

Early Response Cytokines and Innate Immunity: Essential Roles for TNF Receptor 1 and Type I IL-1 Receptor During *Escherichia coli* Pneumonia in Mice¹

Joseph P. Mizgerd,² Matt R. Spieker, and Claire M. Doerschuk³

The early response cytokines, TNF and IL-1, have overlapping biologic effects that may function to propagate, amplify, and coordinate host responses to microbial challenges. To determine whether signaling from these early response cytokines is essential to orchestrating innate immune responses to intrapulmonary bacteria, the early inflammatory events induced by instillation of *Escherichia coli* into the lungs were compared in wild-type (WT) mice and mice deficient in both TNF receptor 1 (TNFR1) and the type I IL-1 receptor (IL1R1). Neutrophil emigration and edema accumulation induced by *E. coli* were significantly compromised by TNFR1/IL1R1 deficiency. Neutrophil numbers in the circulation and within alveolar septae did not differ between WT and TNFR1/IL1R1 mice, suggesting that decreased neutrophil emigration did not result from decreased sequestration or delivery of intravascular neutrophils. The nuclear translocation of NF- κ B and the expression of the chemokine macrophage inflammatory protein-2 did not differ between WT and TNFR1/IL1R1 lungs. However, the concentration of the chemokine KC was significantly decreased in the bronchoalveolar lavage fluids of TNFR1/IL1R1 mice compared with that in WT mice. Thus, while many of the molecular and cellular responses to *E. coli* in the lungs did not require signaling by either TNFR1 or IL1R1, early response cytokine signaling was critical to KC expression in the pulmonary air spaces and neutrophil emigration from the alveolar septae. *The Journal of Immunology*, 2001, 166: 4042–4048.

The early response cytokines, TNF and IL-1, are generated in response to microbial challenges. These cytokines amplify, propagate, and coordinate proinflammatory signals, resulting in the synchronized expression of effector molecules that mediate diverse aspects of innate immunity (for review, see Refs. 1–3). Especially important for the initial responses to bacterial infections, TNF and IL-1 are capable of eliciting expression of chemokines and adhesion molecules and thus may be critical to the recruitment of neutrophils from the blood. In mice the chemokines that mediate neutrophil emigration in response to bacterial stimuli in the lungs include KC and macrophage inflammatory protein-2 (MIP-2).⁴ Both are ELR-containing CXC chemokines that can elicit neutrophil emigration in vitro (4, 5), and each is independently essential to maximal neutrophil emigration elicited by *Escherichia coli* LPS in the lungs (4, 6). The expression of chemokines and adhesion molecules induced by early response cytokines is mediated by the NF- κ B family of transcription factors (7–9).

TNF- α signals through two different receptors, TNFR1 and TNFR2. TNFR1 induces proinflammatory signaling, as evidenced by the activities of specific agonists of TNFR1 (10, 11) and by overexpression of TNFR1 induced by transfection (12). TNFR2 is also capable of generating proinflammatory signals, as evidenced by TNFR2-specific agonists (13) and by TNF-mediated activation of cells that do not express TNFR1 (14), but this receptor requires higher doses of ligand and/or nonsoluble forms of ligand (15). The gene-targeted deletion of TNFR1 compromises cellular responses to soluble TNF- α , including NF- κ B translocation in fibroblasts (16) and adhesion molecule expression on endothelial cells (17), and results in an inability to control bacterial infections (18–20). In contrast, deficiency of TNFR2 has only modest effects on TNF-induced NF- κ B translocation in cultured fibroblasts (16), and TNFR2-deficient mice do not demonstrate compromised antibacterial defenses (21). Thus, although TNFR2 is capable of eliciting proinflammatory signaling, TNFR1 appears to function as the primary signaling receptor for TNF- α .

IL-1 cytokines (IL-1 α and IL-1 β) bind to two distinct receptors, IL1R1 and IL1R2, but IL1R2 contains a minimal cytoplasmic tail and is incapable of conveying intracellular signals from extracellular IL-1 molecules (for review, see Ref. 22). IL1R1 interacts with a different set of adapter molecules from TNFR1, but the downstream pathways (including NF- κ B) and effects (transcription of chemokines and adhesion molecules) of IL1R1 activation are largely overlapping with those of TNFR1 (discussed in Refs. 1–3, 23, and 24).

The similar biologic effects of TNF and IL-1 suggest that these cytokines share important functions. In the present studies signaling by both TNF and IL-1 was interrupted by combined genetic deficiencies of TNFR1, the primary signaling receptor for TNF- α , and IL1R1, the only signaling receptor for IL-1 α and IL-1 β . To determine whether early response cytokine functions were essential to orchestrating innate immune responses to pulmonary infection, multiple parameters of acute inflammation were compared in

Physiology Program, Harvard School of Public Health, Boston, MA 02115

Received for publication July 27, 2000. Accepted for publication January 2, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by U.S. Public Health Service Grants HL48160 and HL52466, a Clinical Scientist Award in Translational Research from the Burroughs Wellcome Fund, and a research grant from the American Lung Association. J.P.M. is a Parker B. Francis Fellow in Pulmonary Research.

² Address correspondence and reprint requests to Dr. Joseph P. Mizgerd, Physiology Program, Harvard School of Public Health, Building I, Room 301, 665 Huntington Avenue, Boston, MA 02115. E-mail address: jmizgerd@hsph.harvard.edu

³ Current address: Division of Integrative Biology, Rainbow Babies Children's Hospital, Cleveland, OH 44106.

⁴ Abbreviations used in this paper: MIP, macrophage inflammatory protein; BALF, bronchoalveolar lavage fluid; IL1R1, type I IL-1 receptor; TNFR1, TNF receptor 1 (p55, CD120a); TNFR2, TNF receptor 2 (p75, CD120b); WT, wild type; i.t., intratracheal.

wild-type (WT) and combined TNFR1/IL1R1-deficient mice (TNFR1/IL1R1 mice) after the intratracheal (i.t.) instillation of *E. coli*.

