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Role and regulation of IL-12 in the *in vivo* response to staphylococcal enterotoxin B

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Abstract

Injection of a staphylococcal superantigen (SAg) such as staphylococcal enterotoxin B (SEB) in adult mice results in cytokine production and cell proliferation which can lead to septic shock. The aim of the present work was to identify the cytokines and co-stimulatory molecules regulating the *in vivo* systemic release of IFN-γ, a cytokine known to play an important role in the pathophysiology associated with bacterial infections. We demonstrate in this study that (i) in contrast to lipopolysaccharide (LPS), SEB administration induces high levels of the p70, bioactive form, of IL-12; (ii) IL-12 production in response to SEB requires both CD40-dependent signals and IFN-γ secretion; (iii) the early systemic release of IFN-γ (3 h post-treatment) in response to SEB is IL-12 independent, while the sustained, late response (6–9 h post-treatment) requires endogenous IL-12 production; (iv) IL-12 produced during the primary SEB response (day 0) is responsible for priming cells *in vivo* to high IFN-γ production upon secondary challenge (day 2); (v) the priming effect of IL-12 is TCR unrelated, as SEB-primed animals secrete high levels of IFN-γ in response to both staphylococcal enterotoxin A and LPS administered 48 h later. The ability of bacterial SAg to induce septic shock and to modulate the immune response to unrelated antigens may therefore be related to their unique capacity to induce systemic IL-12 production *in vivo.* These observations also help to explain why SEB-primed animals, known to express an anergic phenotype 48 h post-treatment (as judged by defective IL-2 production and proliferation), nevertheless display an increased capacity to secrete the inflammatory cytokine IFN-γ.

Introduction

Bacterial superantigens (SAg) comprise a large group of proteins produced by several bacterial strains, including *Staphylococcus aureus* and *Staphylococcus pyrogenes*, that have long been implicated in the pathogenesis of toxic shock-like syndromes in both human (1,2) and animal models (3). The pathophysiology of *S. aureus* infections is related to the ability of these SAg to interact simultaneously with selected V_β regions of the clonally distributed TCR and with a conserved domain of the class II molecule (MHC II), leading to polyclonal T cell activation *in vivo* and systemic release of proinflammatory cytokines (3). The current interest in bacterial SAg stems not only from their role in septic shock, but also because of their profound effect on the immune system. In particular, the *in vivo* immune response to the *S. aureus* SAg staphylococcal enterotoxin B (SEB) represents a useful model for studying *in vivo* cytokine regulation and has given insight into the phenomenon of peripheral T cell unresponsiveness (4,5). Indeed, following their initial clonal expansion and cytokine production, SEB-reactive T cells are thought to become unresponsive to further TCR stimulation. Lack of *in vivo* anamnestic response to SEB has been considered to represent a fail-safe mechanism limiting the development of dangerous inflammatory responses. However, it has been recently recognized that, although lymphocytes previously exposed to SEB displayed a reduced proliferative response, they retained the ability to respond to TCR stimulation by expressing several cytokines. In a recent study, lymphocytes from SEB-treated animals were found to produce reduced levels of the IL-2, IL-3 and IL-4 mRNA in response to *in vitro* stimulation, while producing normal levels of IL-6, IL-10, IFN-γ and tumor necrosis factor-α under the same experimental setting (6). This study confirmed previous observations reporting that the proinflammatory cytokine IFN-γ was insensitive to the SEB-induced *in vivo* anergy, as it was produced at normal (7) or even at increased levels (8) upon secondary SEB exposure. This finding is particularly intriguing as

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numerous observations indicate that IFN-γ is critically involved in the pathogenesis of the inflammatory response induced by bacterial SAg (8).

The important role of IFN-γ in the pathophysiology of septic shock and the finding that production of this cytokine is not down-regulated during the SEB-induced phase of T cell unresponsiveness prompted us to study the regulation of IFN-γ secretion in response to SEB. Our observations indicate that SEB induces *in vivo* the systemic release of IL-12, a cytokine known to promote IFN-γ production. We demonstrate herein that endogenous IL-12 up-regulates IFN-γ production during primary SEB sensitization and is responsible for the enhanced IFN-γ response upon secondary SEB challenge. Unexpectedly, this priming effect was not restricted to SEB-reactive cells, suggesting that SEB-induced IL-12 may act in a non-cognate fashion to promote *in vivo* inflammatory responses to unrelated antigens.

Methods

Mice

Female BALB/c mice, 6–8 weeks old, were purchased from Charles River Wiga (Sulzfeld, Germany) and maintained in a pathogen-free environment in our own animal facility. Mice genetically deficient for IFN-γ were purchased from the Jackson Laboratory (Bar Harbor, ME). Mice genetically deficient for the p40 chain of IL-12 (9) were kindly provided by J. Magram (New Jersey). All knockout mice used in this study were backcrossed into the BALB/c background.

