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Distinct functions of interferon-γ for chemokine expression in models of acute lung inflammation

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SUMMARY

Challenge of the immune system with bacterial superantigens or endotoxin induces the systemic release of cytokines followed by lethal septic shock. The lung is particularly susceptible to systemic toxin exposure resulting in acute leucocyte infiltration and vascular damage. In the present study, the functions of interferon-γ (IFN-γ) and tumour necrosis factor (TNF) for chemokine regulation during acute lung inflammation were examined. Following administration of the superantigen, staphylococcal enterotoxin B (SEB), lung mRNA levels of the chemokines cytokine-induced neutrophil chemo-attractant (KC), lipopolysaccharide-induced CXC chemokine (LIX), macrophage chemotactic protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1α and MIP-2 were increased to a similar extent both in controls and in mice deficient for the IFN-γ or 55 000 MW TNF receptors. In contrast, interferon-inducible protein-10 (IP-10) and monokine induced by IFN-γ (Mig) mRNA expression was markedly reduced in mice deficient for IFN-γ or IFN-γ receptor, but not in 55 000 MW TNF receptor knockout mice. *In situ* hybridization experiments demonstrated that IP-10 was highly expressed in lung interstitial macrophages of C57BL/6, but not of IFN-γ receptor-deficient mice. In contrast to SEB administration, treatment with lipopolysaccharide resulted in a strong induction of IP-10 and Mig in IFN-γ receptor-deficient mice. Together, these results establish a critical function of IFN-γ for chemokine induction in acute lung inflammation that is dependent on the nature of the inflammatory stimulus.

INTRODUCTION

A group of bacterial and viral proteins (termed superantigens) associate with T-cell-receptor Vβ gene segments and major histocompatibility complex (MHC) class II proteins, resulting in poly-clonal T-cell activation.[1–3] Challenge of the immune system with bacterial superantigens such as staphylococcal enterotoxin B (SEB) results in acute hyperactivation of $CD4^+$ and $CD8^+$ T cells followed by systemic release of cytokines, including tumour necrosis factor-α (TNF-α), interleukin (IL)-1, IL-2, IL-4, IL-6 and interferon-γ (IFN-γ), and lethal septic shock.[4–6] Independent studies have demonstrated that lethal SEB shock is dependent on the presence of T-cell-derived TNF-α, the 55 000 MW receptor for TNF and intracellular adhesion molecule-1 (ICAM-1).[5,7–9]

Systemic administration of SEB results in acute inflammatory lung injury that is characterized by marked leucocyte infiltration, endothelial cell injury and increased vascular permeability.[10] Thus, pathological alteration of lungs following superantigen exposure is similar to that observed after administration of endotoxin.[11] Regardless of the superantigen applied, lung-infiltrating leucocytes consist of granulocytes, mononuclear phagocytes and natural killer (NK) cells.[10] Recent studies have indicated that recruitment of $V\beta8^+$ T cells, but not of neutrophils and mononuclear phagocytes, into the peritoneal cavity of SEB-treated mice is dependent on CD44 and hyaluronate.[11,12] SEB challenge of mice also results in activation of circulating granulocytes, as indicated by enhanced production of reactive oxygen metabolites and altered expression of cell-surface receptors such as macrophage marker-1 (Mac-1) and L-selectin.[10] Interestingly, in trauma patients, developing adult respiratory distress syndrome (ARDS) neutrophils circulating in the pulmonary artery also exhibit increased oxygen radical production and elevated Mac-1 expression.[11,13]

Chemokines are involved in a variety of immune and inflammatory responses and are subdivided into CXC, CC, C and CX_3C families. Several lines of evidence indicate a central

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Abbreviations: ARDS, adult respiratory distress syndrome; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IP-10, interferon-inducible protein-10; KC, cytokine-induced neutrophil chemoattractant; LIX, lipopolysaccharide-induced CXC chemokine; MCP-1, macrophage chemotactic protein-1; Mig, monokine induced by interferon-γ; MIP-1α, macrophage inflammatory protein-1α; MIP-2, macrophage inflammatory protein-2; SEB, staphylococcal enterotoxin B.

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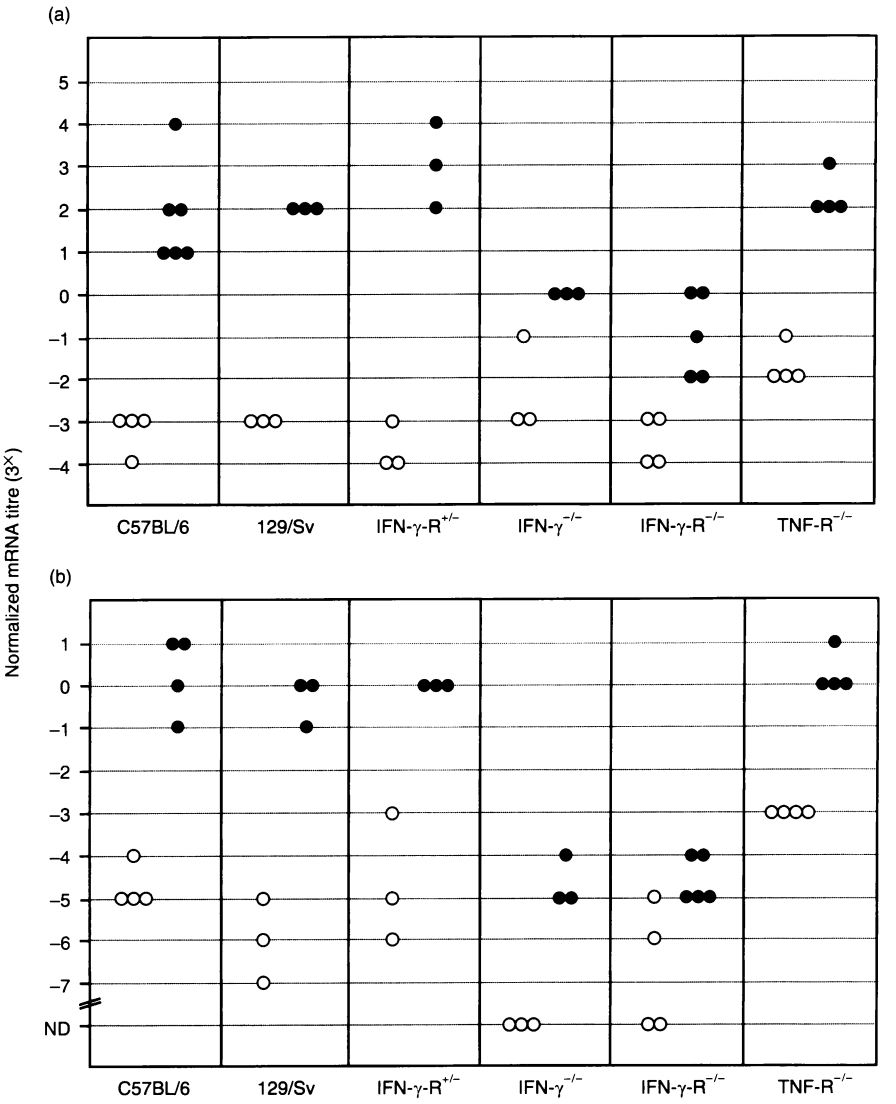

