Role of Endogenous Interleukin-12 in Immune Response to Staphylococcal Enterotoxin B in Mice

FANNY N. LAUW,1,2 SANDRINE FLORQUIN,3 PETER SPEELMAN,2 SANDER J. H. VAN DEVENTER,1 AND TOM VAN DER POLL1,2*

Laboratory of Experimental Internal Medicine, 1Department of Infectious Diseases, Tropical Medicine and AIDS, 2and Department of Pathology, 3University of Amsterdam, Amsterdam, The Netherlands

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In the present study, the roles of interleukin 12 (IL-12) and IL-18 and their possible interaction during superantigen-induced responses were studied by injection of staphylococcal enterotoxin B (SEB) into mice. These data suggest that the role of IL-12 in SEB-induced responses is limited to sustaining gamma interferon release by an IL-18-independent mechanism.

Bacterial superantigens (SAgs) are a unique group of proteins that activate both antigen-presenting cells and T cells (9, 12), resulting in rapid activation and proliferation of Vβ8-specific T cells in vivo and the release of cytokines. Staphylococcal enterotoxin B (SEB) is a product of Staphylococcus aureus and is one of the best-characterized and most-studied SAgs. Previous studies have demonstrated the important role of such cytokines as tumor necrosis factor (TNF), interleukin 2 (IL-2), IL-10, and gamma interferon (IFN-γ) in SEB-induced pathology (1, 4, 5, 14).

IL-12 is a proinflammatory heterodimeric cytokine, formed by a p35 and a p40 subunit, which is produced mainly by antigen-presenting cells (6, 20) and is a potent stimulator of T-cell functions, including proliferation, cytotoxicity, and the release of cytokines, IFN-γ in particular. In addition, IL-12 promotes the differentiation of naive CD4+ T cells into Th1-type cells. Like IL-12, IL-18 is a proinflammatory cytokine derived from activated monocytes/macrophages which is an important costimulus for optimal production of IFN-γ, especially in the presence of IL-12 (2, 17). In the present study we investigated the roles of IL-12 and IL-18 and their possible interaction during SEB-induced pathology in mice.

To investigate the role of IL-12 in SAg-induced responses, we compared the effects of a single intraperitoneal (i.p.) injection of SEB (100 µg dissolved in 200 µl of saline) (Sigma, St. Louis, Mo.) in IL-12p40 gene-deficient (IL-12p40−/−) (Jackson Laboratory, Bar Harbor, Maine) and wild-type (WT) (Harlan Spague Dawley Inc., Horst, The Netherlands) mice. The SEB was endotoxin free as verified by stimulation of whole blood with SEB with or without polymyxin B, a well-known inhibitor of lipopolysaccharide (LPS) activity. There was no difference in TNF production when polymyxin B was added (data not shown). Control mice received saline only. All experiments were approved by the Institutional Animal Care and Use Committee of the Academic Medical Center.

Splens and blood were collected at different time points for determination of IL-12p35 and IL-12p40 mRNA expression by reverse transcription (RT)-PCR and plasma concentrations of IL-12p70, TNF, IL-2, IFN-γ, and IL-18 (R&D Systems, Abingdon, United Kingdom) and total IL-12p40 and IL-10 (Pharmingen, San Diego, Calif.) by enzyme-linked immunosorbent assay.

The following primers were used: for IL-12p35 (520 bp), 5′-AAACCTGTGAAGACCCAC-3′ (sense) and 5′-AGCTCA GATAGCCCATCAC-3′ (antisense); for IL-12p40 (277 bp), 5′-ACTCAATCTGCTGCTCACC-3′ (sense) and 5′-CCTCT GTCTCCTTCATTTTC-3′ (antisense); and for β-actin (617 bp), 5′-GTCGAGAGAGGTCTATGTG-3′ (sense) and 5′-GCTCTTGCCAATAAGTGATG-3′ (antisense). No detectable IL-12p35 and IL-12p40 mRNA was noted in spleens of control mice, while administration of SEB induced the expression of both IL-12p35 and IL-12p40 mRNA (Fig. 1A). Injection of SEB resulted in transient increases in concentrations of IL-12p70 and total IL-12p40 in plasma, peaking after 4 to 8 h (Fig. 1B). TNF and IL-2 concentrations in plasma increased rapidly after SEB injection, reaching peak levels after 2 h, and did not differ between IL-12p40−/− and WT mice (Fig. 2). Concentrations of IFN-γ and IL-10 increased gradually after SEB challenge, peaking at 4 to 8 h. The initial increases in IFN-γ concentrations were similar in IL-12p40−/− and WT mice; however, IFN-γ concentrations quickly decreased in IL-12p40−/− mice after 4 h. Also, concentrations of IL-10 were slightly decreased in IL-12p40−/− mice, although this difference was not significant.

To determine whether IL-12 contributes to the expansion of SEB-reactive T cells, the percentage of CD4+ Vβ8+ and CD8+ Vβ8+ cells in splenocytes and mesenteric lymph node cells was assessed in IL-12p40−/− and WT mice by FACScan analysis using fluorescein isothiocyanate- or phycoerythrin-labeled (control) monoclonal antibodies (Pharmingen). At 2 days after SEB injection, the percentages of CD4+ Vβ8+ cells and CD8+ Vβ8+ cells were almost twofold higher than the percentages found in control mice, and the number of CD4+ Vβ8+ cells returned to normal after 4 days (Fig. 3). The changes in the number of Vβ8+ cells in response to SEB were not different in IL-12p40−/− mice or in WT mice, suggesting that IL-12 does not play a role in SEB-induced proliferation and death of peripheral T cells.