Materials and Methods

Neutrophil emigration and edema accumulation

TNFR1/IL1R1 mice (21), WT mice of similar random hybrid genetic background (C57BL/6 × 129/Sv), and IL1R1-deficient mice (25) that were backcrossed five generations onto a C57BL/6 background were maintained under specific pathogen-free conditions in a full-barrier facility. C57BL/6 mice were purchased from Taconic Farms (Germantown, NY). All experiments used mice at 6–10 wk of age. Lungs from additional sets of mice at ≥52 wk of age were histologically examined for evidence of spontaneous inflammatory processes in the absence of experimental infection, but these older mice were not included in the no instillation control groups. Mice were anesthetized by i.m. injection of ketamine hydrochloride (100 mg/kg) and acepromazine maleate (5 mg/kg), and ¹²⁵I-labeled human albumin (Mallinckrodt, Hazelwood, MO) was injected i.v. as a marker for plasma content. The trachea was surgically exposed, and an angiogatheter was inserted via the trachea into the left bronchus. Fifteen minutes after the injection of ¹²⁵I-labeled albumin, a suspension of *E. coli* (10⁸ CFU/ml) and colloidal carbon (5%), to mark the site of deposition, was instilled into the left lung lobe at a dose of 2.3 μl/g body weight. After 5 h and 58 min mice received i.v. injections of ⁵¹Cr-labeled murine RBC as a marker for blood content. Mice were killed 6 h after bacterial instillation by inhalation of a lethal overdose of halothane. The hearts were tied off to maintain pulmonary blood, and peripheral blood samples were collected from the inferior vena cava. Lungs were excised and fixed by i.t. instillation of 6% glutaraldehyde at a pressure of 23 cm H₂O. Emigrated and sequestered neutrophils were quantified by morphometry in histologic lung sections, as previously described (26, 27).

Pulmonary edema, as measured by the vascular leakage of ¹²⁵I-labeled albumin, was quantified before dissection of the lungs for morphometry, as previously described (26, 27). The specific activities of ¹²⁵I-labeled albumin and ⁵¹Cr-labeled RBC were measured for blood and plasma samples and for excised, fixed lungs from each mouse. The hematocrit of each mouse was calculated from the ¹²⁵I-labeled albumin activities in the blood and plasma samples. The pulmonary blood volume was derived from the ⁵¹Cr-labeled RBC activity in the lungs and blood sample. The total volume of plasma equivalents in the lungs was calculated from the ¹²⁵I-labeled albumin activities in the lungs and the plasma sample. The volume of intravascular plasma in the lungs was derived from the hematocrit and the pulmonary blood volume. The volume of extravascular plasma equivalents in the lungs was calculated as the difference between the total volume of plasma equivalents and the volume of intravascular plasma. Edema fluid accumulation was expressed as microliters of extravascular plasma equivalents per lung.

Circulating neutrophils were quantified in peripheral blood samples. After RBC lysis, leukocytes were counted using a hemacytometer, and differential distributions were assessed in blood smears stained with LeukoStat (Fisher Scientific, Pittsburgh, PA).

NF-κB translocation

WT and TNFR1/IL1R1 mice were anesthetized and instilled with bacteria as described above. After 6 h mice were killed by halothane overdose. Colloidal carbon-containing lung lobes from mice instilled with *E. coli*, left lung lobes of mice that did not receive bacterial instillation, and liver lobes from the same mice, were excised, snap-frozen in liquid nitrogen, and stored at –80°C until protein extraction. Nuclear proteins were collected from frozen tissue samples, and protein concentrations were measured using a bicinchoninic acid assay with BSA as the standard. Nuclear proteins were incubated at 0.5 mg/ml with 3.5 nM [γ -³²P]ATP-labeled NF-κB consensus oligonucleotide (Promega, Madison, WI). Protein-oligonucleotide complexes were separated from protein-free oligonucleotides by PAGE, detected by autoradiography, and quantitated by densitometry using Scion ImagePC software (Scion, Frederick, MD).

Chemokine expression

WT and TNFR1/IL1R1 mice were anesthetized and instilled with bacteria as described above. After 6 h mice were killed by halothane overdose. The chest cavity was opened, a catheter was tied into the trachea, and the airways to the right lung lobes were clamped closed. The left lung lobe was lavaged nine times with 0.5 ml of PBS. After centrifugation to rid the bronchoalveolar lavage fluids (BALF) of cells and debris, the BALF was snap-frozen in liquid nitrogen and stored at

–80°C until KC and MIP-2 concentrations were measured by ELISA (R&D Systems, Minneapolis, MN).

Statistics

Data were presented as the mean ± SE for four to six mice per group. Comparisons among multiple groups used one-way ANOVA and post hoc Scheffé tests. Comparisons between two groups used Student's *t* test. Differences were considered significant when *p* < 0.05.

Results

Spontaneous pulmonary inflammation in the absence of TNFR1 and IL1R1

A characteristic pattern of patchy pulmonary inflammation spontaneously developed in TNFR1/IL1R1 mutant mice. Although most of the lung tissue from each of the TNFR1/IL1R1 mice appeared to be normal, focal inflammatory infiltrates were observed in histologic sections from three of four TNFR1/IL1R1 mice examined at 10 wk of age and six of nine mice examined at 52 wk of age (Fig. 1). Infiltrates contained mixed populations of emigrated leukocytes. They typically localized to the pleura, subpleural alveoli, and perivascular tissue, but in the most severe example, an entire cross-section from one of the lung lobes of a 52-wk-old mouse was involved. Eosinophilic crystalline deposits were observed in the alveolar air spaces of affected regions. Apart from these regions that suggested chronic inflammatory processes, which were sparse and focal, the lung tissue from TNFR1/IL1R1 mice did not appear histologically distinct from that in age-matched WT mice. No leukocytic infiltrates, crystalline deposits, or other evidence of infection and inflammation were evident in four WT mice examined at 9 wk of age or six WT mice examined at >52 wk of age.

E. coli-induced pulmonary inflammation in the absence of TNFR1 and IL1R1

Apart from the patchy and localized infiltrates described above, which were readily differentiated from acute pneumonia (Fig. 1) and were excluded from morphometric analyses, the alveolar air spaces of WT and TNFR1/IL1R1 mice that did not receive bacterial instillations were devoid of emigrated neutrophils (Fig. 2A). The i.t. instillation of *E. coli* induced neutrophil emigration in the lungs of both WT and TNFR1/IL1R1 mice (Figs. 1 and 2A). Significantly less emigration was induced in the mutant mice compared with WT mice (Fig. 2A).

