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Anti-Gamma Interferon and Anti-Interleukin-6 Antibodies Affect Staphylococcal Enterotoxin B-Induced Weight Loss, Hypoglycemia, and Cytokine Release in D-Galactosamine-Sensitized and Unsensitized Mice

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Administration of staphylococcal enterotoxin B (SEB) to BALB/c mice was found to induce a cytokine release syndrome hallmarked by weight loss and hypoglycemia. A neutralizing monoclonal antibody against gamma interferon (IFN-γ) given before SEB counteracted weight loss and prevented hypoglycemia. This protective effect of anti-IFN-γ antibody was associated with decreased IFN-γ levels in serum; tumor necrosis factor (TNF) and interleukin-6 (IL-6) levels remained unchanged. A monoclonal anti-IL-6 antibody, known for its ability to cause accumulation of biologically active IL-6 in the circulation, did not modify SEB-induced body weight loss or hypoglycemia. Levels of TNF, IFN-γ, and IL-6 in serum were all more elevated in anti-IL-6-treated mice than in corresponding SEB-challenged control mice. In D-galactosamine-sensitized mice, SEB-induced weight loss but not hypoglycemia was more severe, resulting mostly in death within 24 h. Higher levels of biologically active TNF and IFN-γ in serum were noted in these mice than in mice receiving SEB only. In D-galactosamine-sensitized mice, anti-IFN-γ antibody did prevent hypoglycemia but failed to reduce the severity of the syndrome. Again, TNF levels in anti-IFN-γ-treated mice remained unchanged. Pretreatment with anti-IL-6 antibody temporarily attenuated SEB-induced hypoglycemia in sensitized mice. Thus, at 6 h post-SEB injection, anti-IL-6-treated mice were less hypoglycemic than corresponding controls. However, at 24 h, hypoglycemia was significantly aggravated. Concomitantly, IL-6 levels were dramatically increased. Neither anti-IFN-γ nor anti-IL-6 antibody treatment modulated mortality levels in D-galactosamine-sensitized mice. The data obtained with anti-IFN-γ antibody clearly indicate that endogenous IFN-γ is instrumental in bringing about hypoglycemia and body weight loss in mice exposed to SEB but also that hypoglycemia is not a crucial determinant of mortality in D-galactosamine-sensitized mice. The data obtained with anti-IL-6 antibody indicate that endogenous IL-6 is involved in regulating the levels of TNF and IFN-γ in serum.

Superantigenic exotoxins of staphylococci and streptococci have been found to exert their often life-threatening actions by activating lymphocytes to release excessive quantities of cytokines which enter the circulation and trigger a general inflammatory response. Although many different cytokines are known to be induced in the process, pathogenic significance has been attributed particularly to one of them, namely, tumor necrosis factor alpha (TNF-α). Blockage of TNF actions in experimental models can prevent the toxic actions of bacterial superantigens (16, 17). However, in toxic syndromes which are similarly associated with cytokine release, cytokines other than TNF have been proven to be equally important in bringing about the disease manifestations. In particular, work in our laboratory has established the important contributions of gamma interferon (IFN-γ) and interleukin-6 (IL-6) in shock syndromes induced by injections of endotoxin or anti-CD3 antibody (7, 8, 15). In addition, IFN-γ has been shown to be produced in large quantities in mice challenged with superantigens (4, 17, 20). We therefore wished to examine whether release of IFN-γ and IL-6 has any role to play in toxic syndromes elicited by bacterial superantigens. As a model for studying this question, we have chosen the syndrome elicited in mice by injection of staphylococcal enterotoxin B (SEB). In unsensitized mice, this toxin induces several acute but transient changes hallmarked by rapid weight loss (12). In mice that are sensitized with the hepatotoxin D-galactosamine, injection of the toxin may lead to death (1, 5, 16, 17). We used both unsensitized and D-galactosamine-sensitized mice to study the influence of anti-IFN-γ and anti-IL-6 antibodies on the manifestations and the severity of the SEB syndrome. Since previous work (7, 9, 11, 13, 15, 21, 23) has demonstrated paradoxical increases of cytokine levels after treatment with homologous anticytokine antibodies, we also measured levels of circulating TNF, IFN, and IL-6 in antibody-treated and corresponding control mice.

MATERIALS AND METHODS

Mice. BALB/c mice (6 to 8 weeks old) were obtained from Charles River Wiga, Sulzfeld, Germany.