Figure 1. Predominant role of IFN-γ for regulation of IP-10 and Mig expression during SEB-induced lung inflammation. Total RNA was isolated from lungs of various mouse strains 4 hr after SEB (filled circles) or PBS treatment (open circles) and reverse transcribed. Serial cDNA dilutions (1:3) were used as template for PCR amplifications to detect the CXC chemokines IP-10 (a) or Mig (b). Normalized cDNA titres of IP-10 and Mig, defined as the final dilutions yielding detectable amplification products divided by the GAPDH titre derived from the same cDNA template, are given. Primers for GAPDH were derived from different exons to control for contamination with genomic DNA. Each symbol represents the result from lungs of a single mouse. (c) Gels representing the results from lungs of a single mouse representative of each group are shown. ND, not detectable.

role of CXC chemokines in inflammatory organ injury. In ARDS patients, concentrations of IL-8 in bronchioalveolar lavage fluid correlate with mortality, while in animal models neutralization of IL-8 receptor-binding chemokines protects from lung vascular damage and death induced by intratracheal instillation of lipopolysaccharide (LPS).[14–18] In the mouse, neutralizing antibodies against cytokine-induced neutrophil chemo-attractant (KC) or macrophage inflammatory protein-2 (MIP-2) markedly inhibit neutrophil accumulation in lungs and reduce vascular leakage following intratracheal administration of endotoxin.[11,15,16,19]

There is also evidence for a role of C–C chemokines in acute inflammatory lung injury. In murine endotoxaemia, pretreatment with macrophage inflammatory protein-1α (MIP-1α) antibodies prevents vascular damage as well as neutrophil and macrophage influx in lungs.[20] Neutralization of macrophage chemo-attractant protein-1 (MCP-1) significantly increases endotoxin-induced mortality as well as serum TNF and IL-12 levels.[21] Conversely, administration of recombinant MCP-1 protein results in elevated IL-10 serum levels and protects mice from lethal endotoxaemia, suggesting *in vivo* crosstalk between cytokine and chemokine networks.[22] In a rat immunoglobulin A (IgA) immune complex model of alveolitis, which is characterized by mononuclear phagocyte-dependent lung injury, however, infusion of neutralizing MCP-1 antibodies reduced both accumulation of mononuclear phagocytes and vascular damage in lung.[23] It therefore appears that chemokines such as MCP-1 may either promote or attenuate organ injury, depending on the inflammatory stimulus.

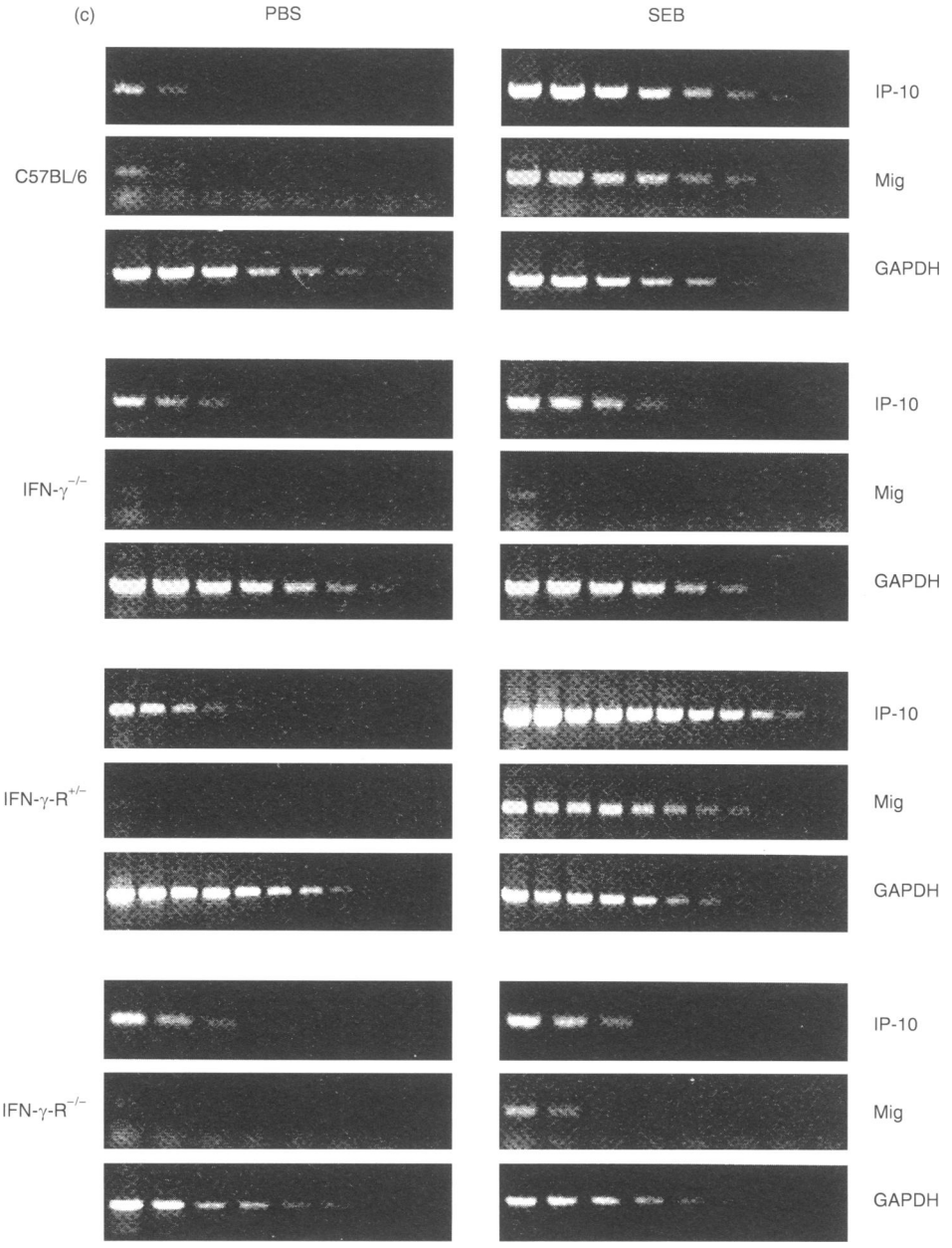

Figure 1. (*Continued*)

In the present study, the regulation of chemokine expression during superantigen- or endotoxin-triggered acute lung inflammation was investigated. We demonstrate that induction of IP-10 and Mig, but not of numerous other chemokines, in lung, is strongly reduced in mice deficient for IFN-γ or the IFN-γ receptor. Stimulated expression of chemokines was not affected by TNF receptor p55 deficiency. In contrast to superantigen-triggered lung inflammation, up-regulation of interferon-inducible protein-10 (IP-10) and monokines induced by IFN-γ (Mig) by systemic application of LPS did not require the presence of IFN-γ or the IFN-γ receptor, suggesting that chemokine regulation during acute lung inflammation varies according to the nature of the inflammatory stimulus.

