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Treatment with Homodimeric Interleukin-12 (IL-12) p40 Protects Mice from IL-12-Dependent Shock but Not from Tumor Necrosis Factor Alpha-Dependent Shock

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The role of interleukin-12 (IL-12) was investigated in different shock models using anti-IL-12 reagents. IL-12 is composed of two disulfide-bonded subunits, p35 and p40. The IL-12 p40 homodimer $(p40)_2$ has been shown to be a potent IL-12 antagonist in vitro. We investigated its in vivo inhibitory capacity in different shock models of mice. We could demonstrate that $(p40)_2$ is able to protect mice from septic shock in primarily IL-12-dependent models such as the Shwartzman reaction and lipopolysaccharide (LPS)-induced shock, whereas $(p40)_2$ has no effect in the tumor necrosis factor alpha-dependent LPS/D-GalN shock model. In IL-12-dependent shock models, $(p40)_2$ inhibits IL-12-induced gamma interferon production and thereby interferes with the cascade of cytokine release, finally leading to death.

Septic shock results from uncontrolled sequential release of cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), IL-6, and gamma interferon (IFN-γ) in response to infection with gram-negative bacteria (1, 4, 5, 7, 23, 25, 26) and in response to endotoxins. Septic shock is of clinical relevance in spite of adequate antibiotic and supportive therapy.

In mice, several different lethal shock syndromes are known. The so-called Shwartzman reaction is induced by two consecutive injections of lipopolysaccharide (LPS) (3). A priming dose of LPS, injected intradermally in the footpad, is followed 24 h later by an intravenous challenge LPS injection. After this challenge injection that is not lethal without the priming injection, the mice die within 48 h from disseminated intravascular coagulation, vascular occlusion, hemorrhage, perivascular accumulation of leukocytes, and necrosis (24). This hypersensitivity reaction occurs only as a consequence of careful dosage and timing of LPS injections and needs specific routes of administration. The Shwartzman reaction is elicited by induced endogenous factors acting in a precise sequence. LPS induces the release of IL-12, which induces the production of IFN-γ, which finally primes macrophages (and other cell types). Upon LPS challenge, the lethal reaction is induced by a massive production of inflammatory cytokines by sensitized macrophages: TNF-α and IL-1 are thought to be the lethal effector molecules acting on target sites already sensitized by IFN-γ.

The second investigated shock model is induced by an intraperitoneal (i.p.) injection of an appropriate dose of LPS (LPS shock). In this model, it was reported that administration of polyclonal anti-IL-12 immunoglobulin G (IgG) substantially reduced LPS induction of IFN-γ production, but effects of anti-IL-12 on mortality were not described (13).

The third investigated shock model is induced by i.p. injection of LPS and D-galactosamine (LPS/D-GalN shock). It has been shown that D-GalN dramatically sensitizes mice to the lethal effect of LPS (8). Studies of TNF receptor (TNFR) p55-deficient mice revealed that lethality in this model depends on TNFR p55-mediated activation (20, 22). The effects of IL-12 blockade in this model have not been previously reported.

The purpose of this study was to characterize the role of IL-12 in shock induction in these various models. Furthermore, we were interested in testing whether IL-12 p40 homodimer $(p40)_2$, which has been shown in vitro to act as an IL-12 receptor antagonist (11, 16), is able to prevent IL-12-dependent activities in vivo. It was previously shown in both binding assays and bioassays in vitro that mouse IL-12 p40 dimer is 25- to 50-fold more potent as an IL-12 antagonist than mouse IL-12 p40 monomer (11). It could not be excluded that the lower levels of antagonist activity observed for preparations of purified p40 monomer were mediated, at least in part, by a small amount of contaminating dimer. In preliminary studies, the pharmacokinetics of mouse p40 dimer in mice appeared to be very similar to those of mouse IL-12 heterodimer, with a terminal elimination serum half-life of 3 to 5 h. Thus, in the present study, we focused on the potential in vivo antagonist activity of recombinant homodimeric IL-12 p40.

MATERIALS AND METHODS

Mice. Specific-pathogen-free female NMRI and C57BL/6 mice, 8 to 10 weeks of age, were purchased from Biological Research Laboratory (Füllinsdorf, Switzerland).