Reagents. SEB was obtained from Sigma Chemical Co. (St. Louis, Mo.). Monoclonal antibody against murine IFN-γ (rat immunoglobulin G2a [IgG2a]) was obtained as ascites fluid from thymusless nude mice inoculated with rat × mouse hybridoma line F3 (8). The antibody was purified by affinity chromatography with a monoclonal anti-rat kappa-chain antibody. The neutralizing titer (endpoint dilution corresponding to 50% neutralization of the antiviral effect of 30 U of mouse IFN-γ per ml on mouse L929 cells infected with mengovirus) of

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the purified antibody was $10^{-5.5}$ (IgG content, 1.4 mg/ml). Irrelevant rat IgG2a was obtained from ascites fluid of a rat plasmacytoma through the courtesy of H. Bazin, University of Louvain Medical School, Brussels, Belgium. Monoclonal antibody against murine IL-6 was prepared from ascites fluid from thymusless nude mice inoculated with the 20F3 (rat × mouse) hybridoma (American Type Culture Collection, Rockville, Md., by courtesy of DNAX, Palo Alto, Calif.) (19). The rat IgG1 antibody was purified by affinity chromatography on an anti-rat kappa-chain antibody-Sepharose column. The neutralizing titer (endpoint dilution corresponding to 50% neutralization of the cell growth effect of 10 U of murine IL-6 per ml [7]) was $10^{-5.5}$ (IgG content, 1.7 mg/ml). Hybridoma GL113 producing irrelevant rat IgG1 was obtained from DNAX. All SEB solutions and batches of anti-IFN-γ, anti-IL-6, and control antibodies were tested for endotoxin content by a chromogenic *Limulus* amoebocyte lysate assay (KabiVitrum, Stockholm, Sweden) and were used only if found to contain less than 2 ng of endotoxin per ml.

Experimental protocol. Mice received intraperitoneal (i.p.) injections of SEB dissolved in 100 μl of pyrogen-free saline on day 0. Mice injected with only 100 μl of saline served as controls. D-Galactosamine (30 mg in 100 μl of saline, administered i.p.) was injected 1 h before the SEB challenge. Some groups of mice were treated (i.p.) with solutions of anti-IFN-γ (1.4 mg/ml) or anti-IL-6 (1.7 mg/ml) antibody on day −1 (0.2 ml) and day 0 (0.1 ml). As controls, mice were injected with similar quantities of irrelevant rat IgG (IgG2a and IgG1, respectively). The treatment schedule and dosing are based on previous work concerning the effects of anti-IFN-γ and anti-IL-6 antibodies in endotoxin- and anti-CD3-induced shock syndromes (7, 8, 15). Animals were scored for the occurrence of piloerection, diarrhea, and weight loss at the times before and after SEB indicated below. Diarrhea was scored as present or absent. Changes in body weight are expressed as percent changes at the indicated days relative to the day of SEB challenge.

Cytokine assays. Blood samples were taken from the orbital sinus and were allowed to clot at room temperature for about 1 h and at 4°C overnight. Serum samples were stored at −20°C until titration. IFN-γ concentrations were determined by titration on first-passage mouse embryo fibroblasts using a cytopathic effect inhibition assay with mengovirus as a challenge. Results are expressed in international units per milliliter (referring to standard preparation NIH Gg 02-901-533), defined as the reciprocal of the dilution corresponding to half-maximal inhibition of cytopathic effect. In neutralization experiments, sera were preincubated for 2 h in triplicate with 30 μl of a solution of anti-IFN-γ or anti-IFN-α/β antibody (both antibodies had a neutralizing titer of $10^{-4.0}$ U/ml against 30 U of mouse IFN-γ or IFN-α/β per ml [6]). TNF levels in sera were determined by using a cytotoxicity assay with WEHI 164 cells (2). Biological activity of IL-6 was assayed by its growth-promoting effect on the 7TD1 B-cell hybridoma (24). IFN-γ, TNF, and IL-6 assays are described in detail elsewhere (7).

Glucose determination. Blood samples were taken from the orbital sinus and collected in tubes that were kept on ice and contained heparin. Plasma was centrifuged within 30 min after collection. Levels of glucose in plasma were measured with a commercially available glucose assay system (Trinder, no. 315; Sigma Chemical Co.).

Statistical evaluation. Unless otherwise indicated, all data are expressed as means ± standard errors (SE). Differences between groups were analyzed statistically by the Mann-Whitney U test. A P of <0.05 is considered significant.