MATERIALS AND METHODS

Acute lung inflammation induced by SEB or LPS challenge of mice

C57BL/6 and 129/Sv mice were purchased from Harlan Winkelmann (Borchen, Germany). IFN-γ-deficient mice backcrossed to the C57BL/6 background were obtained from The Jackson Laboratory (Bar Harbor, ME). TNF receptor p55-deficient mice (C57BL/6 background),[7] IFN-γ receptor-deficient mice,[24] and IFN-γ receptor hemizygous mice (both C57BL/6-129/Sv background) were bred in a conventional animal facility. Mice were injected intraperitoneally (i.p.) with a single dose of 50 μg SEB (Toxin Technology, Sarasota, FL) in 200 μl of phosphate-buffered saline (PBS). The SEB preparation used contained <1 ng LPS/mg SEB as determined by

Table 1. Primers for reverse transcription–polymerase chain reaction analysis of lung chemokine expression

Target	Product (bp)	Sequence
GAPDH sense	471	CAA TGC ATC CTG CAC CAC CAA
GAPDH antisense		GTC ATT GAG AGC AAT GCC AGC
IP-10 sense	431	CCT ATC CTG CCC ACG TGT TG
IP-10 antisense		CGC ACC TCC ACA TAG CTT ACA
KC sense	530	AAC GGA GAA AGA AGA CAG ACT G
KC antisense		GAC GAG ACC AGG AGA AAC AG
LIX sense	344	AGC TCG CCA TTC ATG CGG ATG
LIX antisense		CTA TTG AAC ACT GGC CGT TCT
MCP-1 sense	319	CTC ACC TGC TGC TAC TCA TTC
MCP-1 antisense		GCT TGA GGT GGT TGT GGA AAA
Mig sense	381	ATG AAG TCC GCT GTT CTT TTC C
Mig antisense		TTA TGT AGT CTT CCT TGA ACG AC
MIP-1α sense	257	GCC CTT GCT GTT CTT CTC TGT
MIP-1α antisense		GGC ATT CAG TTC CAG GTC AGT
MIP-2 sense	285	ACC CTG CCA AGG GTT GAC TTC
MIP-2 antisense		GGC ACA TCA GGT ACG ATC CAG
RANTES sense	321	CAT CCT CAC TGC AGC CGC C
RANTES antisense		CCA AGC TGG CTA GGA CTA GAG

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IP-10, interferon-inducible protein-10; KC, cytokine-induced neutrophil chemo-attractant; LIX, lipopolysaccharide-induced CXC chemokine; MCP-1, macrophage chemotactic protein-1; Mig, monokine induced by interferon-γ; MIP, macrophage inflammatory protein; RANTES (regulated upon activation, normally T-cell expressed and secreted).

the *Limulus* amebocyte lysate assay. In separate experiments, mice were injected i.p. with 10 μg LPS from *Escherichia coli* serotype 055:B5 (Sigma Chemical Co., St. Louis, MO). The lungs were removed at various time-points after SEB or LPS challenge and examined after extensive perfusion with PBS.

Quantification of mRNA levels by reverse transcription–polymerase chain reaction (RT–PCR)

After challenge of mice with SEB, LPS or PBS, lungs were extensively perfused, removed and snap-frozen in liquid nitrogen. Total cellular RNA was extracted and first-strand cDNA was synthesized from 20 μg of total RNA using a mixture of oligo $(dT)_{12-18}$ and random hexamer primers and Superscript reverse transcriptase (BRL, Paisley, UK). The reaction was incubated for 75 min at 37° and terminated by heating to 95° for 5 min. Thereafter, serial 1 : 3 dilutions were prepared from the cDNA and chemokine fragments were specifically amplified using sense and antisense primers as listed in Table 1. As a control, a 471-bp fragment of murine glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified. Primer sequences for GAPDH were separated by introns to control for contamination with genomic DNA. The amplification reactions were allowed to proceed for 30 cycles, each consisting of a 1-min denaturation step at 94°, a 30-second annealing step at 63° and a 90-second extension step at 72°. The identity of amplification products was confirmed in each case by restriction enzyme analysis. The final cDNA dilution yielding detectable amplification products was scored for each sample. To normalize mRNA levels, the cDNA titres for chemokines were divided by the GAPDH titres obtained from the same cDNA template.

In situ *hybridization*

Chemokine expression in lungs was also investigated by *in situ* hybridization of organs removed 4 hr after injection of SEB. Lungs were perfused extensively with PBS and subsequently with PBS containing 1% paraformaldehyde. The technique used for *in situ* hybridization has been described previously.[10] Briefly, single-stranded ^{35}S-labelled antisense or sense RNA probes were generated by *in vitro* transcription using T3 or T7 RNA polymerases, as described by the manufacturer (Boehringer, Mannheim, Germany). The murine cDNA spanning the entire coding region of IP-10 was subcloned into the pBK–CMV vector (Invitrogen, Carlsbad, CA) and was used as a probe for *in situ* hybridization analysis. Sense RNA probes were used as controls in all experiments and did not reveal specific hybridization signals. After hybridization, slides were coated with Kodak NTB-2 photographic emulsion (Kodak, New Haven, CT) and exposed for 17 days. Finally, sections were fixed, counterstained with toluidine blue, dehydrated and mounted.

RESULTS

SEB-induced expression of IP-10 and Mig chemokines in lung is specifically dependent on IFN-γ receptor-mediated signals

To examine the regulation of chemokines by inflammatory cytokines during acute lung inflammation, RNA was extracted from lungs of mutant mice deficient for IFN-γ, the IFN-γ receptor or the 55 000 MW TNF receptor, and various control mice, 4 hr after challenge with SEB. Following reverse transcription, the final cDNA dilution yielding detectable amplification products was scored for each chemokine using the

primers listed in Table 1. Chemokine mRNA levels were normalized by dividing the cDNA titre for each chemokine by the GAPDH titre obtained from the same cDNA template. Primers for GAPDH were separated by introns to control for contamination with genomic DNA. The identity of amplification products was confirmed, in each case, by restriction enzyme analysis. The results depicted in Fig. 1 demonstrate that mRNA levels of the CXC chemokines IP-10 and Mig were elevated by two to three orders of magnitude in SEB-treated C57BL/6 and 129/Sv control mice as well as in 55 000 MW TNF receptor-deficient mice. In contrast, following administration of SEB, expression of IP-10 and Mig was strongly reduced in mice deficient for IFN-γ or the IFN-γ receptor (Fig. 1). Induction of IP-10 and Mig was normal, however, in mice hemizygous for IFN-γ receptor deficiency. These results indicate that in SEB-induced acute lung inflammation, expression of IP-10 and Mig is dependent on signals mediated by the IFN-γ receptor but not by the 55 000 MW receptor for TNF. It should be noted that suppression of IP-10 and Mig induction in IFN-γ and IFN-γ receptor knockout mice was not complete, suggesting the presence of alternate, but weak agonists.

