Interleukin-1 receptor antagonist transiently impairs antibacterial defense but not survival in murine pneumococcal pneumonia

Anita W. Rijneveld[1,2*], Sandrine Florquin[3], Peter Speelman[2,4], Carl k. Edwards[5], Charles A. Dinarello[6], Tom van der Poll[2,4]

Academic Medical Center, University of Amsterdam, the Netherlands; Departments of [1] Experimental Internal Medicine, [2] Internal Medicine, [3] Pathology, [4] Infectious Diseases, Tropical Medicine and AIDS, [5] Amgen, Incorporated, Thousand Oaks, California 91320, [6] University of Colorado Health Sciences Center, Denver, Colorado 80262

Correspondence: Anita W. Rijneveld, Academic Medical Center, University of Amsterdam, G2-105 Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands. Tel.: + 31-20-5666034; Fax: + 31-20-6977192
E-mail: a.w.rijneveld@amc.uva.nl

ABSTRACT. The inhibition of the biological activity of IL-1 by recombinant human IL-1 receptor antagonist (IL-1ra) has been investigated in several, controlled clinical trials. Encouraging results have been reported, in particular in patients with rheumatoid arthritis. In the present study, we investigated the influence of treatment of wild type mice with IL-1ra, which resulted in an incomplete and transient inhibition of IL-1 activity. Treatment with recombinant human IL-1ra resulted in an enhanced bacterial outgrowth in the lungs of BALB/c and C57BL/6 mice early after induction of pneumococcal pneumonia, without influencing survival or the pulmonary inflammatory response. The effect of IL-1ra on the host response to *S. pneumoniae* pneumonia is modest and transient. The present data, together with the findings in IL-1R−/− mice in earlier work, suggest that IL-1 occupies a role in the pulmonary immune response to *S. pneumoniae* that is substantially less prominent than that of TNF-α.

Keywords: interleukin 1 receptor antagonist, pneumonia, *S. pneumoniae*, mouse, cytokines

INTRODUCTION

Interleukin (IL)-1 and tumor necrosis factor (TNF)-α are potent proinflammatory cytokines that are targets of therapeutic intervention in a variety of inflammatory and autoimmune conditions [1]. Recombinant human IL-1 receptor antagonist (IL-1ra) has been evaluated in several, controlled clinical trials investigating its capacity to inhibit the biological activity of IL-1, and promising results have, in particular, been reported in patients with rheumatoid arthritis (RA) [2-5], this resulted in approval of IL-1ra as a treatment of RA in the US and several European countries. Approaches to block endogenous TNF-α activity, such as with monoclonal antibodies or soluble TNF-α receptor constructs, have yielded beneficial effects in inflammatory disorders such as RA, Crohn's disease, psoriasis and ankylosing spondylitis [6-12]. However, concern has been raised about the possibility of enhanced susceptibility to infections in patients treated with anti-cytokine strategies.

This concern originated from experimental data showing that blocking either TNF-α or IL-1 in animals reduced host defense against bacterial infections [13-16]. Indeed, since the approval of antibodies against TNF-α, a higher incidence of disseminated and extrapulmonary tuberculosis in patients treated with an anti-TNF-α antibody has been reported [17]. An increased incidence of opportunistic infections has been observed in patients receiving soluble TNF receptor p75 [7, 12]. Although the number of patients treated with anti-TNF-α antibody or soluble TNF receptors is large compared to those receiving IL-1ra in controlled clinical trials, so far no mycobacterial or opportunistic infections have been reported in patients treated with recombinant IL-1ra [18]. In addition, the number of cases of bacterial pneumonia in IL-1ra-treated RA patients is within the range expected for RA patients receiving conventional therapy. However, experimental evidence indicates that such treatment may hamper antibacterial defense mechanisms. Indeed, we recently found that IL-1 receptor type I gene-deficient (IL-1R−/−) mice, which completely lack the ability to transfer IL-1 signals into the cell, have a reduced ability to mount a pulmonary inflammatory response during pneumonia caused by *Streptococcus pneumoniae*, which was associated with an increased outgrowth of pneumococci in the lungs during the first two days after the infection [15]. *Streptococcus pneumoniae* is the most frequently isolated pathogen in community-acquired pneumonia [19]. The present study was initiated to investigate the influence of IL-1ra treatment in immunologically normal wild type mice during on-going pneumonia. The treatment used was expected to result in an incomplete and transient inhibition of IL-1 activity, therefore more appropriately mimicking the clinical situation of patients with a pharmacologically reduced bioavailability of IL-1.