Reagents and antibodies

The following antibodies to murine determinants were used in this study: GL1 (anti-CD86, rat IgG2a) (10), C17.8.20.15 and C15.1 (anti-p40IL-12, rat IgG2a, a kind gift of Dr Trinchieri), MR1 (anti-CD40L, hamster Ig) (11), and PARSI-19 (hamster anti-ARS, produced in our laboratory). Highly purified staphylococcal enterotoxin A (SEA) and SEB were purchased from Toxin Technology (Sarasota, FL). Lipopolysaccharide (LPS) (*Escherichia coli* serotype 0127:B8) was from Sigma (St Louis, MO).

Treatment protocols

Mice were injected i.p.(pretreatment) with the indicated doses of SEB, LPS, purified recombinant mIL-12 (100 ng, kindly provided by S. Wolf, Cambridge), ascitic fluids or purified antibodies (1 mg) 1 h before i.v. injection of the indicated doses of SEB or LPS.

Determination of cytokine levels

Serum IL-2 content was determined by ELISA, using the rat anti-mIL-2 mAb S4B6 (available through the ATCC, Rockville, MD) as capture reagent and a rabbit anti-mIL-2 serum (produced in our laboratory) as revealing reagent. Standard curves were generated using supernatant containing murine IL-2 (produced in our laboratory) and results are expressed as arbitrary U/ml. IFN-γ levels were determined by two-site ELISA using anti-IFN-γ mAb F1 and Db-1, as previously described (12). IL-12-related molecules were identified by ELISA using antibodies specific for p40 (clone 5D9 and SC3) and p70 (clone 9A5), and provided by D. Presky (13). Standard curves were generated using purified recombinant murine IFN-γ (kindly provided by Dr Billiau, KUL, Leuven) and supernatant containing recombinant murine IL-12 (kindly provided by Dr Thielemans, VUB, Brussels). Results are expressed as arbitrary U/ml.

Results

Systemic production of IL-12 p70 heterodimer in response to SEB

To evaluate the presence and thus the possible regulatory role of IL-12 during the *in vivo* response to bacterial SAg, adult BALB/c mice were injected with SEB, and the systemic production of IL-2, IFN-γ and both p40 and p70 immunoreactive forms of IL-12 assayed by ELISA. The *in vivo* response to LPS, a bacterial compound known to cause septic shock in a T cell-independent fashion, has been studied in parallel and will serve as an internal control. As expected from previous studies, SEB induced high levels of IL-2 and IFN-γ (8), while LPS injection only led to a marginal production of IFN-γ (14) (Fig. 1). SEB also induced detectable levels of both p40 and p70 molecules, while LPS led to the strong accumulation of p40 molecules. Of note, SEB-induced serum IL-2 appeared early during the response and peaked 2–4 h after injection, while IFN-γ and IL-12-related p70 heterodimer displayed a slower kinetics (peak production at 6–9 h). The low IFN-γ response induced by LPS also correlated with the weak *in vivo* secretion of potentially bioactive IL-12 p70 heterodimer (15). This analysis revealed therefore that in contrast to LPS, SEB administration led to the production of significant levels of potentially bioactive p70 IL-12 heterodimers.

Complex co-stimulatory requirement for SEB-induced IFN-γ production during primary sensitization

The role of the accessory cell-derived molecules CD86 and IL-12 in the *in vivo* response of naive BALB/c mice to SEB was determined by injecting saturating doses of blocking antibodies before SEB administration, and by monitoring systemic IL-2 and IFN-γ production in the serum. As previously demonstrated, antibodies to CD80 did not interfere with the *in vivo* immune response to SEB and have been omitted for simplicity (16 and data not shown). Antibodies to CD86 inhibited the *in vivo* IL-2 production in response to SEB (Fig. 2A), but only moderately affected the production of IFN-γ (Fig. 2B). Antibodies to IL-12 inhibited IFN-γ production to SEB (in particular the late response at 6 and 9 h, see Fig. 2B), while leading to increased IL-2 serum levels (see Fig. 2A). Antibodies to IL-12, but not to CD86, strongly inhibited the *in vivo* production of IFN-γ following LPS administration (Fig. 2C). A combination of antibodies to CD86 and IL-12 led to a marked, although not complete, inhibition of IFN-γ production in response to SEB (Fig. 2B). These observations were corroborated by studies conducted in mice genetically deficient for the IFN-γ or the IL-12 p40 gene. Mice deficient for the IL-12 p40 gene were able to produce IL-2 in response to SEB, while their production of IFN-γ rapidly decayed (Fig. 3). IFN-$\gamma^{-/-}$ mice failed to produce high levels of IL-12 in response