To determine the cellular source of IP-10, lung samples were examined by *in situ* hybridization. Both C57BL/6 and IFN-γ receptor-deficient mice were challenged with SEB, lungs were removed 4 hr later and cryosections were incubated with ^{35}S-labelled sense and antisense probes of IP-10 mRNA. The results presented in Figs 2 and 3 show that IP-10 mRNA was not detected in lungs of PBS-treated wild-type or mutant mice. Following administration of SEB to C57BL/6 mice, however, a strong and specific increase of IP-10 mRNA was demonstrated (Fig. 2d). Histological examination revealed that in lungs of SEB-treated C57BL/6 mice, IP-10 mRNA was strongly expressed on interstitial macrophages and some alveolar macrophages (Fig. 2e and 2f). In SEB-challenged C57BL/6 mice, lung interstitial macrophages were found to be concentrated in the vicinity of major bronchial tubes. In contrast, in lungs of IFN-γ receptor-deficient mice challenged with SEB, induction of IP-10 mRNA was strongly reduced (Fig. 3). Examination of lung sections from IFN-γ receptor-deficient mice revealed only rare scattered macrophages (fewer than five cells per section) that were positive for IP-10 mRNA expression. Control experiments revealed that the differences in IP-10 expression between wild-type and mutant mice were not caused by a reduced number of mononuclear phagocytes in lungs of mice deficient for IFN-γ or the IFN-γ receptor (data not shown). Together, these results indicate that challenge of mice with SEB strongly increases IP-10 mRNA levels on interstitial lung macrophages and that up-regulation of IP-10 is dependent on IFN-γ receptor-mediated signals.

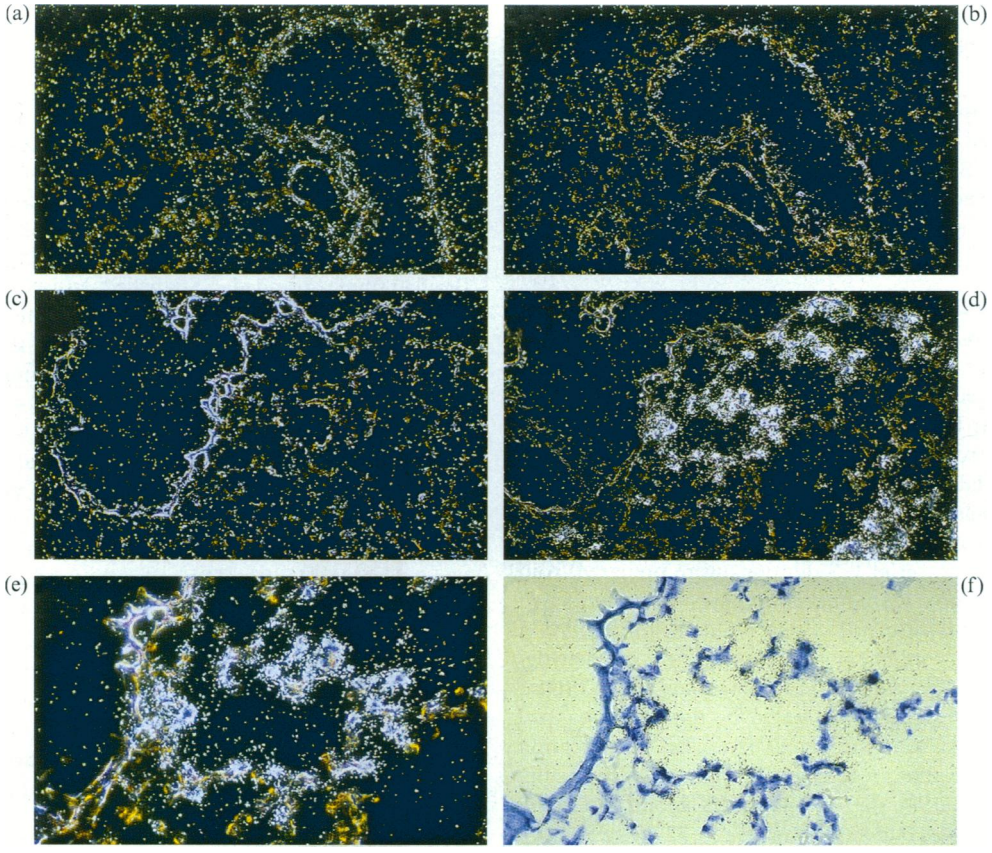

Figure 2. IP-10 mRNA is induced in lung interstitial macrophages of SEB-challenged wild-type mice. IP-10 mRNA expression was examined by an *in situ* hybridization technique 4 hr after injection of PBS (a, b) or SEB (c–f). For each group, lungs from four mice were analysed and gave similar results. Specific mRNA expression is demonstrated on sections incubated with the antisense probe (b, d–f). As a control, serial sections were hybridized with the sense probe (a, c). Tissue sections were counterstained with toluidine blue and analysed microscopically using dark-field (a–e) or bright-field (f) illumination (original magnification ×200 for a–d, and ×400 for e, f).