Reagents. (i) LPS. *Serratia marcescens* LPS (Sigma Chemical Co., St. Louis, Mo.) and *Salmonella abortus-equi* LPS (SEBAK, Aidenbach, Germany) were dissolved in pyrogen-free saline to 10 mg/ml and sterilized by filtration through 0.22-μm-pore-size filters. Aliquots were stored frozen at −20°C.

(ii) IL-12 p40 homodimer. Dimeric p40 was produced by CHO cells stably transfected with mouse IL-12 p40 cDNA and purified as described previously (9). Purified p40 homodimer was >95% pure, as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; endotoxin contamination was <10 IU/mg of p40 protein, as measured by the *Limulus* amebocyte lysate assay.

(iii) Antibodies. Rat monoclonal antibodies (MAbs) XMG1,2 and AN18 are IgG1 antibodies against mouse IFN-γ (mIFN-γ) (6, 21). Rat MAb GR20 is an IgG2a antibody specific for the mIFN-γ receptor (2). MAbs C15.6.7 and 10F6 neutralize mIL-12 activity (10, 27). Irrelevant rat IgG was from Sigma. All antibodies contained <0.05 IU/mg of protein, as detected by the *Limulus* amebocyte lysate assay. TNFR p55 IgG was produced and purified as described previously (14).

Shwartzman shock. The generalized Shwartzman reaction was elicited by two consecutive injections of *Serratia marcescens* LPS in female NMRI mice, 8 to 10

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TABLE 1. Homodimeric IL-12 p40 prevents mortality in the Shwartzman reaction

Treatment	No. of expt	No. of survivors/no. tested	% Survival
PBS	7	3/35	9
Anti-IFN-γ[a]	7	35/35	100[e]
Anti-IL-12[b]	1	4/5	80[e]
Rat IgG	2	0/10	0
$(p40)_2$[c]			
100 μg	4	19/20	95[e]
10 μg	2	2/10	20
1 μg	1	0/5	0
TNFR IgG[d]	2	2/10	20

[a] 10 μg of rat anti-mIFN-γ MAb XMG1,2 was administered i.p. at time of priming.

[b] 100 μg of rat anti-mIL-12 MAb 10F6 was administered i.p. at time of priming and 10 and 23 h later.

[c] Indicated doses of homodimeric p40 were administered i.p. at time of priming and 10 and 23 h later.

[d] 20 μg of TNFR IgG was administered i.p. at time of priming and 22 and 24 h later.

[e] Significantly different ($P < 0.002$) from values for PBS- or IgG-treated control groups as calculated by Fisher's exact test.

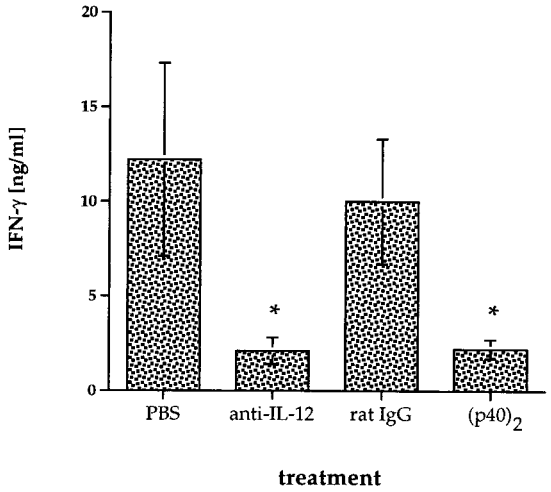

FIG. 1. Homodimeric p40 decreases IFN-γ serum levels in the Shwartzman reaction. Groups of five NMRI mice received two consecutive injections of LPS. For priming, 5 μg of LPS was given in the footpad, followed 24 h later by a challenge with 400 μg of LPS. Furthermore, mice were injected i.p. with 100 μg of anti-IL-12 MAb C15.6.7 (or irrelevant rat IgG) at time of priming or with 100 μg of $(p40)_2$ (or PBS) at time of priming and 10 and 23 h later. IFN-γ serum levels were determined 6 h after the challenge LPS injection. To facilitate IFN-γ determination, all mice were injected i.p. with MAb GR20 (200 μg) directed against the IFN-γ receptor at the time of LPS challenge. *, significantly different ($P < 0.001$) from values for PBS- or IgG-treated control groups as calculated by Student's *t* test.

weeks old (19). The priming injection was given in the footpad and was followed 24 h later by a challenge LPS injection given intravenously. The optimal doses of LPS were determined for each batch of LPS and ranged between 1 and 5 μg for the footpad injection and between 200 and 400 μg for the intravenous injection. Mortality was monitored after 24, 48, 72, and 96 h. No further mortality was seen after 96 h. In all experiments described, MAbs and IL-12 p40 homodimer were given i.p.