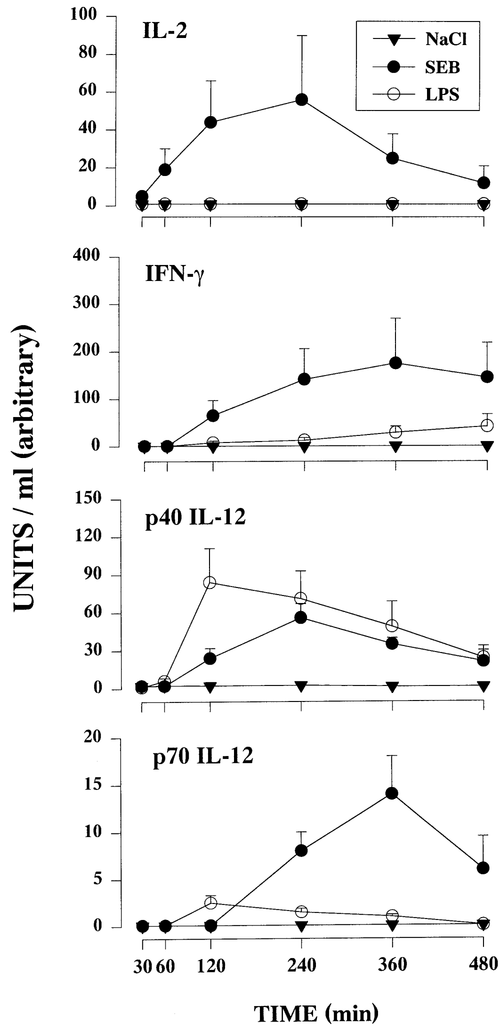

Fig. 1. SEB-induced p40 and p70 IL-12 production *in vivo*. Animals (six per group) were injected i.v. with 50 μg of SEB or LPS, as indicated. Control mice received an equivalent amount of pyrogen-free saline. Mice were bled at various times, and the serum IL-2, IL-12 and IFN-γ levels determined by ELISA as described in Methods. Results are expressed as arbitrary U/ml ± SD of individual determinations. These results are representative of three independent experiments.

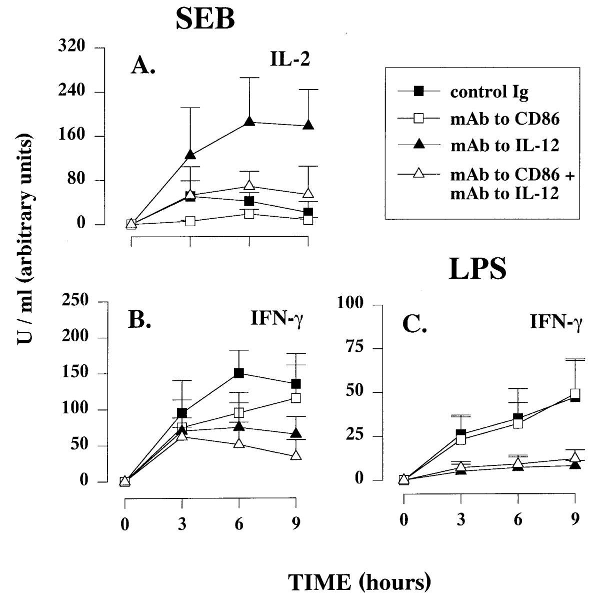

Fig. 2. Role of CD86 and IL-12 in the *in vivo* immune response to SEB. Animals (six per group) were pretreated (i.p. 1 mg) with a mAb to CD86 or with a combination of mAb to IL-12 (clones C15.1 and C17.8, 0.5 mg each), 1 h before i.v. challenge with 50 μg of SEB or LPS. Control mice were treated with an equivalent amount of a control hamster mAb (clone PARSI 19) or with a polyclonal source of rat Ig. Mice were bled 3, 6 and 9 h later, and the serum IL-2 and IFN-γ content determined by ELISA. Results are expressed as arbitrary U/ml ± SD of individual determinations. These results are representative of three independent experiments.

to SEB, while secreting optimal levels of IL-2 (Fig. 3). Note that IFN-γ-deficient mice retained the ability to produce p40-containing molecules during the early phase of the response.