RESULTS

Diarrhea, weight loss, and death in BALB/c mice challenged with SEB or with D-galactosamine and SEB. Groups of unsensitized and D-galactosamine-sensitized BALB/c mice were injected with different doses of SEB and were observed for occurrence of piloerection, diarrhea, weight loss, and mortality. As shown in Table 1, SEB-challenged mice suffered a dose-dependent weight loss. All mice injected with doses of 40 μg or more showed piloerection. Diarrhea was observed at 6 h post-SEB injection, but its incidence was not dose related. None of the unsensitized mice died, not even those challenged with the highest dose of 300 μg of SEB. The injection of D-galactosamine 1 h before the administration of SEB dramatically lowered the dose of SEB needed to induce severe weight loss. The combined injection of D-galactosamine and SEB also caused mortality which was not seen in mice receiving SEB only. From these data, the 50% lethal doses of SEB were calculated and found to be >300 and 16.9 μg for unsensitized and D-galactosamine-sensitized mice, respectively.

Influence of anti-IFN-γ and anti-IL-6 treatment on body weight loss and mortality in SEB-challenged mice. Four groups of five mice each were injected with 100 μg of SEB.

TABLE 1. Weight loss and mortality in BALB/c mice challenged with SEB or D-galactosamine and SEB

Challenge[a]		No. of mice with diarrhea/total[b]	Body weight change[c] at:		Mortality at 48 h (no. dead/total)
D-Gal (mg)	SEB (μg)		24 h	48 h	
		0/3	− 0.2 ± 1.9	3.6 ± 0.8	0/3
	20	0/2	− 2.1 ± 0.5	4.3 ± 0.3	0/2
	40	0/2	− 5.1 ± 0.9	1.6 ± 2.2	0/2
	80	1/3	− 5.5 ± 0.2	− 4.0 ± 2.4	0/3
	100	2/4	− 6.8 ± 1.0	− 6.3 ± 1.0	0/4
	200	0/2	− 7.1 ± 2.8	− 9.3 ± 1.7	0/2
	300	1/2	− 8.4 ± 0.1	− 10.5 ± 1.0	0/2
30	12	2/4	− 7.7 ± 1.9	− 10.3 ± 1.8	1/4
30	20	0/3	− 9.0	− 9.5	2/3[d]
30	30	1/3	− 8.7 ± 2.8		3/3[e]

[a] Groups of BALB/c mice were injected with SEB (in 100 μl of saline, i.p.) at 0 h. D-Galactosamine (D-Gal) (in 100 μl of saline, i.p.) was injected 1 h before SEB. The first group of mice received 100 μl of saline (i.p.) instead of SEB.
[b] Diarrhea was noted as present or absent at 6 h.
[c] Expressed as percent change (mean ± SE) at the indicated time relative to 0 h.
[d] Two mice died within 24 h.
[e] One mouse died within 24 h.

Prior to this injection, two of the groups were treated with either anti-IFN-γ or anti-IL-6 antibody. A third group was treated with irrelevant rat IgG2a. Mice injected with saline instead of SEB were included as further controls. Body weight changes measured during the 7-day course of the experiment are shown in Fig. 1. Control mice receiving SEB started to lose weight within 24 h and lost weight until day 2. Thereafter, the animals regained body weight. Pretreatment of the mice with anti-IFN-γ antibody protected against body weight loss, whereas treatment with anti-IL-6 antibody had no effect. The results of this experiment were confirmed by a series of additional experiments in which not only the effects of anti-IFN-γ antibody but also those of anti-IL-6 antibody on SEB-induced body weight loss were studied (Table 2). In addition, to study

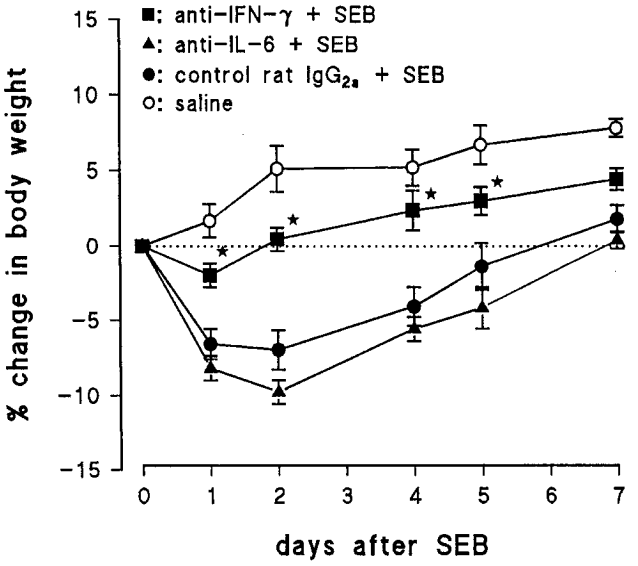

FIG. 1. Body weight loss in mice given 100 μg of SEB (in 100 μl of saline i.p. on day 0) and modulation by anti-IFN-γ and anti-IL-6 antibodies (200 and 100 μl i.p. on days −1 and 0, respectively; see Materials and Methods). Points are averages ± SE for five mice. ★, $P < 0.05$ for comparison with corresponding value for control rat IgG2a group.