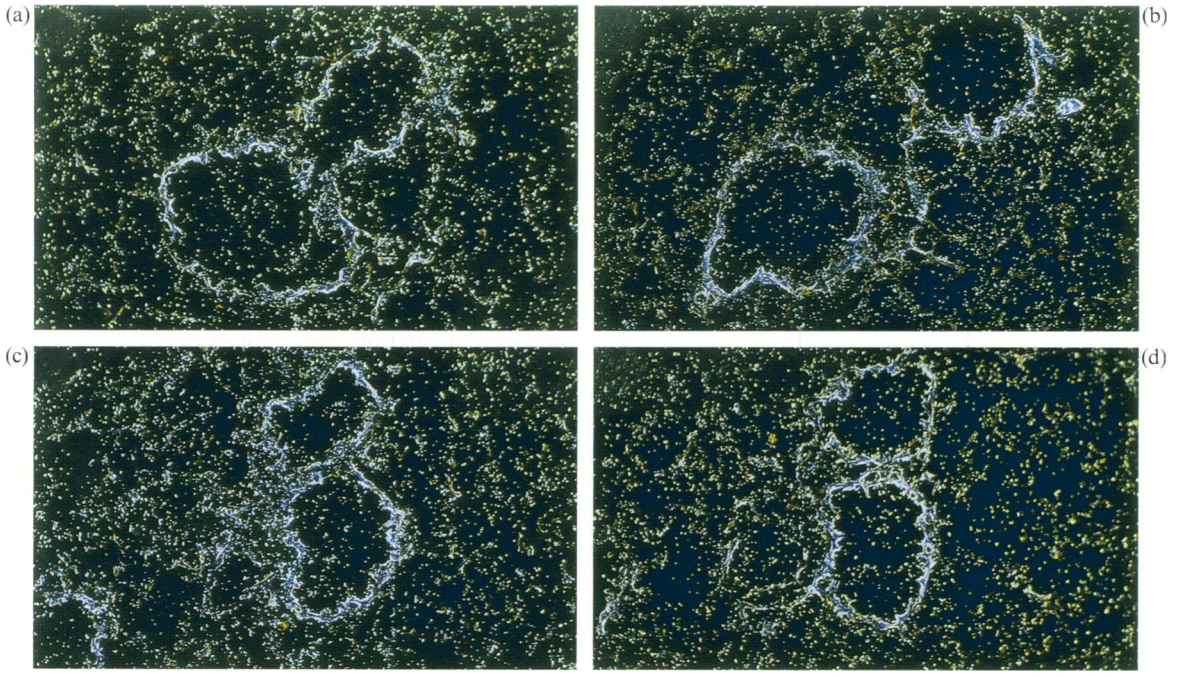

Figure 3. Lack of IP-10 mRNA induction in lungs of SEB-treated mice deficient for the IFN-γ receptor. IP-10 mRNA expression was examined by an *in situ* hybridization technique 4 hr after injection of PBS (a, b) or SEB (c, d). For each group, lungs from four mice were analysed and gave similar results. Specific mRNA expression is demonstrated on sections incubated with the antisense probe (b, d). As a control, serial sections were hybridized with the sense probe (a, c). Tissue sections were counterstained with toluidine blue and analysed microscopically using dark-field illumination (original magnification ×200).

IFN-γ is not required for LPS-induced expression of IP-10 and Mig in lung

LPS is a potent activator of mononuclear phagocytes and systemic application of LPS induces cytokine release and acute lung inflammation similar to that observed with bacterial superantigens. We therefore analysed the role of LPS for IP-10 and Mig mRNA induction in lung using semiquantitative RT–PCR. The results presented in Fig. 4 show that mRNA levels of IP-10 and Mig were highly elevated in lung 4 hr after challenge of wild-type C57BL/6 and 129/Sv mice with LPS. The magnitude of mRNA induction by LPS was comparable to that observed after SEB treatment (Fig. 1). In contrast to SEB, however, LPS strongly up-regulated IP-10 and Mig mRNA expression in lungs of IFN-γ receptor-deficient mice. Whereas induction of Mig (Fig. 4b) was not affected by IFN-γ receptor deficiency, IP-10 mRNA levels were only weakly reduced in LPS-stimulated IFN-γ receptor knockout mice (Fig. 4a). These results therefore indicate that expression of both IP-10 and Mig during LPS-induced acute lung inflammation may occur in the absence of IFN-γ receptor-mediated signals.

Regulation of chemokine expression by IFN-γ during SEB-induced acute lung inflammation is selective for IP-10 and Mig

To determine the overall pattern of chemokines regulated by IFN-γ or TNF in acute lung inflammation, RNA was extracted from lungs of mice deficient for the IFN-γ receptor or the 55 000 MW TNF receptor, and from C57BL/6 control mice, 4 hr after administration of SEB. Expression of the CC chemokines MIP-1α, MCP-1 and RANTES and of the CXC chemokines MIP-2, KC and lipopolysaccharide-induced CXC chemokine (LIX) was determined by RT–PCR analysis. The results presented in Fig. 5 demonstrate that CC and CXC chemokines were differentially regulated in lungs following SEB administration. MCP-1 mRNA levels were increased about 80-fold in C57BL/6 mice and to a similar extent in mutant mice deficient for the IFN-γ receptor or the 55 000 MW TNF receptor (Fig. 5). Induction of MIP-1α mRNA by SEB treatment was weak, but similar in the wild-type and mutant mouse strains examined. Whereas lung mRNA levels of RANTES were not increased after SEB challenge, constitutive expression of RANTES was elevated in 55 000 MW TNF receptor knockout mice (Fig. 5). When compared with untreated mice, induction of MIP-2 by SEB was weak, but of a similar magnitude in IFN-γ receptor-deficient, 55 000 MW TNF receptor-deficient and C57BL/6 mice (Fig. 5). Transcripts for LIX were induced by approximately 10-fold after SEB treatment and KC mRNA levels increased by approximately 20-fold in lungs of both knockout and wild-type mice (Fig. 5). These results therefore demonstrate rapid and intense induction of a distinct panel of CXC and CC chemokines in lung following SEB challenge. However, regulation of mRNA levels of the chemokines tested was not affected by genetic ablation of the IFN-γ receptor or the 55 000 MW receptor for TNF.

DISCUSSION

Activation of T lymphocytes by bacterial superantigens *in vivo* induces a cytokine release syndrome resulting in septic shock and death of sensitized mice.[5,6] Administration of endotoxin to mice provokes a similar cascade of cytokines, although it

