen such as cholera toxin is used (8, 25). A low titer of antibodies in the intestine may be induced after long-term immunization but not by short-term immunization (25). An intriguing finding in the present study is that an i.t. booster following a priming dose via various routes was capable of conferring immunity to SEB aerosol. Monkeys boosted i.t. generally had the highest antibody levels in both the lung and the circulation. Presumably because of the high antibody levels, they had the best survival rate and the lowest illness rate. However, how the i.t. booster elicits the immunity is not clear. It is possible that the airway of the lung is relatively clean and sterile while the intestinal tract is full of food antigens and normal flora. In the lung, the antigen has essentially no competitors for access to the bronchus-associated lymphoid tissue, while in the gut, antigen has various competitors for access to the gut-associated lymphoid tissue. Therefore, i.t. immunization is more efficient in delivering antigens than intestinal immunization. This explanation is consistent with the relative ease of inducing intestinal antibodies in germfree mice (12, 16). Nevertheless, the mechanism by which an i.t. booster elicits immunity requires further study.

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