Endotoxin-induced shock. The LPS shock was induced in female C57BL/6 mice, 8 to 10 weeks old, by the i.p. injection of *S. abortus-equi* LPS (350 μg in 0.2 ml of sterile PBS per mouse). With a dose of 350 μg per mouse, the 100% lethal dose is almost reached (see Table 2). Mortality was monitored 24, 48, and 72 h after LPS injection.

LPS/D-GalN-induced shock. Female C57BL/6 mice, 8 to 10 weeks old, were injected i.p. with a mixture of *S. abortus-equi* LPS (0.1 μg in 0.2 ml of sterile PBS per mouse) and D-GalN (10 mg in 0.2 ml of sterile PBS per mouse; Roth, Karlsruhe, Germany) in order to sensitize to the lethal effects of LPS (8). Mortality was monitored after 24, 48, and 72 h.

Detection of serum IFN-γ levels. IFN-γ was measured by enzyme-linked immunosorbent assay using rat IgG1 MAbs AN18 and XMG1,2 (6, 21). The detection limit of this assay was 40 pg/ml.

RESULTS

Homodimeric p40 prevents mortality in the Shwartzman reaction. Groups of five NMRI mice received two consecutive injections of LPS. The priming injection was given in the footpad and was followed 24 h later by the intravenous challenge injection. The reaction was lethal only if the mice received both the preparative and the challenge injections. A single footpad or a single intravenous injection was ineffective in inducing shock.

It had been shown earlier that one single injection of MAb neutralizing mIFN-γ activity protects mice from lethality when given i.p. at time of priming (19). Similar results were obtained with MAb against mIL-12. Confirmatory data are presented in Table 1: MAb directed either against IFN-γ or against IL-12 rescued mice from the shock reaction. Furthermore, Table 1 shows that $(p40)_2$ was able to protect mice in a dose-dependent manner. Intraperitoneal injection of $(p40)_2$ (100 μg/mouse) at the time of priming and 10 and 23 h later resulted in 95% survival of the animals. Application of less $(p40)_2$ (Table 1) and application of $(p40)_2$ fewer than three times (not shown) resulted in a decreased percentage of survival.

The TNFR IgG was shown to be protective in the LPS/D-GalN-induced shock syndrome (14) (see below). Table 1 demonstrates that TNFR IgG had no positive effect on survival in the experimental Shwartzman shock syndrome when given at the time of priming and during challenge.

IFN-γ has been shown to be necessary for the manifestation of lethality in the generalized Shwartzman reaction. Since $(p40)_2$ inhibits IL-12-induced IFN-γ production in vitro (11, 16), we examined the effect of $(p40)_2$ application on IFN-γ serum levels. For determination of IFN-γ, mice were sacrificed and heart blood was collected. Peak IFN-γ levels were reached 6 h after the LPS challenge injection. Figure 1 demonstrates that $(p40)_2$ decreased IFN-γ serum levels comparably to a MAb neutralizing IL-12. The average amount of IFN-γ serum level compared to the control group was reduced about fivefold in mice treated with anti-IL-12 reagents. These results clearly indicate that $(p40)_2$ is a potent IL-12 antagonist in vivo.

Homodimeric p40 prevents mortality in endotoxin-induced shock. To further confirm that $(p40)_2$ inhibited IL-12 activity in vivo, we investigated its influence in another LPS shock syndrome (LPS shock). In this model, groups of five C57BL/6 mice were injected i.p. with 350 μg of LPS (*S. abortus-equi*) per animal.