Plasma extravasation was measured in total pulmonary tissue, which included the focal infiltrates observed in histologic sections. However, plasma extravasation in the absence of bacterial instillation did not differ between WT and TNFR1/IL1R1 mice (Fig. 2B), suggesting no differences in vascular permeability between noninfected WT and TNFR1/IL1R1 mice over the 6-h period in which they were examined. The instillation of *E. coli* resulted in an increased accumulation of extravascular plasma, consistent with pulmonary edema, in both WT and TNFR1/IL1R1 mutant mice (Fig. 2B). Similar to neutrophil emigration, the *E. coli*-induced plasma extravasation was reduced, but not eliminated, by the deficiency of TNFR1 and IL1R1 (Fig. 2B).

Extravascular neutrophils in the absence of TNFR1 and IL1R1

To determine whether the decreased neutrophil emigration and edema accumulation resulted from a paucity of circulating neutrophils in TNFR1/IL1R1 mice, circulating neutrophil counts were compared in WT and mutant mice. There were no significant differences in the numbers of neutrophils per milliliter of peripheral blood between WT and TNFR1/IL1R1 mice, with or without *E. coli* pneumonia (Fig. 3A). Thus, decreased inflammatory responses

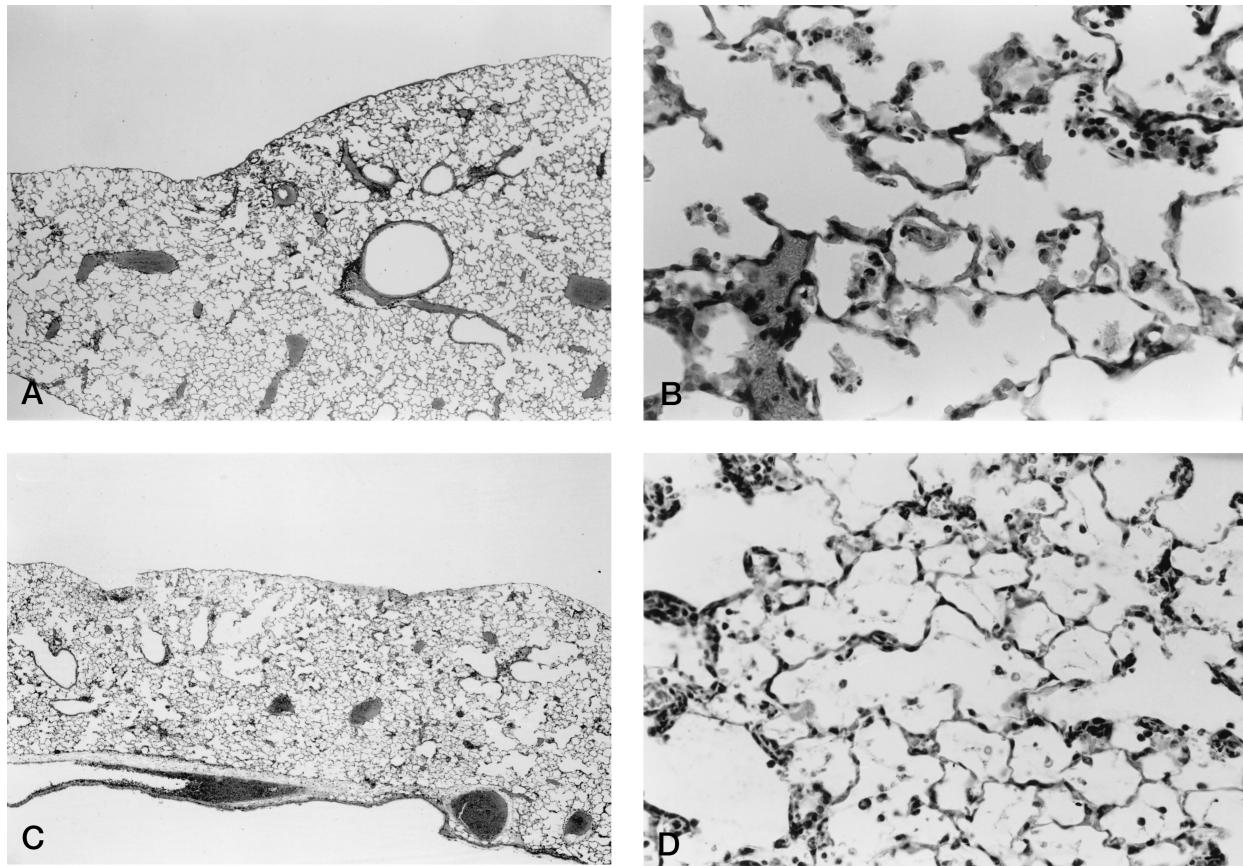


FIGURE 1. Photomicrographs of histologic sections from lungs of TNFR1/IL1R1 mice collected after no instillation (A and B) and 6 h after i.t. instillation of *E. coli* (C and D). A, A representative patch of spontaneous inflammation. Although most of the tissue is not inflamed, the upper middle region of the micrograph demonstrates leukocyte infiltrates in the pleural wall, subpleural alveoli, and perivascular tissue. B, A higher magnification from this region, demonstrating organized infiltrates of mixed leukocytes in the alveolar air spaces associated with eosinophilic crystalline deposits. C, Representative section of inflammation induced by *E. coli* in lungs from TNFR1/IL1R1 mice. A diffuse infiltration of leukocytes is apparent throughout the alveolar air spaces. D, A higher magnification from this section demonstrates emigrated neutrophils and staining suggestive of edema accumulation in the alveolar air spaces. All sections were stained with hematoxylin and eosin. Original magnification: A and C, $\times 40$; C and D, $\times 400$.

in the lungs of TNFR1/IL1R1 mice did not result from peripheral blood neutropenia.