Interaction of antigen-presenting cell (APC)-derived CD40 molecules with CD40 ligand (CD40L) proteins (gp39) expressed by activated T cells has been shown to play an important role in IL-12 production by accessory cells interacting with antigen-specific T cells (17). The role of CD40–CD40L interaction in SAg-induced IL-12 release has been ascertained by pretreating naive mice with blocking antibodies to CD40L before SEB and LPS administration. Figure 4 shows that in mice injected with SEB, antibodies to CD40L strongly inhibited the production of both IL-12-related molecules (p40 and p70 immunoreactive forms) and IFN-γ, while leaving IL-2 production unaffected. As expected, the

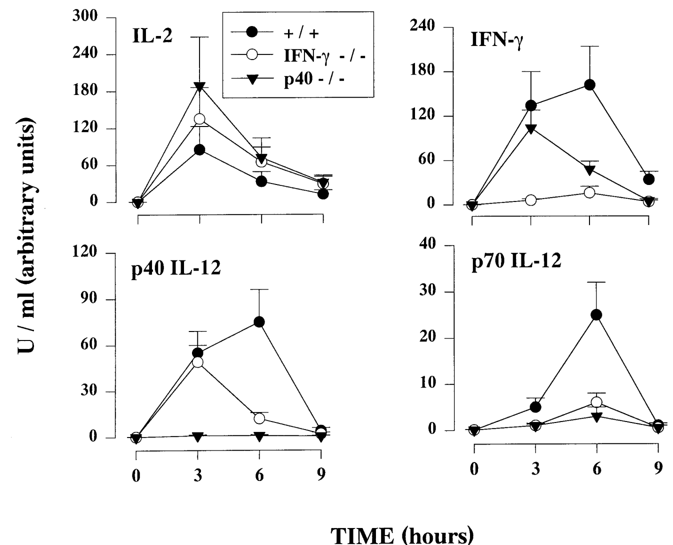

Fig. 3. IFN-γ and IL-12 cross-regulation during the *in vivo* response to SEB. Genetically modified and control animals (three per group) were injected i.v. with 50 μg SEB and the systemic release of IL-2, IFN-γ, p40 and p70 IL-12 molecules determined by ELISA. Results are expressed as arbitrary U/ml ± SD of individual determinations. These results are representative of three independent experiments.

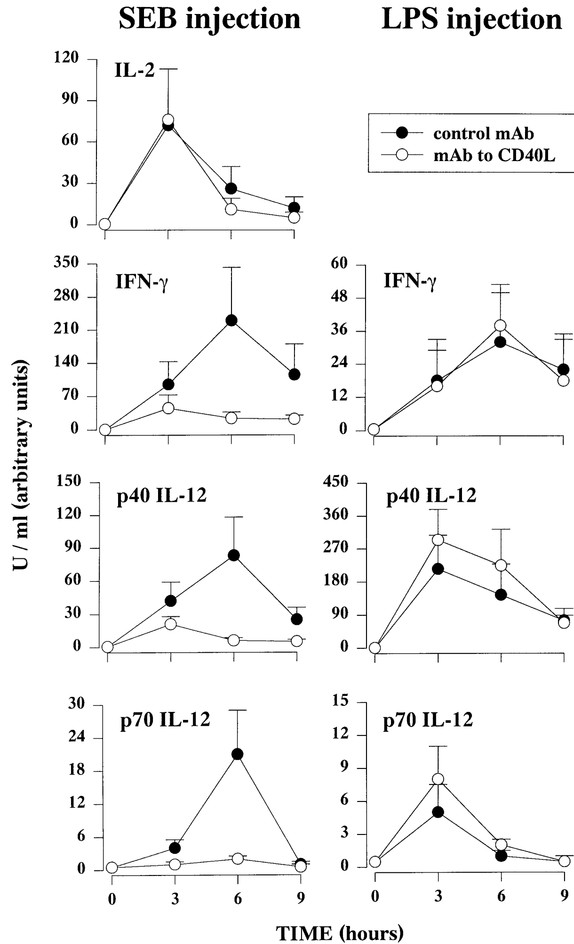

Fig. 4. CD40L-dependent IL-12 p70 heterodimer production in response to SEB. Animals (six per group) were pretreated with mAb to CD40L or isotype-matched hamster control Ig (1 mg i.p), 1 h before i.v challenge with 50 μg of SEB or LPS. Mice were bled 3, 6 and 9 h later, and the serum IL-2, IL-12 and IFN-γ content determined by ELISA. Results are expressed as arbitrary U/ml ± SD of individual determinations. These results are representative of three independent experiments.

response to LPS appeared to be insensitive to CD40–CD40L antibody-mediated blockade.

Collectively, these observations indicate that optimal and sustained *in vivo* IFN-γ production in response to SEB required APC-derived CD86 and IL-12 signals. IL-12 secretion was itself dependent upon CD40–CD40L interactions and required prior IFN-γ synthesis.