For pretreatment, mice received MAb neutralizing IL-12 activity or $(p40)_2$ 24, 12, and 1 h before LPS application. For determination of IFN-γ serum levels, blood was taken from the tail vein. As can be seen in Table 2, application of anti-IL-12 MAb 10F6 or $(p40)_2$ raised the survival rate to 90 and 93%, respectively, whereas only 12% of the mice in the PBS-treated group survived. In this model, TNFR IgG was found to have a partial positive effect on survival, because the proportion of survivors was raised to 70%. In addition, combined administration of TNFR IgG and IL-12 p40 homodimers was able to completely protect mice from LPS-induced shock (data not shown).

Anti-IL-12 MAb 10F6 as well as $(p40)_2$ were able to decrease dramatically peak serum IFN-γ levels, measured 8 h after LPS application (Fig. 2). IFN-γ serum levels were five- to

TABLE 2. Homodimeric IL-12 p40 prevents mortality in the endotoxin-induced shock syndrome

Treatment	No. of expt	No. of survivors/no. tested	% Survival
PBS	5	3/25	12
Anti-IL-12[a]	2	9/10	90[d]
$(p40)_2$[b]	3	14/15	93[d]
TNFR IgG[c]	2	7/10	70[d]

[a] 100 μg of rat anti-mIL-12 MAb 10F6 was administered i.p. 24, 12, and 1 h before LPS challenge.
[b] 100 μg of homodimeric p40 was administered i.p. 24, 12, and 1 h before LPS challenge.
[c] 20 μg of TNFR IgG was administered i.p. 1 h before LPS challenge.
[d] Significantly different ($P < 0.002$) from value for PBS-treated control group as calculated by Fisher's exact test.

TABLE 3. Homodimeric IL-12 p40 does not prevent mortality in the LPS/D-GalN-induced shock syndrome

Treatment	No. of expt	No. of survivors/no. tested	% Survival
PBS	4	0/20	0
Anti-IL-12[a]	2	0/10	0
$(p40)_2$[b]	2	2/10	20
TNFR IgG[c]	4	18/20	90[d]

[a] 100 μg of rat anti-mIL-12 MAb 10F6 was administered i.p. 24, 12, and 1 h before LPS/D-GalN challenge.
[b] 100 μg of homodimeric p40 was administered i.p. 24, 12, and 1 h before LPS/D-GalN challenge.
[c] 20 μg of TNFR IgG was administered i.p. 1 h before LPS/D-GalN challenge.
[d] Significantly different ($P < 0.0001$) from value for PBS-treated control group as calculated by Fisher's exact test.

sixfold lower in animals treated with IL-12 antagonists than in control mice. It should be mentioned that IFN-γ levels correlated well with survival or death of individual animals (data not shown). The potency of $(p40)_2$ as IL-12 antagonist in vivo was demonstrated again.

Homodimeric p40 does not prevent mortality in LPS/D-GalN-induced shock. The next investigated shock syndrome was induced by the application of LPS (0.1 μg/mouse) together with D-GalN (10 mg/mouse) to groups of five C57BL/6 mice.

For pretreatment, mice were injected i.p. with IL-12-neutralizing MAb or $(p40)_2$ 24, 12, and 1 h before, or with TNFR IgG 1 h before, application of LPS/D-GalN. TNFR IgG has been shown to be protective in this model (14). Table 3 shows that TNFR IgG raises the proportion of surviving animals to 90%, whereas no animal in the control group survived. Neither anti-IL-12 MAb 10F6 nor $(p40)_2$ had a protective effect in this model. In addition, MAbs neutralizing IFN-γ had no effect (not shown). IL-12 seems to play a minor role, if any, in the LPS/D-GalN-induced shock syndrome.