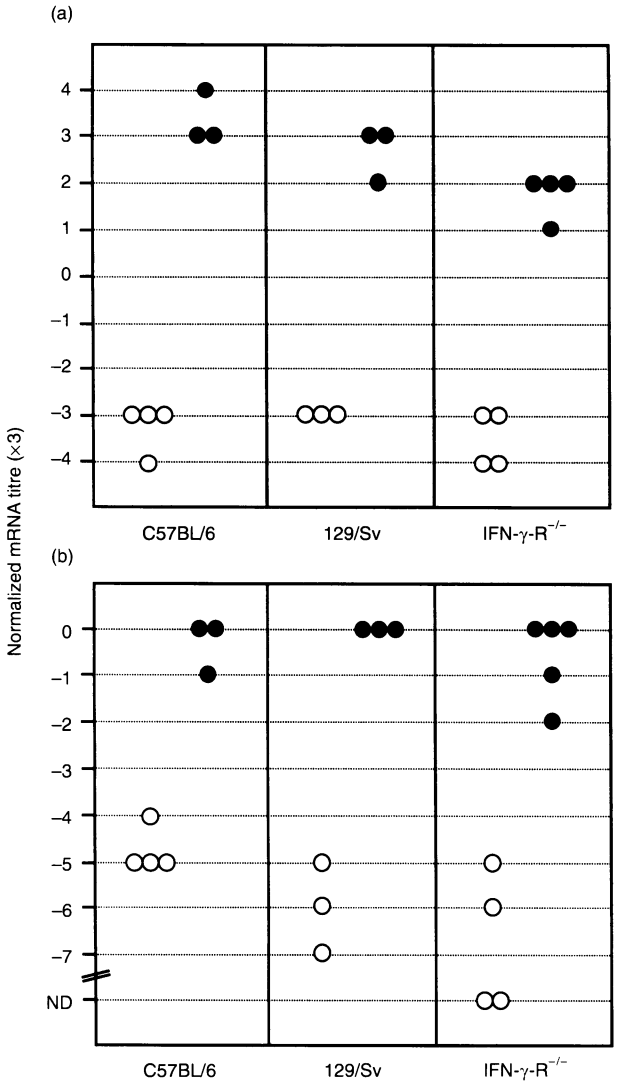

Figure 4. Induction of IP-10 and Mig mRNA during LPS-stimulated lung inflammation does not require the IFN-γ receptor. Total RNA was isolated from lungs of various mouse strains 4 hr after LPS (filled circles) or PBS treatment (open circles) and reverse transcribed. Serial cDNA dilutions (1 : 3) were used as template for PCR amplifications to detect IP-10 (a) or Mig (b). Normalized cDNA titres, as defined in the legend to Fig. 1, are presented. Each symbol represents the result from lungs of a single mouse. ND, not detectable.

primarily activates mononuclear phagocytes.[25,26] Septic shock induced by either bacterial superantigens or endotoxin is dependent on TNF and IFN-γ as principal mediators.[5–8,27–29] Systemic exposure to superantigens or endotoxin results in acute lung inflammation that is associated with leucocyte recruitment and activation, up-regulation of endothelial cell adhesion molecules and increased vascular permeability.[10,11] In the present study, systemic application of the superantigen, SEB, or LPS served as models for analysis of the roles of IFN-γ and the 55 000 MW TNF receptor for chemokine induction during acute lung inflammation. We demonstrated that SEB-induced production of IFN-γ was required to stimulate lung expression of IP-10 and Mig but not other chemokines, including MIP-1α, MCP-1, RANTES, MIP-2, KC and LIX. When acute lung inflammation was induced by LPS, however, up-regulation of IP-10 and Mig was not dependent on IFN-γ or the IFN-γ receptor. In contrast to the IFN-γ receptor, genetic ablation of the 55 000 MW receptor for TNF did not affect SEB-triggered chemokine expression in the lung.

It has been reported that IFN-γ induces IP-10 and Mig in monocytic cells both *in vitro* and *in vivo*.[30–34] Moreover, IP-10 mRNA levels are up-regulated by systemic application of TNF-α, IFN-β, or LPS in liver, spleen and kidney, but only poorly in lung and skin.[32] IP-10 expression of murine peritoneal macrophages is enhanced by IFN-γ as well as IFN-β and the combination of either type of interferon with IL-2 shows synergistic effects on IP-10 induction.[32] Similarly, TNF and IL-1β synergize with IFN-γ to augment IP-10 production of vascular smooth muscle cells.[35,36] In contrast to IP-10, type I interferons and LPS fail to up-regulate Mig mRNA levels in monocytic cell lines.[37,38] There is also evidence that, in addition to IFN-γ, recombinant TNF induces Mig in endothelial cells, whereas only IFN-γ appears to be effective with keratinocytes and fibroblasts.[33] These reports therefore indicate that many of the cytokines released in response to SEB are potential inducers of IP-10 and Mig. However, using mutant mouse strains we demonstrated that a single cytokine, IFN-γ, plays a predominant role for induction of IP-10 and Mig during SEB-triggered acute lung inflammation. Nonetheless, production of IP-10 and Mig was not completely suppressed in mice deficient for IFN-γ or the IFN-γ receptor, suggesting that alternate, but weak, agonists are present in the lungs of SEB-challenged mice. Although the biochemical nature of these mediators remains to be identified, SEB-induced up-regulation of IP-10 and Mig in 55 000 MW TNF receptor-deficient mice indicates that signalling through this receptor is not required.

In the present study, we demonstrated that LPS is a potent stimulus not only for IP-10, but also for Mig mRNA expression in lung. In direct contrast to SEB exposure, however, genetic ablation of IFN-γ or the IFN-γ receptor did not affect induction of IP-10 and Mig mRNA by LPS, suggesting different mechanisms of IP-10 and Mig regulation during lung inflammation triggered by SEB or LPS. Consistent with these observations, previous work has demonstrated that during infection of mice with *Toxoplasma gondii* or vaccinia virus, IP-10 mRNA was augmented in multiple organs, but only in mice infected with *Toxoplasma gondii* induction of IP-10 was dependent on IFN-γ.[34] Stimulated expression of Mig, however, was abrogated by neutralization of IFN-γ in both infection models.[34] It therefore appears that IFN-γ independent induction of Mig *in vivo* is unique to lung inflammation triggered by endotoxin. Collectively, these results suggest that the role of IFN-γ for regulation of IP-10, as well as Mig, at sites of inflammation is critically dependent on the nature of the inflammatory stimulus.

Expression of IP-10 mRNA has been observed in a number of different cell types, including monocytes and macrophages, neutrophils, endothelial cells, fibroblasts, hepatocytes and keratinocytes. Interestingly, different subsets of mononuclear phagocytes were shown to produce IP-10, depending on the route of agonist administration.[39] After local administration of IFN-γ by aerosol, IP-10 mRNA was up-regulated in alveolar macrophages but not in peripheral blood monocytes. Conversely, following systemic administration of IFN-γ