To determine whether the decreased neutrophil emigration resulted from an inability of intravascular neutrophils to sequester within the pulmonary capillaries before emigration, the number of neutrophils within the alveolar septae of WT and TNFR1/IL1R1 mice were quantified. Similar numbers of septal neutrophils were present in the lungs of noninfected WT and TNFR1/IL1R1 mice (Fig. 3B). *E. coli* instillation resulted in increased numbers of septal neutrophils in both WT and TNFR1/IL1R1 mice, consistent with neutrophil sequestration (Fig. 3B). The numbers of neutrophils in alveolar septae of *E. coli*-instilled TNFR1/IL1R1 mice did not significantly differ from those in WT mice (Fig. 3B), suggesting that neutrophil sequestration was not impaired by the deficiency of TNFR1 and IL1R1.

NF- κ B translocation in the absence of TNFR1 and IL1R1

Both TNFR1 and IL1R1 induce the nuclear translocation of NF- κ B transcription factors (23), and NF- κ B mediates the transcription of many genes that regulate inflammatory responses (28). To determine whether NF- κ B was differentially activated in the presence or the absence of TNFR1 and IL1R1, the nuclear translocation of NF- κ B was examined in the lungs of WT and TNFR1/IL1R1 mice. Levels of NF- κ B proteins in the nuclear fractions from noninfected lungs did not significantly differ between genotypes (Fig. 4A). The instillation of *E. coli* resulted in the accumu-

lation of NF- κ B proteins in the nuclear fractions, consistent with nuclear translocation of these transcription factors (Fig. 4A). There were no significant differences in the net nuclear accumulation of NF- κ B proteins in the lungs of WT and TNFR1/IL1R1 mice (Fig. 4B).

In addition to local inflammatory responses, bacterial infection of the lungs induces systemic acute phase responses (29, 30). The transcription of acute phase proteins by the liver is regulated by NF- κ B (31–33). To determine whether systemic responses to intrapulmonary *E. coli* were affected by deficiency of early response cytokine receptors, the nuclear translocation of NF- κ B in the liver was compared in WT and TNFR1/IL1R1 mice. Similar levels of NF- κ B proteins were present within the nuclei of livers from noninfected WT and TNFR1/IL1R1 mice (Fig. 4, C and D). The instillation of *E. coli* resulted in the nuclear accumulation of NF- κ B proteins in the livers of WT mice (Fig. 4, C and D). In contrast, no accumulation of NF- κ B proteins was detected in the livers of TNFR1/IL1R1 mice instilled with *E. coli* (Fig. 4, C and D), suggesting that these early response cytokine receptors are essential for activation of NF- κ B in the liver in response to pulmonary *E. coli* infection.

Chemokine expression in the absence of TNFR1 and IL1R1

Chemokines direct the migration of neutrophils, and the rodent chemokines KC and MIP-2 are essential for maximal neutrophil emigration in response to i.t. instillation of *E. coli* LPS (4, 6). To

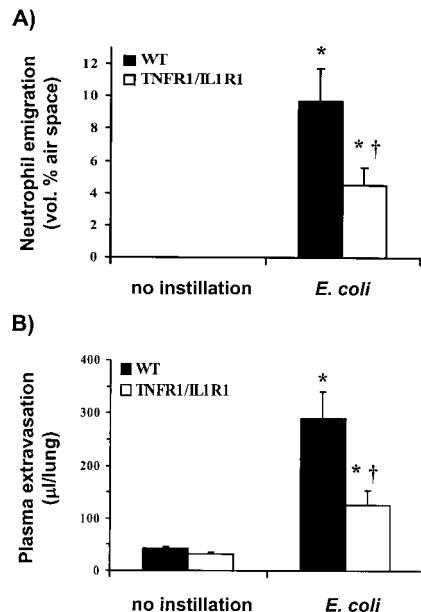


FIGURE 2. Neutrophil emigration (A) and edema accumulation (B) in the lungs of WT and TNFR1/IL1R1-deficient mice with *E. coli* pneumonia. Emigrated neutrophils were quantified in histologic sections of lungs using morphometric methods. Edema was quantified as the volume of extravascular plasma equivalents based on radiotracer distribution. *, Significant differences compared with no instillation in same genotype; †, Significant differences compared with WT after same instillation.

determine whether KC and/or MIP-2 expression required the early response cytokine receptors TNFR1 and IL1R1, KC and MIP-2 concentrations were compared in BALF collected 6 h after *E. coli* instillation to WT and TNFR1/IL1R1 mice. KC expression was detected in all mice examined, but KC concentrations in BALF of *E. coli*-instilled TNFR1/IL1R1 mice were significantly less than those in WT mice (Fig. 5). MIP-2 expression was detected in all mice examined, but, unlike KC, there were no significant differences between MIP-2 concentrations in BALF from WT and TNFR1/IL1R1 mice (Fig. 5).

Inflammatory responses in the absence of IL1R1 alone

To determine whether the deficiency of IL1R1 alone was sufficient to compromise these inflammatory processes, C57BL/6 mice and IL1R1-deficient mice on C57BL/6 backgrounds received i.t. instillations of *E. coli*. After 6 h there were no statistically significant differences in the numbers of emigrated, sequestered, or circulating neutrophils in C57BL/6 and IL1R1-deficient mice (Table I). There were no significant differences in *E. coli*-induced plasma extravasation in C57BL/6 and IL1R1-deficient mice (Table I). Thus, in mice with uninterrupted expression of TNFR1, the deficiency of IL1R1 did not compromise neutrophil emigration or edema accumulation 6 h after the instillation of *E. coli*.