MATERIALS AND METHODS

Animals

Since the effect of IL-1ra may differ in mice with different genetic backgrounds [20, 21], we used 10 week-old female

BALB/c and C57BL/6 mice (Harlan Sprague Dawley Inc., Horst, the Netherlands). These strains were chosen as our laboratory has used these strains previously in the model of pneumococcal pneumonia, and because the vast majority of genetically-modified mice are backcrossed to either a BALB/c or a C57BL/6 background, thus facilitating comparison with other studies. All experiments were approved by the Institutional Animal Care and Use Committee of the Academic Medical Center, University of Amsterdam, the Netherlands.

Reagents

Recombinant human IL-1ra in a hyaluronic acid vehicle (as a sustained delivery system) was provided by Amgen (Thousand Oaks, CA, USA) and was given intraperitoneally at 0, 24 and 48 h after induction of pneumonia at a dose of 100 mg/kg of body weight. This dose had been previously shown to exert biologically significant effects, as it improved the course of arthritis in animal models [22, 23]. Control mice received hyaluronic acid vehicle only.

Experimental design

For intranasal inoculation, mice were anesthesized by inhalation of isoflurane (Upjohn, Ede, the Netherlands), and pneumococcal pneumonia was induced by intranasal inoculation of 10^5 CFU *S. pneumoniae* serotype 3 in 50 µl sterile saline (ATCC 6303; Rockville, MD, USA), exactly as described previously [15]. At 24 and 42 h after inoculation, mice were anesthetized by intraperitoneal injection with Hypnorm® (Janssen Pharmaceutica, Beerse, Belgium) and midazolam (Roche, Mijdrecht, the Netherlands), and blood was collected from the inferior vena cava. Whole lungs were harvested and homogenized at 4 °C in 5 volumes of sterile isotonic saline, with a tissue homogenizer (Biospect Products, Bartlesville, OK, USA), which was carefully cleaned and desinfected with 70% ethanol after each homogenization. Serial 10-fold dilutions in sterile saline were made from these homogenates (and blood), and 50 µl volumes were plated onto sheep-blood agar plates and incubated at 37 °C: CFU was determined after 16 hours. For cytokine measurements, lung homogenates were lysed in buffer (300 mM NaCl, 15 mM Tris, 2 mM MgCl, 2 mM Triton (X-100), pepstatin A, leupeptin, aprotinine (20 ng/ml), pH 7.4) and followed by centrifugation at 1 500 × *g* at 4 °C for 15 minutes; the resulting supernatant was frozen at – 20 °C until assayed for cytokine levels.

Cytokine and chemokine determination

TNF-α (R&D systems, Abingdon, United Kingdom), IL-6 (Pharmingen, San Diego, CA, USA) and cytokine-induced neutrophil chemoattractant (KC)(R&D systems), were measured by ELISA. Detection limits were 150 pg/ml (TNF), 37 pg/ml (IL-6), 55 pg/ml (KC). Myeloperoxidase activity was measured as described [24].

Histologic examination

Lungs were fixed in 10% formalin. After paraffin embedding, 4 µm sections were cut and stained with haematoxylin and eosin. Slides were coded before assessment by one pathologist without knowledge of the type of mice or treatment.

Statistical analysis

All values are expressed as mean ± SEM. Comparisons were conducted with Mann-Whitney U test. Survival curves were compared by log-rank test. $P < 0.05$ was considered statistically significant.

RESULTS

Mortality

IL-1ra injection did not influence mortality in either BALB/c or C57BL/6 mice up until ten days after infection (Figure 1).

Pulmonary clearance

To evaluate whether the early phase of antibacterial defense was influenced by IL-1ra, we determined the number of pneumococci in the lungs 24 and 48 h after inoculation (Figure 2). BALB/c mice treated with IL-1ra displayed an increased outgrowth of *S. pneumoniae* in the lungs compared to vehicle-treated mice at both timepoints ($P < 0.05$). After 24h, 50% of vehicle-treated and 75% of IL-1ra-treated BALB/c mice were bacteraemic, whereas 100% and 88% respectively were bacteraemic after 48 h. C57BL/6 mice injected with IL-1ra had more bacteria in their lungs after 24 h, but not after 48 h. After 24 h, 75% of