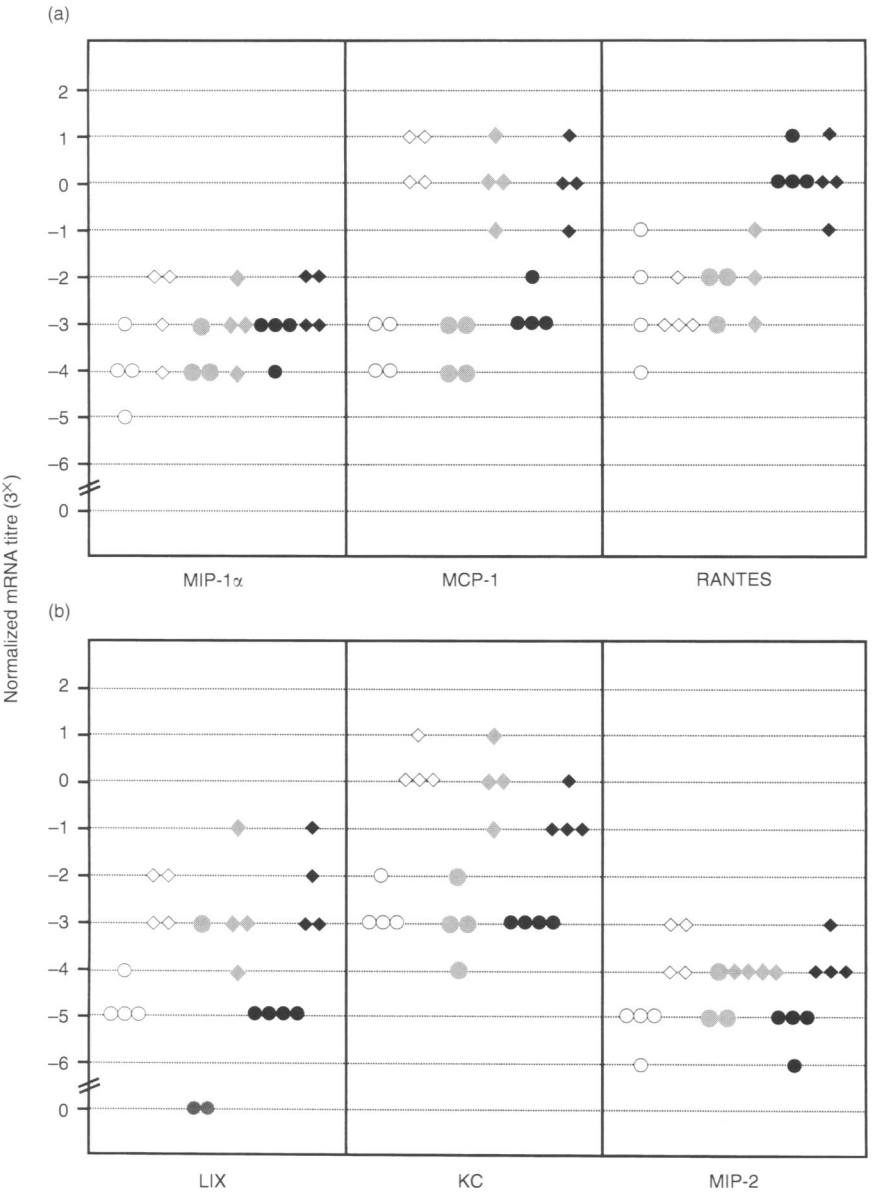

Figure 5. Induction of lung chemokine expression after SEB challenge. Total RNA was isolated from the lungs of C57BL/6 mice (open symbols), IFN-γ receptor-deficient mice (grey symbols) and 55 000 MW TNF receptor-deficient mice (filled symbols), 4 hr after treatment with SEB (triangles) or PBS (circles), and reverse transcribed. Serial cDNA dilutions (1:3) were used as template for PCR amplifications to detect expression of the chemokines KC, LIX, MCP-1, MIP-1α, MIP-2 and RANTES. Normalized cDNA titres, as defined in the legend to Fig. 1, are presented. Each symbol represents the result from lungs of a single mouse.

peripheral blood monocytes, but not alveolar macrophages, expressed IP-10. In lungs of SEB-treated wild-type, but not IFN-γ receptor knockout mice, IP-10 was strongly expressed on interstitial macrophages and on some alveolar macrophages. These observations therefore suggest that in SEB-induced lung inflammation, IP-10 may be induced by IFN-γ released from cells within the lung rather than by circulating IFN-γ derived from distant sites.

Mig and IP-10 are chemotactic for activated T cells, but not for neutrophils or unstimulated T lymphocytes.[40–43] IP-10 was also reported to stimulate monocyte and NK cell chemotaxis and to augment NK cell-mediated cytolytic responses.[41,42,44,45] Together with the results of the present report, these data are consistent with a role of IP-10 and Mig in leucocyte recruitment to the lung during septic shock. However, despite similar induction of IP-10 and Mig in superantigen- and endotoxin-triggered lung inflammation, it is important to note that the mechanisms of IP-10 and Mig regulation clearly differ, depending on the nature of the inflammatory stimulus.