Discussion

TNFR1/IL1R1 mice developed patchy inflammation within their lungs, which was associated with eosinophilic crystal deposits. Such crystal deposits may be a hallmark of chronic pulmonary inflammation in mice (34–37). Although C57BL/6 mice and hybrid mice of mixed C57BL/6 × 129/Sv backgrounds are susceptible to developing idiopathic pulmonary inflammation as they age (see discussions in Refs. 34 and 37), we observed eosinophilic crystals and leukocytic infiltrates in TNFR1/IL1R1 mice, but not in age-matched WT mice in our barrier facility. The development of

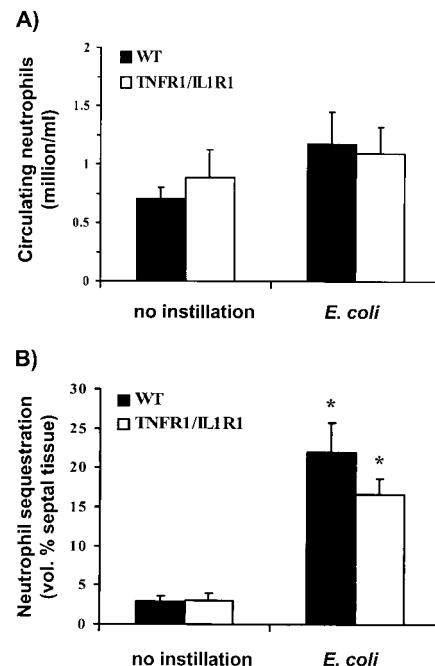


FIGURE 3. Circulating (A) and sequestered (B) neutrophils in WT and TNFR1/IL1R1-deficient mice with *E. coli* pneumonia. Circulating neutrophils were enumerated in blood samples drawn from the inferior vena cava. Sequestered neutrophils were quantified in histologic sections of lungs using morphometric methods. *, Significant differences compared with no instillation in same genotype. TNFR1/IL1R1 mice and WT mice did not significantly differ.

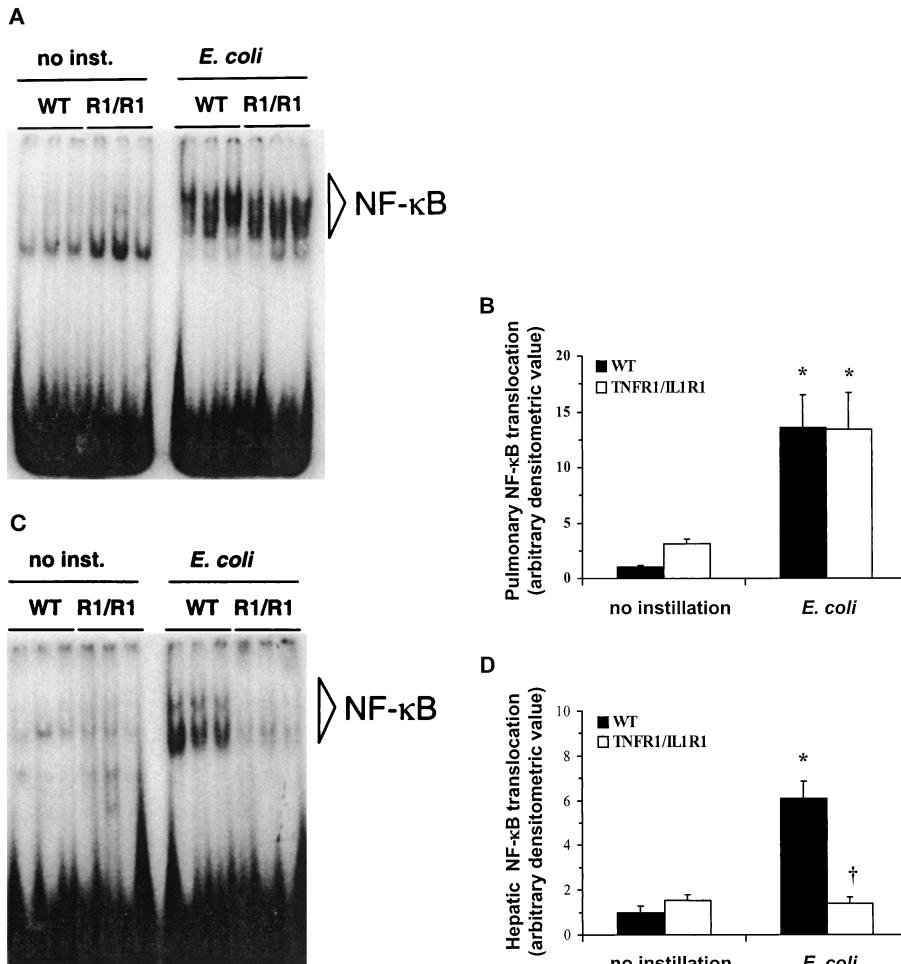
this cellular and crystalline accumulation in young TNFR1/IL1R1 mice probably represents a compromise in pulmonary host defenses resulting from gene-targeted mutations in the TNFR1 and IL1R1 genes.

The combined deficiencies of TNFR1 and IL1R1 compromised neutrophil emigration and edema accumulation in response to *E. coli* in the alveolar air spaces, indicating an essential role for early response cytokine signaling in coordinating these innate immune responses. In contrast, neither the combined deficiency of both TNFR1 and TNFR2 (27) nor the deficiency of IL1R1 alone compromised neutrophil emigration or edema accumulation 6 h after the i.t. instillation of *E. coli*. Altogether these data suggest that TNFR1 and IL1R1 serve essential signaling functions in eliciting acute inflammatory responses to *E. coli* in the lungs, but the essential functions mediated by these receptors are shared and can be elicited by either receptor in the other's absence.

The mechanisms by which the deficiency of TNFR1 and IL1R1 compromises neutrophil emigration are not entirely clear. Neutrophil numbers were significantly decreased in the alveolar air spaces, but not in the alveolar septae, suggesting that signaling from these receptors is not required for neutrophil sequestration, but is essential to coordinating neutrophil migration across the endothelial or epithelial barriers or through the interstitium.

Deficiency of TNFR1 and IL1R1 significantly decreased KC expression, but not MIP-2 expression, elicited by *E. coli* in the lungs. Differential regulation of these two chemokines is surprising and has not been previously reported to our knowledge. Expression of each of these chemokines is regulated transcriptionally, but the dominant factor mediating LPS- or TNF-induced expression from the promoters of each of these genes is common to both, NF- κ B (8, 38). It is possible that other transcription factors, not yet identified, amplify the transcription of KC but not MIP-2, and these other