Down-regulation of IL-12 production following in vivo administration of SEB

We next wished to analyze the role of IL-12 during a secondary response to SEB. Based on published observations relating the differential regulation of IL-2 and IFN-γ secretion during the *in vivo* secondary response to SEB (8), we chose to inject mice after a 48 h interval. Mice were primed *in vivo* with SEB or LPS, and subsequently challenged with SEB, an unrelated SAg (SEA) or LPS. As previously described, SEB-treated mice displayed an increased IFN-γ response upon secondary challenge with SEB (Fig. 5). Unexpectedly, however, this priming effect was found to be V_β non-specific, as SEB-primed animals produced high levels of IFN-γ in response to both SEA or LPS (Fig. 5B and C respectively). In spite of the increased IFN-γ production, SEB-primed animals failed to secrete detectable levels of IL-12 *in vivo* upon secondary SEA or SEB administration (Fig. 5D, E, G and H). This unresponsive phase was transient, as SEB-primed animals recovered the ability to secrete IL-12 when challenged 5 days post-treatment. Note also that SEB priming selectively down-regulated the p70 response while priming for p40-containing molecules in response to LPS (Fig. 5I). Of interest, lack of endogenous IL-12 production in SEB-primed animals correlated with their inability to sustain a high IFN-γ production over time (compare the kinetics of IFN-γ release in naive versus SEB-primed animals; Fig. 5A).

The priming properties of endogenous IL-12

SEB-treated animals produced high levels of IFN-γ in response to all inflammatory stimuli tested, in the absence of concomitant IL-12 production. To test whether endogenous IL-12 produced upon primary exposure to SEB *in vivo* might be responsible for this V_β-unrelated, IFN-γ-specific priming effect, naive animals were pretreated with exogenous recombinant IL-12 48, 24 or 2 h before a secondary challenge with SEB. Control groups included mice pretreated with SEB or saline, 48 h before a secondary exposure to SEB. All mice were bled and tested for systemic IL-2 and IFN-γ production. Exogenous IL-12 administered 48 or 24 h before SAg challenge led to a specific increase in serum IFN-γ production in response to both SEB or SEA, leaving IL-2 secretion unaffected. Of note, IFN-γ production was only marginally affected by exogenous IL-12 administered 2 h before SEB injection (Fig. 6).

To study the role of endogenous IL-12 produced during the priming phase, naive mice were primed on day 0 with rIL-12 or SEB and treated 2 days later with SEB or SEA, as indicated in Fig. 7. In keeping with previous observations, administration of rIL-12 led to a selective priming of the IFN-γ response to SEB administered 2 days later which was completely abrogated by concomitant administration of anti-IL-12 mAb (Fig. 7A). Similarly, injection of blocking anti-IL-12 antibodies during the priming phase (day 0) counteracted the SEB-dependent priming effect on the subsequent IFN-γ response to both SEB (Fig. 7B) and SEA (Fig. 7C).

Discussion

The primary response to SEB

We demonstrate in this study that administration of SEB into naive animals leads to the systemic release of bioactive IL-12 heterodimers. As expected from previous studies performed with T cells specific for conventional protein antigens (17), IL-12 production upon *in vivo* SEB exposure was found to be dependent on both CD40L–CD40 interaction (see Fig. 4) and prior endogenous IFN-γ secretion (Figs 2 and 3). Of note, the production of p40-containing molecules by IFN-γ-deficient animals was normal at 3 h post-treatment and then rapidly decayed. The observation that systemic secretion of heterodimeric p70 IL-12 molecules but not p40 molecules in

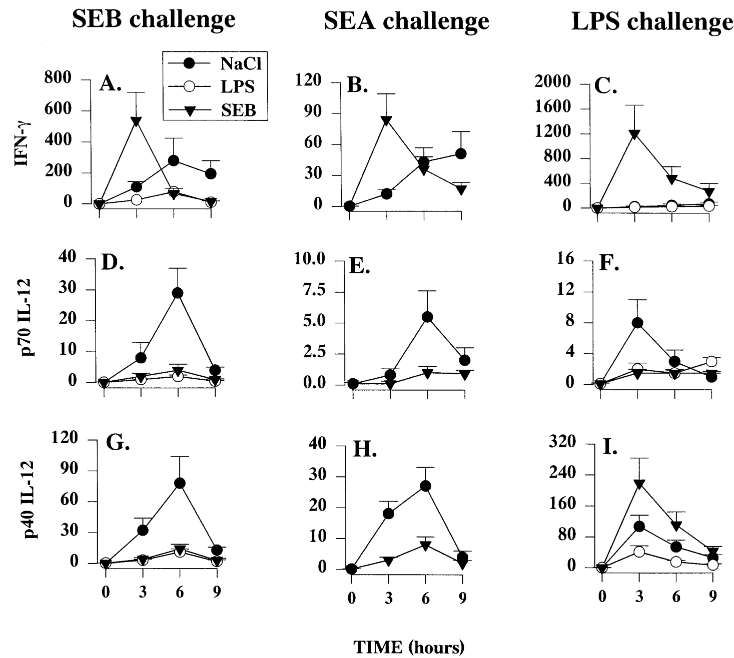

Fig. 5. Altered cytokine profile in SEB or LPS-primed animals upon secondary *in vivo* challenge. Animals (six per group) were pretreated (i.p.) with a pyrogen-free solution, SEB (50 μg) or LPS (50 μg) and challenged (i.v.) 48 h later with the same doses of SEB, SEA or LPS, as indicated. Mice were bled 3, 6 and 9 h later, and the serum IFN-γ and IL-12 content tested by ELISA. Results are expressed as arbitrary U/ml ± SD of individual determinations. These results are representative of three independent experiments.