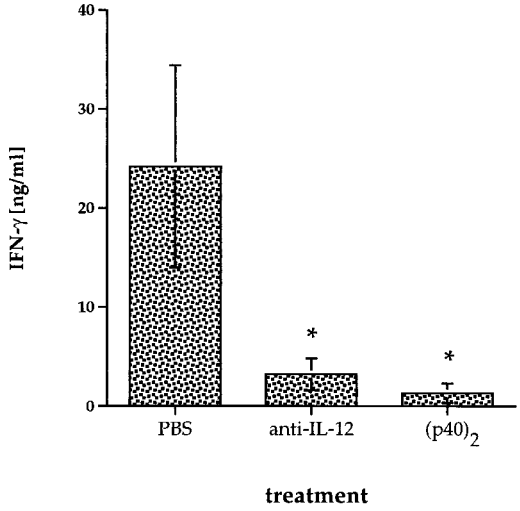

FIG. 2. Homodimeric p40 decreases IFN-γ serum levels in endotoxin-induced shock syndrome. Groups of 5 C57BL/6 mice were injected i.p. with 350 μg of LPS (*S. abortus-equi*). For pretreatment, mice received MAb neutralizing IL-12 (clone 10F6, 200 μg) or $(p40)_2$ (100 μg) 24, 12, and 1 h before LPS application. IFN-γ serum levels were determined 8 h after LPS injection. *, significantly different ($P < 0.001$) from value for PBS-treated control group as calculated by Student's *t* test.

DISCUSSION

Table 4 summarizes our findings. In experimental shock syndromes with IL-12 playing the central role, such as the Shwartzman reaction and endotoxin-induced shock, $(p40)_2$ is able to prevent mortality from mice, thus demonstrating its potency as an IL-12 antagonist in vivo. In both models, $(p40)_2$ strongly reduced IL-12-induced IFN-γ. In these models, TNFR IgG does not provide full protection even though a certain effect can be seen in endotoxin-induced shock. In contrast, TNFR IgG can rescue mice from LPS/D-GalN-induced shock, a model in which anti-IL-12 or anti-IFN-γ reagents have no effect.

Recently a new cytokine called IFN-γ-inducing factor (now called IL-18) was described (18). This factor was found to be critical in the induction of liver injury caused by endotoxin exposure of mice treated with *Propionibacterium acnes*. Treatment of mice with anti-IL-18 antibodies was able to prevent liver damage (18). In mice sensitized to the lethal effect of LPS by treatment with the avirulent bacille Calmette-Guérin (BCG) vaccine strain of *Mycobacterium bovis*, neutralizing anti-IL-12 antibodies were able to protect from shock-induced death (28). These studies suggest that IL-18 and/or IL-12 plays a critical role in development of septic shock. Potentially they act in synergy (17), and neutralizing one of both cytokines is sufficient for protection.

Our results obtained with MAb against IL-12 very closely parallel data published by Wysocka et al. for BCG-primed mice challenged with LPS (28). In this study, neutralizing anti-IL-12 antibodies were shown to inhibit IFN-γ production and mortality elicited by LPS. These results were confirmed by a recent report showing a fivefold reduction of serum IFN-γ levels in IL-12-deficient mice compared to wild-type mice upon challenge with LPS (15).

We have found significant reduction of serum IFN-γ levels after LPS challenge of mice treated with either anti-IL-12 MAb or homodimeric IL-12 p40. Preliminary data indicated that serum TNF-α was not reduced following treatment (data not shown). Similar results were published very recently by

TABLE 4. Prevention of shock by TNFR-IgG, homodimeric IL-12 p40, and anti-IFN-γ in different models

Shock model	Prevention of shock by:		
	TNFR-IgG	$(p40)_2$	Anti-IFN-γ
Shwartzman	No	Yes	Yes
LPS	Partial	Yes	Yes
LPS/D-GalN	Yes	No	No

Heinzel et al., who found significantly reduced serum levels of IFN-γ, but no change in the concentration of TNF-α and IL-12 heterodimer, in mice challenged with LPS and treated with recombinant homodimeric IL-12 p40 (12). These data suggest that homodimeric IL-12 p40 and anti-IL-12 MAb specifically block the interaction of IL-12 with its receptor, thereby antagonizing cellular activation, which results in selective inhibition of IFN-γ production. Interestingly, treatment with anti-IL-12 antibodies and homodimeric IL-12 p40, thereby targeting the ligand or the receptor, respectively, is similarly effective. Proinflammatory cytokines other than IFN-γ, such as TNF-α, appear to be regulated independently of IL-12. Moreover, our data show that selective inhibition of IL-12-induced IFN-γ production is sufficient to protect mice from the lethal effects of LPS.