FIGURE 4. NF- κ B translocation in the lungs (*A* and *B*) and livers (*C* and *D*) of WT and TNFR1/IL1R1-deficient mice with *E. coli* pneumonia. *A*, Translocated NF- κ B proteins were assessed by EMSAs. Nuclear proteins were isolated from the lungs of WT or TNFR1/IL1R1 (R1/R1) mice with or without *E. coli* instilled intratracheally 6 h previously. Each lane contains proteins from an individual mouse. *B*, Translocated NF- κ B proteins in the lungs were quantified by densitometry. Each bar represents the mean and SEM from six mice. *, Significant differences compared with no instillation in same genotype. *C*, Translocated NF- κ B proteins detected with EMSAs using nuclear proteins isolated from the livers of WT or TNFR1/IL1R1 (R1/R1) mice with or without *E. coli* instilled intratracheally 6 h previously. Each lane contains proteins from an individual mouse. *D*, Translocated NF- κ B proteins in the livers were quantified by densitometry. Each bar represents the mean and SEM from six mice. *, Significant differences compared with no instillation in same genotype; †, Significant differences compared with WT after same instillation.



factors may require TNFR1 and IL1R1 signaling for their activation in the lungs during *E. coli* pneumonia. Furthermore, transcriptional regulation of the genes encoding KC and MIP-2 could require distinct coactivators, differentially dependent on TNFR1 and IL1R1 for their activation during *E. coli* pneumonia, to link NF- κ B and/or other transcription factors with the transcriptional machinery. Finally, the divergent effects of TNFR1/IL1R1 deficiency on the concentrations of these chemokines in the BALF of mice with *E. coli* pneumonia could result from differential posttranscriptional regulation of KC and MIP-2.

Decreased KC expression could be the means by which the deficiency of TNFR1 and IL1R1 compromised neutrophil emigration in the present studies, since KC function is essential to neutrophil emigration in response to *E. coli* LPS in the alveolar air spaces (4). Although only two chemokines were measured in these studies, many pro- and anti-inflammatory factors are regulated by the early response cytokines and may be affected by TNFR1/IL1R1 deficiency. The combined deficiency of these two receptors almost certainly resulted in complex disturbances in the balance of inflammatory mediators in the lungs of mice infected with *E. coli*, and the resulting shift in this balance is most likely responsible for

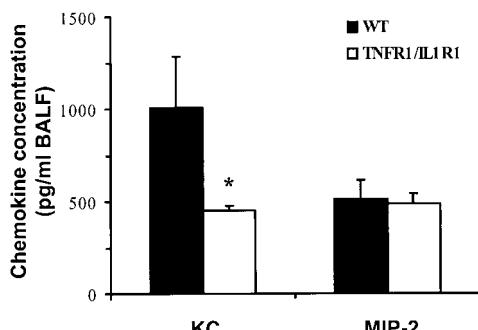


FIGURE 5. Chemokine expression in the lungs of WT and TNFR1/IL1R1-deficient mice with *E. coli* pneumonia. KC and MIP-2 concentrations were measured by ELISAs in BALF collected 6 h after instillation of *E. coli*. *, Significant difference compared with WT mice.

Table I. Inflammatory responses during *E. coli* pneumonia in IL1R1-deficient mice^a

	IL1R1 Deficient	
	C57BL/6	IL1R1 Deficient
Emigrated neutrophils (vol % air space)	3.7 ± 1.0	6.4 ± 1.2
Sequestered neutrophils (vol % septal tissue)	16 ± 2	25 ± 4
Circulating neutrophils (million/ml)	0.7 ± 0.1	1.4 ± 0.3
Plasma extravasation (μl/lung)	98 ± 32	146 ± 43

^a *E. coli* were instilled i.t. to C57BL/6 and IL1R1-deficient mice, and lungs were excised and fixed after 6 h. Emigrated and sequestered neutrophils were quantified in histological sections of lungs using morphometric methods. Circulating neutrophils were enumerated in blood samples drawn from the inferior vena cava. Plasma extravasation was quantified as the volume of extravascular plasma equivalents based on radiotracer distribution. There were no statistically significant differences between values from C57BL/6 and IL1R1-deficient mice.

the observed decreases in neutrophil emigration and edema accumulation.

Both TNFR1 and IL1R1 induce NF- κ B translocation, and NF- κ B is essential to neutrophil emigration in response to *E. coli* LPS in the lungs.⁵ However, in the present studies NF- κ B translocation in the lungs was not decreased by TNFR1/IL1R1 deficiency when examined using EMSAs and nuclear protein extracts from whole lung lobes. Thus, receptors other than TNFR1 and IL1R1 are sufficient for propagating NF- κ B signaling in the lungs. TNFR2 (13–15) and Toll-like receptors 2 and 4 (39–42) are capable of inducing NF- κ B translocation, are probably activated in the lungs during *E. coli* pneumonia, and may be mediating this signaling. Despite this evidence of NF- κ B activation, neutrophil emigration and edema accumulation were significantly decreased by deficiency of TNFR1 and IL1R1. Therefore, the signaling by TNFR1 and IL1R1 which is essential to neutrophil emigration and edema accumulation may be mediated by factors other than NF- κ B. Alternatively, TNFR1/IL1R1 deficiency may decrease essential NF- κ B translocation in specific subsets of lung cells, which may be indiscernible with the methods used in these studies.

NF- κ B translocation was inhibited by TNFR1/IL1R1 deficiency in the livers of pneumonic mice, suggesting that at least some extrapulmonary transmission of proinflammatory signaling requires these receptors. Whether hepatic activation of NF- κ B and the acute phase response contribute to neutrophil emigration in the lungs is unknown. The transcription of the acute phase proteins serum amyloid A (32) and complement C3 (33) is NF- κ B dependent, and these proteins can influence neutrophil activation and recruitment (43–46). If the acute phase response contributes to innate immune responses in pneumonic lungs, then the decreased pulmonary inflammation in TNFR1/IL1R1 mutant mice may be due in part to a lack of activation of NF- κ B in the liver.

Although neutrophil emigration and edema accumulation were compromised in TNFR1/IL1R1 mice compared with WT mice, these processes were not completely inhibited. Approximately half of the neutrophil emigration and a third of the edema accumulation occurred despite the complete absence of TNFR1 and IL1R1. Other mediators and receptors are clearly capable of propagating and coordinating signals required to elicit acute inflammatory responses to bacteria in the lungs. Some of these signaling events might be transmitted by TNF- α , particularly membrane-bound TNF- α (15), through TNFR2, which remains functional in the TNFR1/IL1R1 mice. Additional studies will be required to determine whether acute inflammatory responses that occur in the absence of TNFR1 and IL1R1 are mediated by TNFR2 or are entirely independent of the early response cytokines TNF and IL-1.