IL-18 is an essential cofactor for IFN-γ production after LPS
FIG. 1. Injection of SEB i.p. (100 µg) induces the expression of IL-12 p35 and IL-12 p40 mRNA in spleens and the systemic release of IL-12 p70 and IL-12 p40. (A) Spleens of three mice at each time point were pooled for IL-12 mRNA and β-actin mRNA expression as determined by RT-PCR. Molecular size markers are shown to the left. (B) Plasma concentrations of IL-12 p70 and total IL-12 p40 (mean ± standard error of the mean of results for six to eight mice per time point) were measured at the indicated time points after injection of SEB. Control mice received saline only.

FIG. 2. Concentrations of TNF, IL-2, IL-10, and IFN-γ in plasma of IL-12p40−/− mice and WT mice after i.p. injection of 100 µg of SEB. Data are means ± standard errors of the means of results for six to eight mice at each time point. Asterisk, P value of <0.05 in comparison of results for IL-12p40−/− mice and WT mice by the Mann-Whitney U test.
challenge (17), but little is known about the role of IL-18 during SEB-induced pathology. Concentrations of IL-18 in plasma increased slightly after injection of SEB in WT mice (at 4 h, 302 ± 36 pg/ml versus levels for controls, 152 ± 33 pg/ml [P value was not significant]). To determine whether IL-18 contributes to SEB-stimulated IFN-γ production and/or interacts with IL-12 to induce IFN-γ release, WT and IL-12p40−/− mice were injected with SEB (100 μg in 200 μl of saline) in combination with rabbit anti-murine IL-18 antiserum or control rabbit serum (200 μl; Sigma) injected intraperitoneally 1 h prior to SEB administration and sacrificed after 8 h for measurements of IFN-γ levels. IL-18 antiserum was prepared as described previously and was kindly donated by C. A. Dinarello (University of Colorado Health Sciences Center, Denver, Colo.) (3). As demonstrated in Fig. 4, IL-12p40−/− mice again had significantly lower plasma concentrations of IFN-γ. Administration of anti-IL-18 did not affect SEB-stimulated IFN-γ production in either WT or IL-12p40−/− mice. Previous studies have shown that IL-18 can stimulate the production of other cytokines and chemokines, such as TNF, IL-1β, IL-8, and macrophage inflammatory protein (MIP) 2 (16, 18). However, administration of anti-IL-18 did not influence SEB-induced peak levels of TNF and IL-12 (data not shown), suggesting that IL-18 is not a central mediator in SAg-induced cytokine responses.

In line with a previous study (15), we showed that injection of SEB into mice stimulated the systemic release of both IL-12p70 and IL-12p40. In addition, we found that IL-12 did not importantly influence the early release of cytokines within 4 h after SEB injection, since IL-12p40−/− and WT mice displayed no significant differences in release of TNF, IL-2, IL-10, and IFN-γ, cytokines which have previously been shown to importantly contribute to SEB-induced lethality (1, 4, 5, 14). However, IL-12 contributes to the sustained increase in SEB-induced IFN-γ production, since IFN-γ concentrations were strongly decreased in IL-12p40−/− mice after 4 h. This may suggest that the initial release of IFN-γ is the result of direct stimulation of T cells by SEB while the subsequent release of IFN-γ is mediated through the production of IL-12 from activated monocytes/macrophages. These results for SEB-stimulated secretion of IFN-γ are in contrast with results reported after injection of LPS, where IFN-γ production is largely dependent on IL-12 and neutralization of IL-12 protects against LPS-induced lethality (7, 11, 21). This illustrates the different mechanisms involved in SEB-induced lethality compared to LPS toxicity, i.e., whereas the former is mediated by T cells, the latter results from the activation of monocytes/macrophages.

IL-18 is an important cofactor for production of IFN-γ (2, 3, 17, 19). In the presence of IL-12, IL-18 synergistically enhances IFN-γ production (13), which involves the upregulation of IL-18 receptor expression by IL-12 (10, 22). During experimental endotoxemia in mice, neutralization of IL-18 protected against LPS-induced liver injury and lethality (16, 17). We found that IL-18 does not contribute to secretion of IFN-γ after SAg challenge in both WT and IL-12p40−/− mice, as demonstrated with neutralizing anti-IL-18 antiserum. This antiserum has previously been shown to potently inhibit LPS-induced production of IFN-γ by splenocytes in vitro (3), to reduce the release of IFN-γ and TNF, and to protect against lethality during experimental endotoxemia in mice (16).
neutralizing capacity of this batch of anti-IL-18 was demonstrated in a previous study (16) and confirmed in our laboratory (data not shown). Therefore, contrary to results found in other infection models, during SAg-induced pathology, IL-12 contributes to the sustained systemic release of IFN-γ independently from IL-18. These results are in line with data from a previous study which reported that IL-18 does not play a role in SEB sensitization of mice to LPS challenge, which is considered to be dependent on T-cell-derived IFN-γ production (8).

In conclusion, we demonstrated that IL-12 is strongly induced after in vivo administration of SEB in mice and that IL-12 contributes to the sustained increase in IFN-γ concentrations without influencing the release of other cytokines, such as TNF-α, IL-2, and IL-10. Although IL-12 is a potent stimulator of T-cell proliferation, IL-12 did not play a role in the clonal expansion of SEB-reactive Vβ 8 T cells. Contrary to its function in other infection models, IL-18 did not play a costimulatory role in the SEB-induced release of IFN-γ.

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REFERENCES