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REFERENCES

1. **Alexander, H. R., G. M. Doherty, C. M. Buresh, D. J. Venzon, and J. A. Norton.** 1991. A recombinant human receptor antagonist to interleukin 1 improves survival after lethal endotoxemia in mice. J. Exp. Med. **173:**1029–1032.
2. **Basu, M., J. L. Pace, D. M. Pinson, M. P. Hayes, P. P. Trotta, and S. W. Russel.** 1988. Purification and partial characterization of a receptor protein for mouse interferon-γ. Proc. Natl. Acad. Sci. USA **85:**6282–6286.
3. **Billiau, A.** 1988. Gamma-interferon: the match that lights the fire? Immunol. Today **9:**37–40.
4. **Car, D. B., V. M. Eng, B. Schnyder, L. Ozmen, S. Huang, P. Gallay, D. Heumann, M. Aguet, and B. Ryffel.** 1994. Interferon γ receptor deficient mice are resistant to endotoxic shock. J. Exp. Med. **179:**1437–1444.
5. **Cerami, A., Y. Ikeda, N. Le Trang, P. J. Hotez, and B. Beutler.** 1985. Weight loss associated with an endotoxin-induced mediator from peritoneal macrophages: the role of cachectin (tumor necrosis factor). Immunol. Lett. **11:** 173–177.
6. **Cherwinski, H. M., J. H. Schumacher, K. D. Brown, and T. R. Mosmann.** 1987. Two types of mouse helper T cell clone. 3. Further differences in lymphokine synthesis between TH1 and TH2 clones revealed by RNA hybridisation, functionally monospecific bioassays and monoclonal antibodies. J. Exp. Med. **166:**1229–1244.
7. **Doherty, G. M., J. R. Lange, H. N. Langstein, H. R. Alexander, C. M. Buresh, and J. A. Norton.** 1992. Evidence for IFN-gamma as a mediator of the lethality of endotoxin and tumor necrosis alpha. J. Immunol. **149:**1666–1670.
8. **Galanos, C., M. A. Freudenberg, and W. Reutter.** 1979. Galactosamine-induced sensitization to the lethal effects of endotoxin. Proc. Natl. Acad. Sci. USA **76:**5939–5943.
9. **Gately, M. K., D. M. Carvajal, S. E. Connaughton, S. Gillessen, R. R. Warrier, K. D. Kolinsky, V. L. Wilkinson, C. M. Dwyer, G. F. Higgins, F. J. Podlaski, D. A. Faherty, P. C. Familletti, A. S. Stern, and D. H. Presky.** 1996. Interleukin-12 antagonist activity of mouse interleukin-12 p40 homodimer in vitro and in vivo. Ann. N. Y. Acad. Sci. **795:**1–12.
10. **Gately, M. K., A. G. Wolitzky, P. M. Quinn, and R. Chizzonite.** 1992. Regulation of human cytolytic lymphocyte responses by interleukin-12. Cell. Immunol. **143:**127–142.
11. **Gillessen, S., D. Carvajal, P. Ling, F. J. Podlaski, D. L. Stremlo, P. C. Familletti, U. Gubler, D. H. Presky, A. S. Stern, and M. K. Gately.** 1995. Mouse interleukin-12 (IL-12) p40 homodimer: a potent IL-12 antagonist. Eur. J. Immunol. **25:**200–206.
12. **Heinzel, F. P., A. M. Hujer, F. N. Ahmed, and R. M. Rerko.** 1997. In vivo production and function of IL-12 p40 homodimers. J. Immunol. **158:**4381–4388.
13. **Heinzel, F. P., R. M. Rerko, P. Ling, J. Hakimi, and D. Schoenhaut.** 1994. Interleukin-12 is produced in vivo during endotoxemia and stimulates synthesis of gamma interferon. Infect. Immun. **62:**4244–4249.
14. **Lesslauer, W., H. Tabuchi, R. Gentz, M. Brockhaus, E. J. Schlaeger, G. Grau, P. F. Piguet, P. Pointaire, P. Vassalli, and H. Loetscher.** 1991. Recombinant soluble tumor necrosis factor receptor proteins protect mice from lipopolysaccharide-induced lethality. Eur. J. Immunol. **21:**2883–2886.
15. **Magram, J., S. E. Connaughton, R. R. Warrier, D. M. Carvajal, C.-Y. Wu, J. Ferrante, C. Stewart, U. Sarmiento, D. A. Faherty, and M. K. Gately.** 1996. IL-12 deficient mice are defective in IFN-γ production and type 1 cytokine responses. Immunity **4:**471–481.