TABLE 2. Influence of treatment with anti-IFN-γ or anti-IL-6 on body weight loss and mortality in mice challenged with SEB or D-galactosamine and SEB[a]

Expt no.[b]	Pretreatment			Challenge[c]		Body weight change[d] at:		Mortality at 48 h (no. dead/total)
	Anti-IFN-γ	Anti-IL-6	Rat IgG	D-Gal (mg)	SEB (μg)	24 h	48 h	
2	+	−	−		50	− 0.5 ± 1.1[e]	3.5 ± 1.7	0/8
	−	−	+		50	− 5.5 ± 0.6	1.9 ± 2.3	0/5
	−	−	−			1.5 ± 0.6	3.9 ± 1.0	0/4
3	+	−	−		100	− 3.0 ± 0.5[e]	− 4.4 ± 0.7[e]	0/5
	−	+	−		100	− 7.1 ± 0.5	− 5.9 ± 1.2	0/5
	−	−	+		100	− 6.0 ± 0.5	− 8.1 ± 0.6	0/5
4	+	−	−		200	− 6.2 ± 0.4[e]	− 7.5 ± 0.7[e]	0/3
	−	+	−		200	− 8.7 ± 0.8	− 14.0 ± 0.5	0/3
	−	−	+		200	− 9.3 ± 0.6	− 13.0 ± 1.0	0/3
5	+	−	−	30	12	− 5.3 ± 1.0	− 2.9 ± 1.8	2/5
	−	+	−	30	12	− 5.7 ± 0.8	− 7.8 ± 1.1	2/5
	−	−	+	30	12	− 5.4 ± 0.6	− 7.8 ± 1.2	1/5
	−	−	−	30		0.4 ± 1.3	0.8 ± 0.9	0/3
	+	−	−			1.0 ± 1.0	− 0.3 ± 1.0	0/3
6	+	−	−	30	20	ND	ND	4/5
	−	+	−	30	20	ND	ND	5/5
	−	−	+	30	20	ND	ND	4/5
	−	−	−	30	20	ND	ND	3/3
7	+	−	−	30	30			2/2[f]
	−	+	−	30	30	− 10.4	− 14.2	1/2
	−	−	+	30	30			2/2[f]

[a] For standard time schedule of injections, see the legend to Fig. 1.
[b] Results of experiment 1 are shown in Fig. 1.
[c] Mice were injected with SEB (in 100 μl of saline, i.p.) at 0 h. D-Galactosamine (D-Gal) (in 100 μl of saline, i.p.) was injected 1 h before SEB.
[d] Percent change (mean ± SE) at the indicated time relative to 0 h. ND, not determined.
[e] Statistical analysis: $P < 0.05$ for comparison with irrelevant rat IgG-treated mice.
[f] All mice died within 24 h.

the influence on mortality scores, some mice were sensitized with D-galactosamine. Data from these experiments are assembled in Table 2. In contrast to its protective effect against body weight loss in SEB-challenged mice, anti-IFN-γ antibody failed to affect weight loss (experiment 5) and mortality (experiments 5 through 7) in mice sensitized with D-galactosamine and challenged with SEB. It can also be seen that neither D-galactosamine nor anti-IFN-γ by itself influenced body weight changes (experiment 5).

Hypoglycemia in mice challenged with SEB: protective effects of anti-IFN-γ antibody. Hypoglycemia is a regularly observed phenomenon in shock syndromes elicited by lipopolysaccharide (LPS) or anti-CD3 antibody (15, 21, 25, 26). In view of the effects of anti-IFN-γ and anti-IL-6 antibodies on the gross manifestations of these syndromes, we wished to analyze blood glucose levels in mice challenged with SEB and treated with anti-IFN-γ or anti-IL-6 antibody. As shown in Fig. 2A, SEB injected into unsensitized mice at a dose of 100 μg caused significant hypoglycemia at 6 h postinjection ($P < 0.01$ for comparison with mice that were not challenged with SEB), and blood glucose levels were completely restored at 24 h. Pretreatment with anti-IFN-γ antibody significantly antagonized development of SEB-induced hypoglycemia. Anti-IL-6 antibody had no such effect.