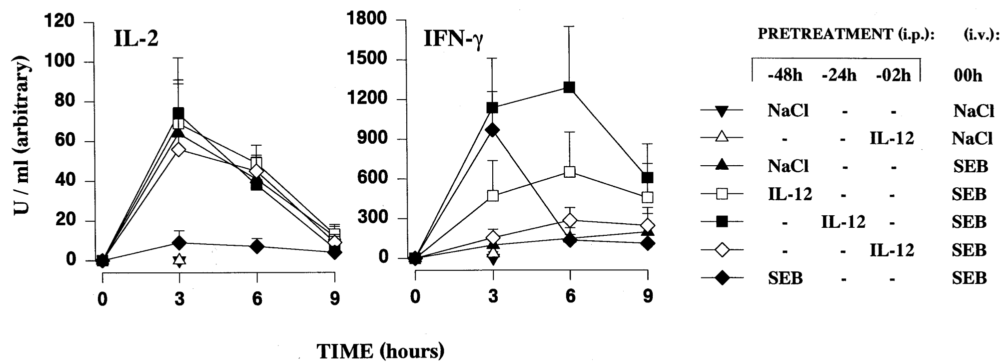

Fig. 6. The priming properties of exogenous rIL-12. Animals (six per group) were pretreated (i.p.) with 100 ng of rIL-12 or pyrogen-free saline when indicated, before i.v. challenge with 50 μg of SEB. Mice were bled 3, 6 and 9 h later, and the serum IL-2 and IFN-γ content determined by ELISA. Results are expressed as arbitrary U/ml ± SD of individual determinations. These results are representative of three independent experiments.

response to SEB required IFN-γ production is in agreement with *in vitro* studies showing that the transcription of the IL-12 p35 mRNA is positively regulated by IFN-γ in cultured macrophages (18). Collectively these data suggest that SEB injection induces the early synthesis of p40-containing molecules in an IFN-γ-independent fashion, followed by the IFN-γ-dependent secretion of p70 IL-12 heterodimers. In support of this contention, our studies performed using blocking anti-IL-12 mAb and p40-deficient animals indicate that the early IFN-γ production induced by SEB *in vivo* is largely IL-12 independent, while the sustained secretion of IFN-γ in the serum requires IL-12 synthesis. These data are also compatible with recent studies demonstrating that IL-12 secretion in response to mycobacterial infections is dependent on prior IFN-γ production (19). In summary, these results suggest that IFN-γ produced early in response to SEB initiates a positive feedback loop that leads to the CD40-dependent secretion of IL-12.

Taking into account previous observations and the data reported herein, the *in vivo* primary immune response to SEB can be envisioned as follows. During a primary sensitization, SEB presented by CD86-expressing APC activates $V_{\beta}8$-bearing T cells to cytokine production. Based on recent immunohistochemical analysis and for the sake of discussion, we tentatively postulate that the cells that present SEB *in vivo* belong to the dendritic cell family. Indeed, these cells constitu-