16. **Mattner, F., S. Fischer, S. Guckes, S. Jin, H. Kaulen, E. Schmitt, E. Rüde, and T. Germann.** 1993. The interleukin-12 subunit p40 specifically inhibits effects of the interleukin-12 heterodimer. Eur. J. Immunol. **23:**2202–2208.
17. **Micallef, M. J., T. Ohtsuki, K. Kohno, F. Tanabe, S. Ushio, M. Namba, T. Tanimoto, K. Torigoe, M. Fujii, M. Ikeda, S. Fukuda, and M. Kurimoto.** 1996. Interferon-gamma-inducing factor enhances T helper 1 cytokine production by stimulated human T cells: synergism with interleukin-12 for interferon-gamma production. Eur. J. Immunol. **26:**1647–1651.
18. **Okamura, H., H. Tsutsui, T. Komatsu, M. Yutsudo, A. Hakura, T. Tanimoto, K. Torigoe, T. Okura, Y. Nukada, K. Hattori, K. Akita, M. Namba, F. Tanabe, K. Konishi, S. Fukuda, and M. Kurimoto.** 1995. Cloning of a new cytokine that induces IFN-γ production by T cells. Nature **378:**88–91.
19. **Ozmen, L., M. Pericin, J. Hakimi, R. A. Chizzonite, M. Wysocka, G. Trinchieri, M. Gately, and G. Garotta.** 1994. Interleukin 12, interferon γ, and tumor necrosis factor α are the key cytokines of the generalized Shwartzman reaction. J. Exp. Med. **180:**907–915.
20. **Pfeffer, K., T. Matsuyama, T. M. Kundig, A. Wakeham, K. Kishihara, A. Shahinian, K. Wlegmann, P. S. Ohashi, M. Kronke, and T. W. Mak.** 1993. Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to L. monocytogenes infection. Cell **73:**457–467.
21. **Prat, M., G. Gribaudo, P. Comoglio, G. Cavallo, and S. Landolfo.** 1984. Monoclonal antibodies against murine gamma interferon. Proc. Natl. Acad. Sci. USA **81:**4515–4519.
22. **Rothe, J., W. Lesslauer, H. Loetscher, Y. Lang, P. Koebel, F. Kontgen, A. Althage, R. Zinkernagel, M. Steinmetz, and H. Bluethmann.** 1993. Mice lacking the tumor necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by Listeria monocytogenes. Nature **364:**798–802.
23. **Starnes, H. F., M. K. Pearce, A. Tewari, J. H. Yim, J. Zou, and J. S. Abrams.** 1990. Anti-IL-6 monoclonal antibodies protect against lethal Escherichia coli infection and lethal tumor necrosis factor-alpha challenge in mice. J. Immunol. **145:**4185–4191.
24. **Thomas, L., and R. A. Good.** 1952. Studies on the generalized Shwartzman reaction. I. General observations concerning the phenomenon. J. Exp. Med. **96:**605–611.
25. **Tracey, K. J., B. Beutler, S. F. Lowry, J. Merryweather, S. Wolpe, I. W. Milsark, R. J. Hariri, T. J. Fahey, A. Zentella, J. D. Albert, G. T. Shires, and A. Cerami.** 1986. Shock and tissue injury induced by recombinant human cachectin. Science **234:**470–474.
26. **Ulich, T. R., K. Guo, B. Irwin, D. G. Remick, and G. N. Davatelis.** 1990. Endotoxin-induced cytokine gene expression in vivo. II. Regulation of tumor necrosis factor and interleukin-I alpha/beta expression and suppression. Am. J. Pathol. **137:**1173–1185.
27. **Wilkinson, V. L., R. R. Warrier, T. P. Truitt, P. Nunes, M. K. Gately, and D. H. Presky.** 1996. Characterization of anti-mouse IL-12 monoclonal antibodies and measurement of mouse IL-12 by ELISA. J. Immunol. Methods **189:**15–24.
28. **Wysocka, M., M. Kubin, L. Q. Vieira, L. Ozmen, G. Garotta, P. Scott, and G. Trinchieri.** 1995. Interleukin-12 is required for interferon-γ production and lethality in lipopolysaccharide-induced shock in mice. Eur. J. Immunol. **25:** 672–676.

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