After sensitization with D-galactosamine (Fig. 2B), a dose of 20 μg of SEB was lethal for almost all mice, including those pretreated with anti-IL-6 or anti-IFN-γ antibody. Remarkably, however, SEB-induced hypoglycemia at 6 h in sensitized mice was less pronounced than that in unsensitized mice. However, no hypoglycemia at all was observed in anti-IFN-γ and anti-IL-6 antibody-treated mice. Since all of these mice died within 24 h, an additional experiment in which mice were sensitized with D-galactosamine and challenged with a still smaller dose of SEB (12 μg) was done. Again, at 6 h, this dose of SEB induced only mild hypoglycemia (119 ± 18.5 mg/dl for mice that were treated with irrelevant antibody versus 151 ± 8.8 mg/dl for unchallenged mice injected with D-galactosamine only). At 24 h, hypoglycemia was still present but was much

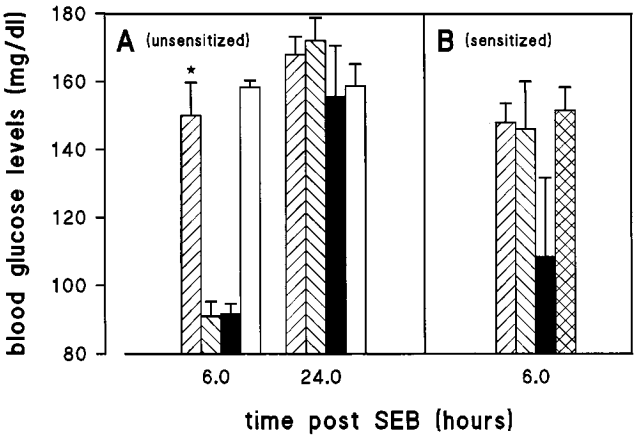

FIG. 2. Hypoglycemia in unsensitized mice given 100 μg of SEB (A) and in mice sensitized with 30 mg of D-galactosamine and given 20 μg of SEB (B) and modulation by anti-IFN-γ and anti-IL-6 antibodies (see the legend to Fig. 1). ★, $P < 0.05$ for comparison with control rat IgG2a group. Symbols: □, saline only; ▨, SEB plus anti-IFN-γ; ▧, SEB plus anti-IL-6; ■, SEB plus control rat IgG2a; ▩, D-galactosamine only.