Acknowledgments

We thank Jacques J. Peschon (Immunex Corp., Seattle, WA) for generously providing TNFR1/IL1R1 mutant mice.

References

- Bazzoni, F., and B. Beutler. 1996. The tumor necrosis factor ligand and receptor families. *N. Engl. J. Med.* 334:1717.
- Dinarello, C. A. 1997. Interleukin-1. *Cytokine Growth Factor Rev.* 8:253.
- Mehrad, B., and T. J. Standiford. 1999. Role of cytokines in pulmonary antimicrobial host defense. *Immunol. Res.* 20:15.
- Frevert, C. W., S. Huang, H. Danaee, J. D. Paulauskis, and L. Kobzik. 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.
- E. A. Alcamo, J. P. Mizgerd, B. H. Horwitz, R. Bronson, A. A. Beg, M. Scott, C. M. Doerschuk, R. O. Hynes, and D. Baltimore. Targeted mutation of tumor necrosis factor receptor 1 rescues the RelA-deficient mouse and reveals a critical role for NF- κ B in leukocyte recruitment. *Submitted for publication.*
- Frevert, C. W., A. Farone, H. Danaee, J. D. Paulauskis, and L. Kobzik. 1995. Functional characterization of rat chemokine macrophage inflammatory protein-2. *Inflammation* 19:133.
- Schmal, H., T. P. Shanley, M. L. Jones, H. P. Friedl, and P. A. Ward. 1996. Role for macrophage inflammatory protein-2 in lipopolysaccharide-induced lung injury in rats. *J. Immunol.* 156:1963.
- Mukaida, N., Y. Mahe, and K. Matsushima. 1990. Cooperative interaction of nuclear factor- κ B- and *cis*-regulatory enhancer binding protein-like factor binding elements in activating the interleukin-8 gene by pro-inflammatory cytokines. *J. Biol. Chem.* 265:21128.
- Ohmori, Y., S. Fukumoto, and T. A. Hamilton. 1995. Two structurally distinct κ B sequence motifs cooperatively control LPS-induced KC gene transcription in mouse macrophages. *J. Immunol.* 155:3593.
- Collins, T., M. A. Read, A. S. Neish, M. Z. Whitley, D. Thanos, and T. Maniatis. 1995. Transcriptional regulation of endothelial cell adhesion molecules: NF- κ B and cytokine-inducible enhancers. *FASEB J.* 9:899.
- Mackay, F., H. Loetscher, D. Stueber, G. Gehr, and W. Lesslauer. 1993. Tumor necrosis factor α (TNF- α)-induced cell adhesion to human endothelial cells is under dominant control of one TNF receptor type, TNF-R55. *J. Exp. Med.* 177:1277.
- Barbara, J. A. J., W. B. Smith, J. R. Gamble, X. Van Ostade, P. Vandenabeele, J. Tavernier, W. Fiers, M. A. Vadas, and A. F. Lopez. 1994. Dissociation of TNF- α cytotoxic and proinflammatory activities by p55 receptor- and p75 receptor-selective TNF- α mutants. *EMBO J.* 13:843.
- Boldin, M. P., I. L. Mett, E. E. Varfolomeev, I. Chumakov, Y. Shemer-Avni, J. H. Camonis, and D. Wallach. 1994. Self-association of the death domains of the p55 tumor necrosis factor (TNF) receptor and Fas/APO1 prompts signaling for, TNF and Fas/APO1 effects. *J. Biol. Chem.* 270:387.
- Laegreid, A., A. Medvedev, U. Nonstad, M. P. Bombara, G. Ranges, A. Sundan, and T. Espenik. 1994. Tumor necrosis factor receptor p75 mediates cell-specific activation of nuclear factor- κ B and induction of human cytomegalovirus enhancer. *J. Biol. Chem.* 169:7785.
- Rothe, M., V. Sarma, V. M. Dixit, and D. V. Goeddel. 1995. TRAF2-mediated activation of NF- κ B by TNF receptor 2 and CD40. *Science* 269:1424.
- Grell, M., E. Doum, H. Wajant, M. Löhdner, M. Clauss, B. Maxeiner, S. Georgopoulos, W. Lesslauer, G. Kollias, K. Pfizenmaier, et al. 1995. The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. *Cell* 83:793.
- Kalb, A., H. Bluethmann, M. W. Moore, and W. Lesslauer. 1996. Tumor necrosis factors (Tnfr) in mouse fibroblasts deficient in Tnfr1 or Tnfr2 are signaling competent and activate the mitogen-activated protein kinase pathway with differential kinetics. *J. Biol. Chem.* 271:28097.
- Neumann, B., T. Machleidt, A. Lifka, K. Pfeffer, D. Vestweber, T. W. Mak, B. Holzmann, and M. Krönke. 1996. Critical role of 55-kilodalton TNF receptor in TNF-induced adhesion molecule expression and leukocyte organ infiltration. *J. Immunol.* 156:1587.
- Rothe, J., W. Lesslauer, H. Lotscher, Y. Lang, P. Koebel, F. Kontgen, A. Althage, R. Zinkernagel, M. Steinmetz, and H. Bluethmann. 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.
- Pfeffer, K., T. Matsuyama, T. M. Kundig, A. Wakeham, K. Kishihara, A. Shahinian, K. Wiegmann, 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.
- Flynn, J. L., M. M. Goldstein, J. Chan, K. J. Triebold, K. Pfeffer, C. J. Lowenstein, R. Schreiber, T. W. Mak, and B. R. Bloom. 1995. Tumor necrosis factor- α is required in the protective immune response against *Mycobacterium tuberculosis* in mice. *Immunity* 2:561.
- Peschon, J. J., D. S. Torrance, K. L. Stocking, M. B. Glaccum, C. Otten, C. R. Willis, K. Charrier, P. J. Morrissey, C. B. Ware, and K. M. Mohler. 1998. TNF receptor-deficient mice reveal divergent roles for p55 and p75 in several models of inflammation. *J. Immunol.* 160:943.
- Sims, J. E., J. G. Giri, and S. K. Dower. 1994. The two interleukin-1 receptors play different roles in IL-1 actions. *Clin. Immunol. Immunopathol.* 72:9.
- Mercurio, F., and A. M. Manning. 1999. Multiple signals converging on NF- κ B. *Curr. Opin. Cell Biol.* 11:226.
- Kopp, E. B., and R. Medzhitov. 1999. The Toll-receptor family and control of innate immunity. *Curr. Opin. Cell Biol.* 11:13.
- Glaccum, M. B., K. L. Stocking, K. Charrier, J. L. Smith, C. R. Willis, C. Maliszewski, D. J. Livingston, J. J. Peschon, and P. J. Morrissey. 1997. Phenotypic and functional characterization of mice that lack the type 1 receptor for IL-1. *J. Immunol.* 159:3364.
- Mizgerd, J. P., H. Kubo, G. J. Kutkoski, S. D. Bhagwan, K. Scharffetter-Kochanek, A. L. Beaudet, and C. M. Doerschuk. 1997. Neutrophil emigration in the skin, lungs, and peritoneum: different requirements for CD11/CD18 revealed by CD18-deficient mice. *J. Exp. Med.* 186:1357.
- Mizgerd, J. P., J. J. Peschon, and C. M. Doerschuk. 2000. Roles of tumor necrosis factor signaling during murine *Escherichia coli* pneumonia in mice. *Am. J. Respir. Crit. Care Med.* 22:85.
- Ghosh, S., M. J. May, and E. B. Kopp. 1998. NF- κ B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu. Rev. Immunol.* 16:225.
- Hansson, L. O., J. U. Hedlund, and A. B. Ortvist. 1997. Sequential changes of inflammatory and nutritional markers in patients with community-acquired pneumonia. *Scand. J. Clin. Lab. Invest.* 57:111.
- Kosmas, E. N., C. N. Baxevanis, M. Papamichail, and T. Kordossis. 1997. Daily variation in circulating cytokines and acute-phase proteins correlates with clinical