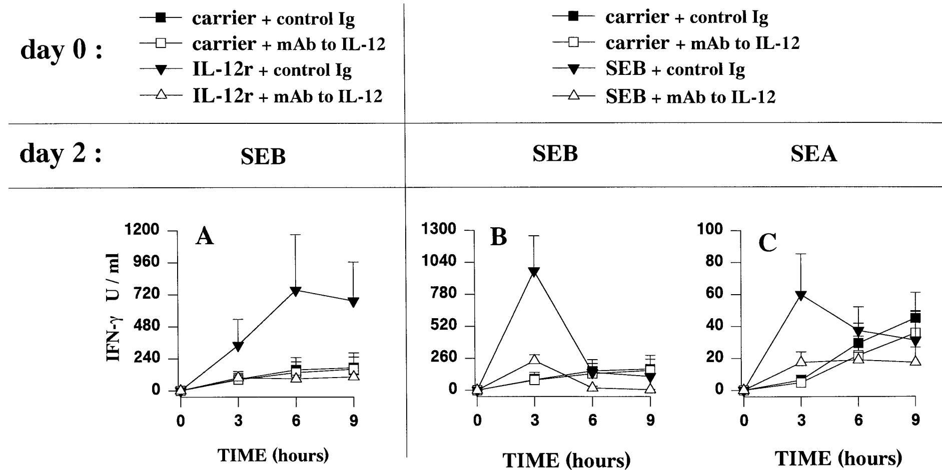

Fig. 7. The immunomodulatory properties of exogenous and endogenous IL-12. Animals (six per group) were pretreated (i.p.) with antibodies to IL-12 (a combination of two anti-IL-12 mAb, clones C15.1 and C17.8, 0.5 mg each) and rIL-12 (100 ng, A) or SEB (50 μg, B and C). After 48 h mice were challenged (i.v.) with 50 μg SEB (A and B) or SEA (C), and bled 3, 6 and 9 h later. Control mice were treated with an equivalent amount of rat Ig. The systemic release of IFN-γ was monitored by ELISA. Results are expressed as arbitrary U/ml ± SD of individual determinations. These results are representative of three independent experiments.

tively express detectable levels of CD86 molecules *in vivo* (20), while they require an *in vitro* or *in vivo* maturation step before expressing CD80 molecules on their membranes. These findings may explain why antibodies to CD86 inhibit the *in vivo* IL-2 production in response to SEB (16) (Fig. 2), and why a combination of antibodies to CD80 and CD86 is required to significantly inhibit *in vitro* responses to the same SAg (21). In agreement with this hypothesis, both B cells (22) and macrophages (23,24) have been found dispensable for bacterial SAg-mediated T cell activation *in vivo*, while dendritic cells are known to optimally stimulate naive T cells *in vitro* in response to SEB (12,25,26). Following interaction with $CD86^+$ accessory cells, SEB-primed lymphocytes produce cytokines (including IL-2 and IFN-γ) and presumably up-regulate CD40L expression, which interacts with APC-expressed CD40 molecules to induce IL-12 production. Accordingly, we demonstrate in this study that antibodies to CD40L do not affect the IL-2 response, but inhibit both IL-12 heterodimer secretion and the sustained IFN-γ production (Fig. 4). Our study indicates that the ability of bacterial SAg to induce the systemic release of bioactive IL-12 is related to their unique capacity to polyclonally stimulate T cells *in vivo*, and therefore to provide both the proper cytokine environment (IFN-γ) and the appropriate signals (CD40L) required for inducing accessory cells to high IL-12 production. Note that we have been unable to detect IL-12 production *in vitro* by unselected spleen cells stimulated by SEB (our own unpublished observations). The discrepancy between *in vivo* and *in vitro* studies may be related to the observation that B cells *in vitro* inhibit IL-12 production in response to T cell mitogens (27).

The secondary response to SEB

As previously reported by others (8), SEB-primed animals respond to a secondary SEB challenge given 48 h after the first by an increased production of IFN-γ and a defective IL-2 secretion. The priming for IFN-γ production has important biological consequences, as it has been shown to be involved in the increased lethality induced by SEB in SEB-pretreated mice (8). In contrast to our expectations, SEB-treated animals were found unable to produce IL-12 in response to a secondary SEB challenge, while producing high levels of IFN-γ (Fig. 5), adding IL-12 to the list of cytokines [including IL-2, IL-3 and IL-4 (6)] sensitive to SEB-induced *in vivo* unresponsiveness. Failure to secrete IL-12 may be related to the selective down-regulation of splenic dendritic cell numbers observed in SEB-primed animals 48 h following treatment (28).

Our study demonstrates that endogenous IL-12 produced upon primary *in vivo* SEB exposure is responsible for the increased production of IFN-γ upon secondary SAg challenge. Endogenous IL-12 appeared to affect secondary responses in a non-cognate, TCR-unrelated fashion, as evidenced by the observation that SEB-primed animals displayed an increased IFN-γ response to SEA. Note that although we have not attempted to identify the cellular source of IFN-γ in this study, previous observations suggested that both $CD4^+$ and $CD8^+$ cells produce IFN-γ in response to a secondary SEB challenge (8). Accordingly, *in vitro* analysis demonstrated that IFN-γ production by spleen cells from SEB-primed animals required $Thy\text{-}1^+$ cells (our own unpublished observations). Furthermore, the observation that SEB-primed animals respond to LPS with increased IFN-γ secretion strongly suggests that all potential IFN-γ producers [including thus NK cells, known to secrete IFN-γ in response to LPS (29)] are primed *in vivo* following SEB administration.