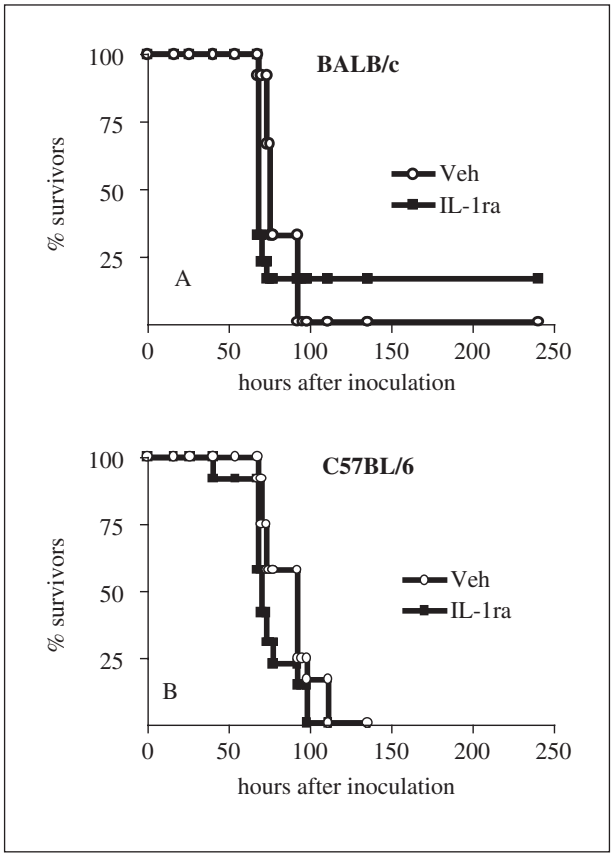

Figure 1
Recombinant human IL-1ra does not influence survival. Survival after intranasal inoculation with *S. pneumoniae* of mice treated with recombinant human IL-1ra (100 mg/kg at 0, 24 and 48 h; closed squares) or vehicle (open circles) in BALB/c (A) and C57BL/6 (B) mice. Mortality was assessed twice daily for 10 days. N = 20 per group.

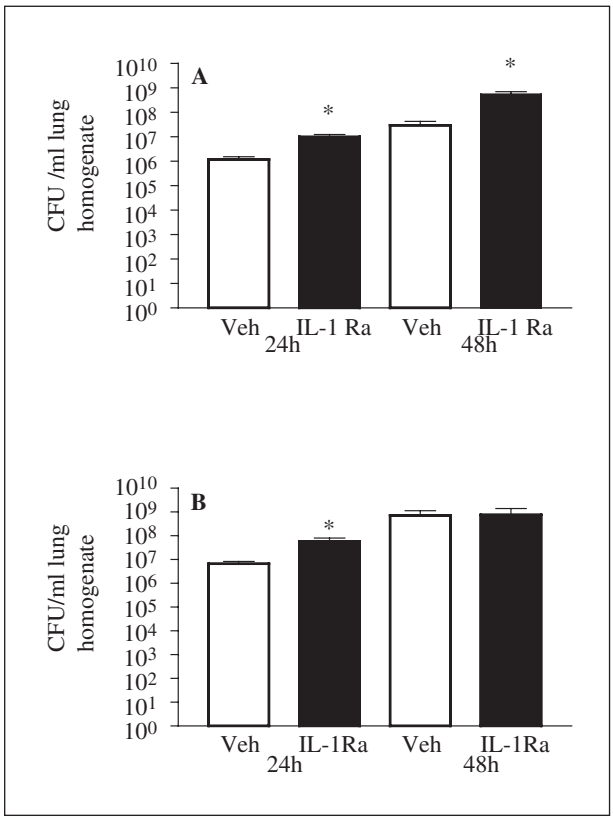

Figure 2
Recombinant human IL-1ra enhances bacterial outgrowth in lungs. *S. pneumoniae* CFU in lungs of BALB/c (A) and C57BL/6 (B) mice treated with recombinant human IL-1ra (100 mg/kg at 0 and 24 h; closed bars) or vehicle intraperitoneally (open bars), 24 and 48 h after i.n. inoculation with *S. pneumoniae*. Data are mean ± SEM. N = 8 per group per time point. * indicates $P < 0.05$ *versus* vehicle-treated mice.