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REFERENCES

1. Heeg K., Miethke T. & Wagner H. (1996) Superantigen-mediated lethal shock: the functional state of ligand-reactive T cells. *Curr Top Microbiol Immunol* **216,** 83.
2. Herman A., Kappler J.W., Marrack P. & Pullen A.M. (1991) Superantigens: mechanism of T-cell stimulation and role in immune responses. *Annu Rev Immunol* **9,** 745.
3. Herrmann T. & MacDonald H.R. (1991) T cell recognition of superantigens. *Curr Top Microbiol Immunol* **174,** 21.
4. Gaus H., Miethke T., Wagner H. & Heeg K. (1998) Superantigen-induced anergy of $V\beta8^+$ $CD4^+$ T cells induces functional but non-proliferative T cells *in vivo*. *Immunology* **83,** 333.
5. Miethke T., Wahl C., Heeg K., Echtenacher B., Krammer P.H. & Wagner H. (1992) T cell-mediated lethal shock triggered in mice by the superantigen staphylococcal enterotoxin B: critical role of tumor necrosis factor. *J Exp Med* **175,** 91.
6. Miethke T., Wahl C., Regele D., Gaus H., Heeg K. & Wagner H. (1993) Superantigen mediated shock: a cytokine release syndrome. *Immunobiology* **189,** 270.
7. Pfeffer K., Matsuyama T., Kündig T.M. *et al.* (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.
8. Rothe J., Lesslauer W., Lötscher H. *et al.* (1993) Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by *Listeria monocytogenes*. *Nature* **364,** 798.
9. Xu H., Gonzalo J.A., Pierre Y. Sr. *et al.* (1994) Leukocytosis and resistance to septic shock in intercellular adhesion molecule 1-deficient mice. *J Exp Med* **180,** 95.
10. Neumann B., Engelhardt B., Wagner H. & Holzmann B. (1997) Induction of acute inflammatory lung injury by staphylococcal enterotoxin B. *J Immunol* **158,** 1862.
11. Brigham K.L. & Meyrick B. (1986) Endotoxin and lung injury. *Am Rev Respir Dis* **133,** 913.
12. DeGrendele H.C., Estess P. & Siegelman M. (1997) Requirement for CD44 in activated T cell extravasation into an inflammatory site. *Science* **278,** 672.
13. Simms H.H. & D'Amico R. (1991) Increased PMN CD11b/CD18 expression following post-traumatic ARDS. *J Surg Res* **50,** 362.
14. Matsumoto T., Yokoi K., Mukaida N. *et al.* (1997) Pivotal role of interleukin-8 in the acute respiratory distress syndrome and cerebral reperfusion injury. *J Leukoc Biol* **62,** 581.
15. Frevert C.W., Huang S., Danaee H., Paulauskis J.D. & Kobzik L. (1995) Functional characterization of the rat chemokine KC and its importance in neutrophil recruitment in a rat model of pulmonary inflammation. *J Immunol* **154,** 335.
16. Schmal H., Shanley T.P., Jones M.L., Friedl H.P. & Ward P.A. (1996) Role for macrophage inflammatory protein-2 in lipopolysaccharide-induced lung injury in rats. *J Immunol* **156,** 1963.
17. Miller E.J., Cohen A.B., Nagao S. *et al.* (1992) Elevated levels of NAP-1/interleukin-8 are present in the airspaces of patients with the adult respiratory distress syndrome and are associated with increased mortality. *Am Rev Respir Dis* **146,** 427.
18. Courtney Broaddus V., Boylan A.M., Hoeffel J.M. *et al.* (1994) Neutralization of IL-8 inhibits neutrophil influx in a rabbit model of endotoxin-induced pleurisy. *J Immunol* **152,** 2960.
19. Ulich T.R., Howard S.C., Remick D.G. *et al.* (1995) Intratracheal administration of endotoxin and cytokines. VI. antiserum to CINC inhibits acute inflammation. *Am J Physiol* **268,** 245.
20. Standiford T.J., Kunkel S.L., Lukacs N.W. *et al.* (1995) Macrophage inflammatory protein-1α mediates lung leukocyte recruitment, lung capillary leak and early mortality in murine endotoxemia. *J Immunol* **155,** 1515.
21. Jansen P.M., van Damme J., Put W., De Jong I.W., Taylor F.B. & Hack C.E. (1995) Monocyte chemotactic protein 1 is released during lethal and sublethal bacteremia in baboons. *J Infect Dis* **171,** 1640.
22. Zisman D.A., Kunkel S.L., Strieter R.M. *et al.* (1997) MCP-1 protects mice in lethal endotoxemia. *J Clin Invest* **99,** 2832.
23. Jones M.L., Mulligan M.S., Flory C.M., Ward P.A. & Warren J.S. (1992) Potential role of monocyte chemoattractant protein 1/JE in monocyte/macrophage-dependent IgA immune complex alveolitis in the rat. *J Immunol* **149,** 2147.
24. Huang S., Hendriks W., Althage A. *et al.* (1993) Immune response in mice that lack the interferon-γ receptor. *Science* **259,** 1742.
25. Freudenberg M.A., Keppler D. & Galanos C. (1986) Requirement for lipopolysaccharide-responsive macrophages in galactosamine-induced sensitization to endotoxin. *Infect Immun* **51,** 891.
26. Beutler B. & Cerami A. (1988) Tumor necrosis, cachexia, shock and inflammation: a common mediator. *Annu Rev Biochem* **57,** 505.
27. Beutler B., Milsark I.W. & Cerami A.C. (1985) Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science* **229,** 869.
28. Tracey K.J., Beutler B., Lowry S.F. *et al.* (1986) Shock and tissue injury induced by recombinant human cachectin. *Science* **234,** 470.
29. Car B.D., Eng V.M., Schnyder B. *et al.* (1994) Interferon γ receptor deficient mice are resistant to endotoxic shock. *J Exp Med* **179,** 1437.
30. Narumi S., Wyner L.M., Stoler M.H., Tannenbaum C.S. & Hamilton T.A. (1992) Tissue-specific expression of murine IP-10 mRNA following systemic treatment with interferon γ. *J Leukoc Biol* **52,** 27.
31. Luster A.D., Unkeless J.C. & Ravetch J.V. (1985) γ-interferon transcriptionally regulates an early-response gene containing homology to platelet proteins. *Science* **315,** 672.
32. Vanguri P. & Farber J.M. (1990) Identification of CRG-2. An interferon-inducible mRNA predicted to encode a murine monokine. *J Biol Chem* **265,** 15049.
33. Farber J.M. (1997) Mig and IP-10: CXC chemokines that target lymphocytes. *J Leukoc Biol* **61,** 246.
34. Amichay D., Gazzinelli R.T., Karupiah G., Moench T.R., Sher A. & Farber J.M. (1996) Genes for chemokines muMig and Crg-2 are induced in protozoan and viral infections in response to IFN-γ with patterns of tissue expression that suggest nonredundant roles *in vivo*. *J Immunol* **157,** 4511.
35. Wang X., Yue T.-L., Ohlstein E.H., Sung C.-P. & Feuerstein G.Z. (1996) Interferon-inducible protein-10 involves vascular smooth muscle cell migration, proliferation and inflammatory response. *J Biol Chem* **271,** 24286.
36. Narumi S., Finke J.H. & Hamilton T.A. (1990) Interferon γ and interleukin 2 synergize to induce selective monokine expression in murine peritoneal macrophages. *J Biol Chem* **265,** 7036.
37. Farber J.M. (1990) A macrophage mRNA selectively induced by γ-interferon encodes a member of the platelet factor 4 family of cytokines. *Proc Natl Acad Sci USA* **87,** 5238.
38. Farber J.M. (1993) HuMIG: a new human member of the chemokine family of cytokines. *Biochem Biophys Res Commun* **192,** 223.
39. Jaffe H.A., Buhl R., Mastrangeli A. *et al.* (1991) Organ specific cytokine therapy. Local activation of mononuclear phagocytes by delivery of an aerosol of recombinant interferon-γ to the human lung. *J Clin Invest* **88,** 297.
40. Loetscher M., Gerber B., Loetscher P. *et al.* (1996) Chemokine

receptor specific for IP10 and Mig: structure, function and expression in activated T lymphocytes. *J Exp Med* **184,** 963.

41. Taub D.D., Lloyd A.R., Conlon K. *et al.* (1993) Recombinant human interferon-inducible protein 10 is a chemoattractant for human monocytes and T lymphocytes and promotes T cell adhesion to endothelial cells. *J Exp Med* **177,** 1809.
42. Taub D.D., Longo D.L. & Murphy W.J. (1996) Human interferon-inducible protein-10 induces mononuclear cell infiltration in mice and promotes the migration of human T lymphocytes into the peripheral tissues of human peripheral blood lymphocytes–SCID mice. *Blood* **87,** 1423.
43. Liao F., Rabin R.L., Yannelli J.R., Koniaris L.G., Vanguri P. & Farber J.M. (1995) Human Mig chemokine: biochemical and functional characterization. *J Exp Med* **182,** 1301.
44. Taub D.D., Sayers T.J., Carter C.R.D. & Ortaldo J.R. (1995) α and β chemokines induce NK cell migration and enhance NK-mediated cytolysis. *J Immunol* **155,** 3877.
45. Maghazachi A.A., Skalhegg B.S., Rolstad B. & Al-Aoukaty A. (1997) Interferon-inducible protein-10 and lymphotactin induce the chemotaxis and mobilization of intracellular calcium in natural killer cells through pertussis toxin-sensitive and -insensitive heterotrimeric G-proteins. *FASEB J* **11,** 765.