- and laboratory indices in community-acquired pneumonia. *Eur. J. Clin. Invest.* 27:308.
31. Vik, D., P. Amiguet, G. Moffat, M. Fey, F. Amiguet-Barras, R. Wetsel, and B. Tack. 1991. Structural features of the human C3 gene: intron/exon organization, transcriptional start site, and promoter region sequence. *Biochemistry* 30: 1080.
 32. Shimizu, H., and K. Yamamoto. 1994. NF- κ B and C/EBP transcription factor families synergistically function in mouse serum amyloid A gene expression induced by inflammatory cytokines. *Gene* 149:305.
 33. Moon, M., A. Parikh, T. Prittis, J. Fischer, S. Cottongim, C. Szabo, A. Salzman, and P. Hasselgren. 1999. Complement component C3 production in IL-1 β -stimulated human intestinal epithelial cells is blocked by NF- κ B inhibitors and by transfection with Ser 32/36 mutant IkB α . *J. Surg. Res.* 82:48.
 34. Murray, A. B., and A. Luz. 1990. Acidophilic macrophage pneumonia in laboratory mice. *Vet. Pathol.* 27:274.
 35. Huffnagle, G. B., M. B. Boyd, N. E. Street, and M. F. Lipscomb. 1998. IL-5 is required for eosinophil recruitment, crystal deposition, and mononuclear cell recruitment during a pulmonary *Cryptococcus neoformans* infection in genetically susceptible mice (C57BL/6). *J. Immunol.* 160:2393.
 36. Zhu, Z., R. J. Homer, Z. Wang, Q. Chen, G. P. Geba, J. Wang, Y. Zhang, and J. A. Elias. 1999. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J. Clin. Invest.* 103:779.
 37. Guo, L., R. S. Johnson, and J. C. L. Schuh. 2000. Biochemical characterization of endogenously formed eosinophilic crystals in the lungs of mice. *J. Biol. Chem.* 275:8032.
 38. Widmer, U., K. R. Manogue, A. Cerami, and B. Sherry. 1993. Genomic cloning and promoter analysis of macrophage inflammatory protein (MIP)-2, MIP-1 α , and MIP-1 β , members of the chemokine superfamily of proinflammatory cytokines. *J. Immunol.* 150:4996.
 39. Yang, R. B., M. R. Mark, A. Gray, A. Huang, M. H. Xie, H. Zhang, A. Goddard, W. I. Wood, A. L. Gurney, and P. J. Godowski. 1998. Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signaling. *Nature* 17:284.
 40. Kirschning, C. J., H. Wesche, T. M. Ayres, and M. Rothe. 1998. Human Toll-like receptor 2 confers responsiveness to bacterial lipopolysaccharide. *J. Exp. Med.* 188:2091.
 41. Poltorak, A., X. He, I. Smirnova, M. Liu, C. Van Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, et al. 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science* 282:2085.
 42. Qureshi, S. T., L. Larivière, G. Leveque, S. Clermont, K. J. Moore, P. Gros, and D. Malo. 1999. Endotoxin-tolerant mice have mutations in Toll-like receptor 4. *J. Exp. Med.* 189:615.
 43. Badolato, R., J. M. Wang, W. J. Murphy, A. R. Lloyd, D. F. Michiel, L. L. Bausserman, D. J. Kelvin, and J. J. Oppenheim. 1994. Serum amyloid A is a chemoattractant: induction of migration, adhesion, and tissue infiltration of monocytes and polymorphonuclear leukocytes. *J. Exp. Med.* 180:203.
 44. Prodeus, A. P., X. Zhou, M. Maurer, S. J. Galli, and M. C. Carroll. 1997. Impaired mast cell-dependent natural immunity in complement C3-deficient mice. *Nature* 390:172.
 45. Furlaneto, C., and A. Campa. 2000. A novel function of serum amyloid A: a potent stimulus for the release of tumor necrosis factor- α , interleukin-1 β , and interleukin-8 by human blood neutrophils. *Biochem. Biophys. Res. Commun.* 268: 405.
 46. Badolato, R., J. Wang, S. Stornello, A. Ponzi, M. Duse, and T. Musso. 2000. Serum amyloid A is an activator of PMN antimicrobial functions: induction of degranulation, phagocytosis, and enhancement of anti-*Candida* activity. *J. Leukocyte Biol.* 67:381.