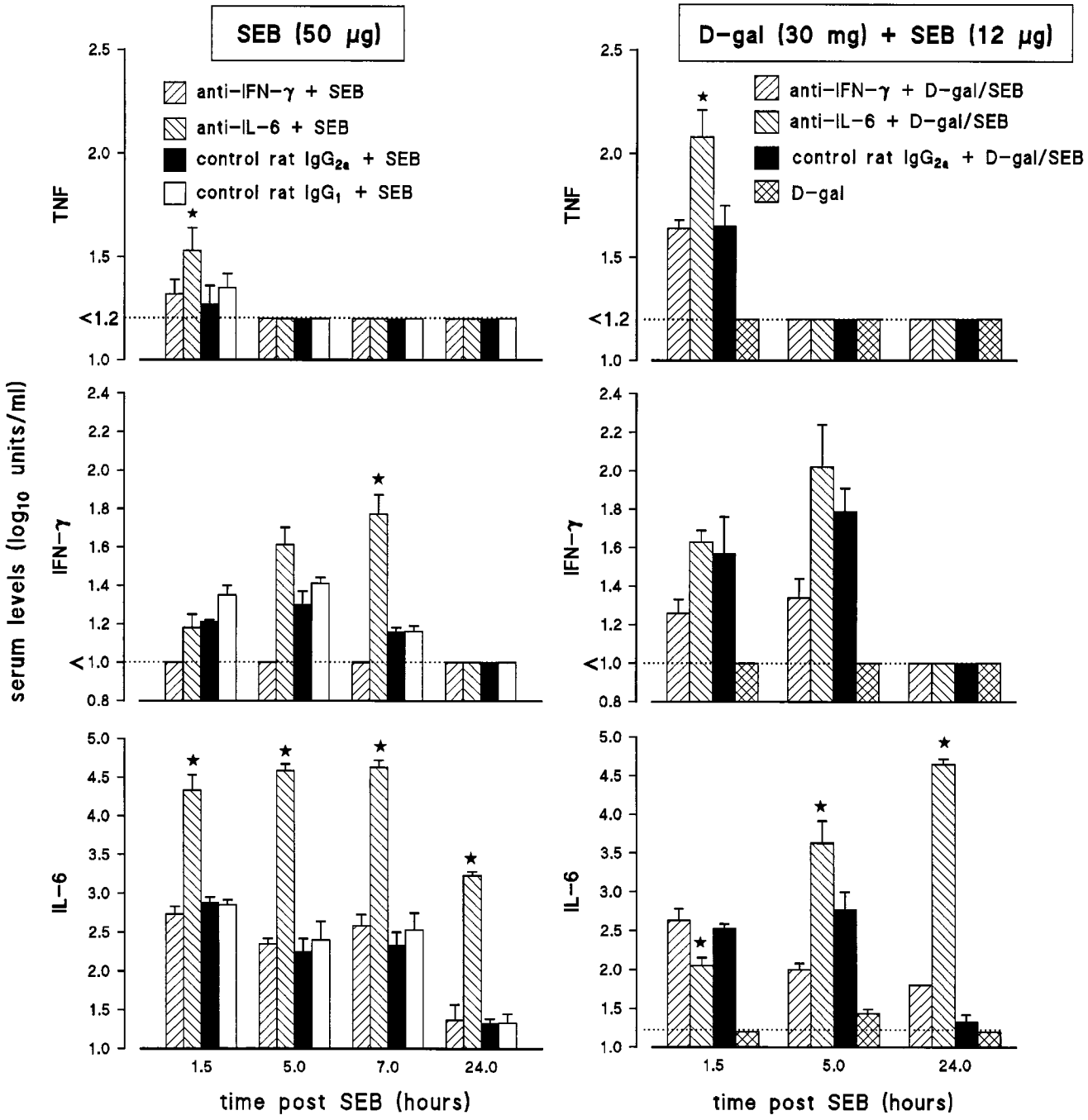

FIG. 3. Levels of cytokines in sera of mice given SEB alone or D-galactosamine (D-gal) plus SEB and pretreated with anti-IFN-γ antibody, anti-IL-6 antibody, or irrelevant rat IgG (for treatment schedule, see the legend to Fig. 1). Bars show averages ± SE for four mice. ★, $P < 0.05$ for comparison with control rat IgG1 + SEB group or control rat IgG2a + D-gal/SEB group.

more pronounced in mice treated with anti-IL-6 antibody (21 ± 2.8 versus 119 ± 21.2 mg/dl for SEB-challenged mice treated with anti-IL-6 and irrelevant antibodies, respectively, and 154 ± 8.7 mg/dl for unchallenged mice injected with D-galactosamine only). In contrast, anti-IFN-γ antibody antagonized development of hypoglycemia at both times (142 ± 3.2 and 131 ± 3.5 mg/dl at 6 and 24 h, respectively).

Modulation of TNF, IFN, and IL-6 levels in serum by anti-IFN-γ and anti-IL-6 antibody treatment in mice challenged with SEB or with D-galactosamine and SEB. Figure 3 shows the results of two experiments in which mice were injected with SEB (50 μg) or with D-galactosamine (30 mg) plus SEB (12 μg). In both experiments, groups of four mice were pretreated with anti-IFN-γ antibody, anti-IL-6 antibody, or control irrelevant IgG. Blood samples were taken at the indicated times and were titrated for TNF, IFN, and IL-6. As can be seen, TNF was detectable only at 1.5 h post-SEB injection. In all D-galactosamine-sensitized groups, the levels of TNF were significantly higher than those in corresponding unsensitized mice ($P < 0.05$). Anti-IFN-γ did not modify TNF levels. In contrast,

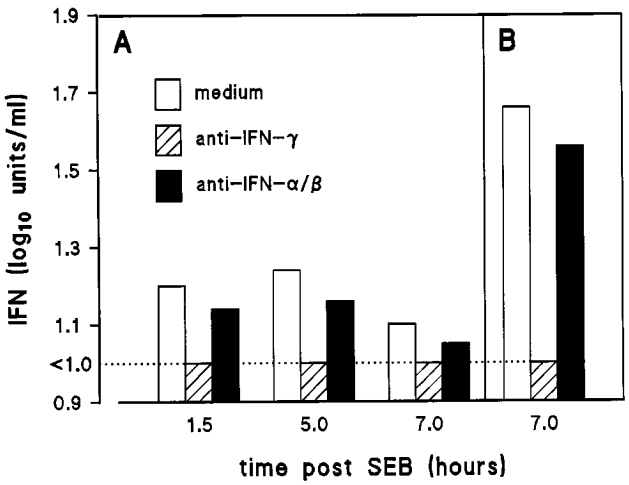

FIG. 4. Characterization of antiviral activity in sera of mice given 50 μg of SEB and pretreated with irrelevant IgG2a (A) or anti-IL-6 antibody (B) (for treatment schedule, see the legend to Fig. 1). Sera in each group of mice were pooled and preincubated with either medium, anti-IFN-γ antibody, or anti-IFN-α/β antibody as described in Materials and Methods.

anti-IL-6 administration was associated with increased titers of TNF in serum.