In agreement with the hypothesis that priming by endogenous IL-12 does not require cognate, TCR-specific, cell interactions, administration of exogenous IL-12 in the absence of TCR-ligands was able to modify the subsequent (24 or 48 h later) response of SEB-reactive cells *in vivo* (see Fig. 6). Of interest, administration of IL-12 2 h before SEB treatment failed to significantly enhance IFN-γ production, suggesting that up-regulation of secondary IFN-γ responses required cell differentiation. Note that, although the ability of IL-12 to promote IFN-γ secretion when present during the antigen-

recognition step has been well documented, the observation that IL-12 is able to prime naive effector cells to higher IFN-γ production when administered before antigen exposure is novel and may explain why IL-12 is more efficient at inducing a protective immune response to *Cryptosporidium parvum* when administered 1 day before rather than the day of infection (30).

In murine T cells, IL-12 has been shown to uniquely activate the transcription factor Stat4 (31,32). The important role of Stat4 in mediating IL-12 proinflammatory properties was demonstrated by the reduced IFN-γ response to IL-12 observed in Stat4-deficient mice (33,34). It would therefore be of interest to investigate whether the priming effect of IL-12 described herein is due to the sustained activation of Stat4 and/or other Stat4-induced transcription factors [such as ERM (35)], possibly explaining the propensity of SEB-primed cells to secrete large amounts of IFN-γ upon secondary stimulation.

Finally, the difference in the kinetics of the IFN-γ response between IL-12 and SEB-primed animals (Fig. 6) can be easily explained by the lack of endogenous IL-12 in SEB-primed animals. As a consequence, these animals cannot sustain IFN-γ production at later times, as demonstrated by studies performed in p40-KO animals (see Fig. 3).

Although we demonstrate in this study that endogenous IL-12 antagonized IL-2 production in response to SEB (see Fig. 2A), we wish to stress that endogenous IL-12 production did not appear to be responsible for the well characterized phenomenon of SEB-induced T cell anergy. Indeed, although anti-IL-12 mAb treatment was found to increase SEB-induced IL-2 secretion (Fig. 2A), they did not prevent induction of IL-2 unresponsiveness and decreased proliferative responses to SEB (our own unpublished observations). It is tempting to speculate that IL-12 produced in response to SEB favors the development of T_h1-like cells *in vivo*. Note that it has been recently recognized that *in vitro* differentiation of CD4 cells towards the T_h1 phenotype is accompanied by the rapid loss of IL-2 secretion and acquisition of high IFN-γ production (36). In light of these considerations, we would speculate that blocking IL-12 (by antibody treatment or genetic invalidation) will inhibit T_h1 differentiation, thus maintaining a high level of IL-2 production by SEB-primed T cells. Note that SEB-induced apoptosis and anergy are impaired in IL-2 KO mice suggesting that IL-2 may play a role in the down-regulation of *in vivo* immune responses (37). Blocking of endogenous IL-12 may therefore counteract the *in vivo* inflammatory response to SEB by both increasing IL-2 production and by down-regulating IFN-γ secretion.

The observation that IL-12 is able to modify the response of SEB-primed animals to further *in vivo* challenge may help explain some of the pathophysiological properties of bacterial SAg. Indeed, susceptibility of mice to SEB-induced death seems to vary according to laboratories and protocols. Our study suggests that IL-12 may represent a possible factor contributing to these disparate results. In particular, endogenous IL-12 produced following infection by unrelated pathogens may sensitize mice to bacterial SAg in a TCR-unrestricted manner. Accordingly, it has been recently demonstrated that while SPF mice appear to be resistant to high doses of SEB, mice infected with an influenza virus became susceptible to SEB-mediated death (38). Note that SEB-primed animals challenged with LPS produce very high levels of systemic IFN-γ (at least 20-fold increase when compared to controls; see Fig. 5). SEB-treated animals also display increased mortality when challenged with LPS (data not shown). IL-12 may therefore play an important role in the increased morbidity to concurrent exposure to viruses and bacteria. Moreover, IL-12 production induced by bacterial SAg may explain several of the antigen non-specific immunomodulatory properties of these compounds. Indeed, it has been shown that stimulation by enterotoxins led to increased non-specific resistance to unrelated microbial (39) or parasitic (40) infections or tumor growth (41). We suggest that SAg-induced IL-12 may favor the development of a protective T_h1-like response *in vivo*.

In conclusion, we demonstrate in this work that SEB induces a robust IL-12 response *in vivo*, which requires prior IFN-γ production and relies on CD40–CD40L interactions. Endogenous IL-12 promotes the production of IFN-γ in a TCR-non-specific fashion, a finding that may explain the adjuvant-like properties of bacterial SAg and their ability to modulate immune responses to unrelated antigens.

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Abbreviations

APC	antigen-presenting cell
CD40L	CD40 ligand
LPS	lipopolysaccharide
SAg	superantigen
SEA	staphylococcal enterotoxin A
SEB	staphylococcal enterotoxin B

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