SEB injection also induced the release of an IFN-like antiviral activity into the circulation that was detectable from 1.5 to 7.0 h post-SEB challenge. Here again, the levels of this activity were higher in mice sensitized with D-galactosamine than in unsensitized mice (Fig. 3; $P < 0.05$ at 5 h). Characterization with neutralizing antibodies against IFN-γ and against IFN-α/β revealed that the serum antiviral activity resulted from IFN-γ (data for sera of unsensitized mice challenged with SEB are shown in Fig. 4; similar results were obtained with samples from D-galactosamine-sensitized mice [data not shown]). Treatment of the mice with anti-IFN-γ antibody led to disappearance of detectable IFN-γ activity in the sera of unsensitized mice but did not do so, or did so to a lesser extent, in D-galactosamine-sensitized mice. Increased serum antiviral activity was noted after anti-IL-6 antibody treatment, particularly at 7 h in mice given 50 μg of SEB. Here again, preincubation of the sera with anti-IFN-γ or anti-IFN-α/β antibody revealed that the antiviral activity was attributable to IFN-γ (Fig. 4B).

IL-6 was detectable in the circulation of SEB-challenged mice at all times tested. In contrast to TNF and IFN-γ levels, the levels of IL-6 were not higher in D-galactosamine-sensitized mice than in mice challenged with only SEB. Anti-IFN-γ did not significantly modify IL-6 levels. However, anti-IL-6 antibody treatment paradoxically resulted in significantly higher IL-6 levels, except at 1.5 h, in D-galactosamine-sensitized mice.

DISCUSSION

We have previously established the existence of a causal relationship between IFN-γ production and body weight loss in tumor-bearing mice (14). In addition, we have observed protective effects of anti-IFN-γ and anti-IL-6 antibodies on the physical signs and mortality associated with the Shwartzman reaction elicited by endotoxin (7, 8) or in the anti-CD3-induced cytokine release syndrome (15). A syndrome somewhat reminiscent of these manifestations is known to develop in mice challenged with superantigens (4, 12, 16, 17, 20). Therefore, in the present study, we investigated the possible involvement of IFN-γ and IL-6 in the pathogenesis of SEB-induced toxicity in mice that are either unsensitized or sensitized with D-galactosamine. We confirmed the finding that injection of SEB in BALB/c mice causes a potentially lethal syndrome and found it to be characterized by piloerection, precipitous weight loss, and hypoglycemia. In accordance with previous reports (1, 5, 16, 17), we also found that pretreatment with D-galactosamine sensitized the mice to these effects of SEB.

In unsensitized mice, administration of anti-IFN-γ antibody prior to SEB attenuated weight loss and prevented hypoglycemia. Concomitantly, levels of circulating IFN-γ were annihilated, but the levels of TNF and IL-6 remained unchanged. These data indicate that endogenous IFN-γ contributes to the syndrome in much the same way as it does in the anti-CD3 syndrome. We observed protective effects of anti-IFN-γ against the metabolic alterations induced by anti-CD3 without effects on the TNF and IL-6 levels in serum (15). In the LPS-induced generalized Shwartzman reaction, anti-IFN-γ has also been described to exert a beneficial effect that is, however, associated with a reduction in the titers of circulating TNF (8). Therefore, in the SEB- and anti-CD3-induced reactions, the contribution of IFN-γ does not seem to consist of augmentation of TNF production but, rather, in activation of some other pathway, for instance, production of more-distal mediators such as NO or stimulation of leukocyte adhesion to endothelia.

In D-galactosamine-sensitized mice, anti-IFN-γ antibody retained its protective effect against hypoglycemia but failed to reduce the severity of the syndrome. This finding contrasts with the results obtained by Nagaki et al. (17), who found anti-IFN-γ antibody to exert a slight protective effect against lethality of SEB in D-galactosamine-sensitized mice. However, in that study, mice were sensitized with a lower dose of D-galactosamine (20 mg) in combination with a higher dose of SEB (100 μg). It thus appears that anti-IFN-γ antibody can provide protection against SEB-induced pathology except in mice that are sensitized with high doses of D-galactosamine. Similarly, we have reported that anti-IFN-γ fails to attenuate LPS-associated reactions in D-galactosamine-sensitized mice despite providing significant protection in unsensitized mice (8). Clearly, galactosamine-sensitized mice develop lethal pathology that is independent of endogenous IFN-γ, either because lethal phenomena other than those in unsensitized mice are triggered or because IFN-γ-triggered pathways are somehow mimicked by galactosamine intoxication. It cannot be questioned that TNF plays a critical role in mediating lethal toxicity in D-galactosamine-sensitized mice: D-galactosamine makes mice exquisitely sensitive to TNF (10); accordingly, D-galactosamine-sensitized mice can be protected against LPS toxicity by anti-TNF antibody (3). Similarly, in the D-galactosamine–SEB syndrome, anti-TNF antibody treatment has been found to exert significant protection (16, 17). Clearly, TNF is the critical cytokine in the lethal toxicity associated with SEB or LPS challenge in D-galactosamine models. Therefore, the failure of anti-IFN-γ antibody to protect against SEB-induced body weight loss in the galactosamine model could be due to the high TNF levels. Indeed, TNF has been shown to be involved in weight loss (18, 22). TNF, however, seems not to be involved in hypoglycemia, since anti-IFN-γ provided complete protection against hypoglycemia in both sensitized and unsensitized mice. Likewise, TNF is not a critical mediator in LPS-mediated hypoglycemia (21, 25, 26).

A second aspect of our investigation was the role of endogenous IL-6. Its contribution was likewise studied by administration of a specific antibody. Although this antibody does possess in vitro neutralizing activity for IL-6, its affinity is

relatively low, so in vivo, its neutralizing potential is easily overrun when endogenous IL-6 production persists. In fact, in different in vivo model systems, we and others have noted that pretreatment with anti-IL-6 antibodies causes paradoxically increased levels of biologically active IL-6, resulting from accumulation of antigen-antibody complexes (7, 9, 11, 13, 15, 21, 23). These complexes are less effectively cleared than uncomplexed IL-6 and continue to release biologically active IL-6 even when endogenous production has subsided. It can be inferred that IL-6 produced in anti-IL-6 antibody-pretreated mice is neutralized only in the very initial phases of the syndrome and becomes available in increased quantities in later phases. Effects of anti-IL-6 antibody on levels of other cytokines (TNF and IFN-γ) in blood, on hypoglycemia, and on severity of the SEB syndrome therefore cannot be unequivocally interpreted in terms of neutralization or enhancement of IL-6 availability. It seems that endogenous IL-6 does not participate in the control of the syndrome. In mice that were not sensitized with D-galactosamine, anti-IL-6 antibody did not modify SEB-induced body weight loss and did not prevent hypoglycemia. IL-6 levels in anti-IL-6-treated mice were enhanced, as expected. However, TNF and particularly IFN-γ levels were also more elevated, and this may have accounted for the failure to protect against SEB-induced hypoglycemia. We did, indeed, previously find that anti-IL-6 antibody provides significant protection in anti-CD3-induced hypoglycemia. However, in that system, it was not associated with increased TNF or IFN-γ levels (15).

In mice that had been sensitized with D-galactosamine, anti-IL-6 antibody caused augmentation of levels of cytokines (TNF, IFN-γ, and IL-6) in addition to the augmentation already caused by D-galactosamine sensitization (TNF and IFN-γ). This added augmentation was not translated in more-manifest disease signs or mortality, conceivably because a maximum level of toxicity had already been reached. Remarkable was the effect of anti-IL-6 antibody on the hypoglycemia, which was initially attenuated but then dramatically aggravated, both in apparent concordance with the height of the IL-6 levels. It thus appears that augmented IL-6 levels (as caused by anti-IL-6 antibody treatment) are associated with more-severe hypoglycemia in galactosamine-sensitized mice but with unaltered (SEB model) or attenuated (anti-CD3 model) hypoglycemia in unsensitized mice. This difference may be accounted for by effects of D-galactosamine on liver metabolism which confound the induction and action of cytokines. Hypoglycemia, although severe, does not seem to be a crucial determinant of mortality in cytokine release syndromes. Indeed, in D-galactosamine-sensitized and SEB-challenged mice, anti-IFN-γ prevented hypoglycemia without protection against mortality. Furthermore, in LPS-challenged mice, anti-TNF antibody was reported to protect against mortality (3) but not against hypoglycemia (21, 26).

In conclusion, our study demonstrates that endogenous IFN-γ and IL-6 are differently involved in superantigen-induced manifestations in D-galactosamine-sensitized and unsensitized mice. It thus appears that anti-IFN-γ antibody can provide protection against SEB-induced body weight loss except in mice that are sensitized with high doses of D-galactosamine. The data obtained with anti-IL-6 antibody indicate that endogenous IL-6 is involved in regulating levels of TNF and IFN-γ in serum. Thus, anti-IL-6 antibody treatment was associated with increased TNF, IFN-γ, and IL-6 levels, and this may have accounted for the failure to provide any significant protection against SEB-induced pathology.

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