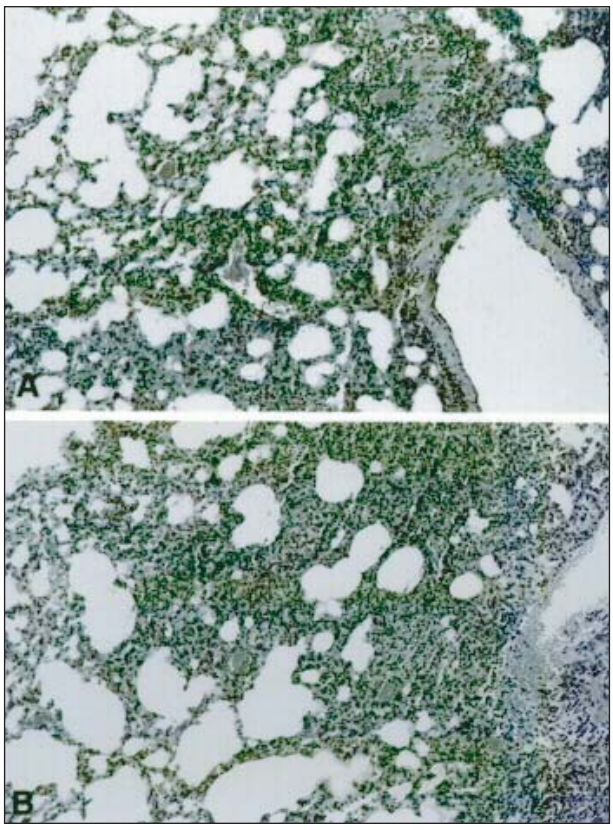

Figure 3
Recombinant IL-1ra does not affect inflammatory infiltrates in lungs. Representative histopathology of lungs of BALB/c mice treated with IL-1ra (A) and vehicle (B) intraperitoneally, 24 h after i.n. inoculation with *S. pneumoniae* showing comparable inflammatory infiltrates. Haematoxylin, eosin staining, magnification × 33.

vehicle-treated C57BL/6 mice had pneumococci in their blood, and 88% of IL-1ra-treated mice; after 48 h, 88% of both groups had positive blood cultures.

Pulmonary inflammatory response

To determine whether IL-1ra treatment affected the pulmonary inflammatory response to pneumococcal infection, we measured MPO activity and the concentrations of TNF-α, IL-6 and KC (a prominent neutrophil-attracting CXC chemokine in rodents) in lung homogenates. None of these parameters were altered by IL-1ra treatment in either BALB/c or C57BL/6 mice (data not shown). In addition, we compared the histopathology of the lungs of each groups at 24 and 48 h after after inoculation with *S. pneumoniae*. IL-1ra-treated mice displayed inflammatory infiltrates to the same extent as vehicle-treated mice (Figure 3).

DISCUSSION

We here report that although IL-1ra treatment impaired the early host defense against pneumococcal pneumonia, it did not influence survival. These data are in line with our recent data obtained in the same model, revealing an increased bacterial outgrowth in lungs of IL-1R–/– mice, relative to lungs of wild type mice during the first two days of the infection, with no difference in mortality [15]. Importantly, whereas the complete absence of an IL-1 signal (as in IL-1R–/– mice) resulted in a profound reduction in the pulmonary inflammatory response to *S. pneumoniae* infection [15], partial inhibition of IL-1 activity by Il-1ra did not result in a clearly altered inflammatory reaction. This latter finding should be viewed in the context of an earlier study in which this dose of IL-1ra, in a slow-release hyaluronic acid vehicle, strongly reduced joint inflammation in a rat model of rheumatoid arthritis [23]. The difference in results between our previous study with IL-1R–/– mice, and the present study, could also, in part, be related to the fact that knockout mice may not only differ from wild type mice with respect to the product of the deleted gene. The hereditary deficiency of IL-1R may result in compensatory changes that are not directly related to the absence of an IL-1 signal in adult life. The effect of IL-1ra did not markedly differ in BALB/c and C57BL/6 mice, although only the former strain had higher bacterial numbers in their lungs at 48 h post-infection.

It should be noted that in the present study, only one bacterial dose was used. Our data therefore do not exclude the possibility that IL-1ra treatment has a more profound effect on the host response to pneumococcal pneumonia when less concentrated bacterial inocula are administered. In addition, our data do not exclude the possibility that IL-1ra may impact survival in experiments using lower challenges with pneumococci. In the survival studies presented here, BALB/c mice displayed a nonsignificant survival benefit after IL-1ra treatment, which further argues against a biologically relevant, adverse effect of IL-1ra on

the innate immune response to respiratory tract infection with *S. pneumoniae*.

Anti-cytokine therapy offers new hope for the management of inflammatory diseases. We have previously demonstrated that TNF-α is of critical importance in host defense against pneumococcal pneumonia [15, 16]. The present data, together with our findings in IL-1R−/− mice [15], suggest that IL-1 occupies a role in the pulmonary immune response to *S. pneumoniae* that is substantially less prominent than that of TNF-α. There are two possible explanations why blocking IL-1 is not as harmful as blocking TNF-α. Firstly, TNF-α induces IFN-γ which plays a protective role against mycobacterial and fungal infections, while IL-1 does not [25]. Secondly, anti-TNF-α antibodies and soluble receptors have a more prolonged TNF inhibitory effect relative to the IL-1 antagonizing effect of IL-1ra. Thus, treatment with IL-1ra results in a partial and transient blockage of IL-1 function, and this might result in a return of host defenses in between the administration of IL-1ra. Further studies are warranted to establish the influence of IL-1ra therapy on host defense against other pathogens.

ACKNOWLEDGEMENTS. We would like to thank I. Kop and J.B. Daalhuijsen for expert technical assistance.

REFERENCES

1. Dinarello CA. 2000. The role of the interleukin-1-receptor antagonist in blocking inflammation mediated by interleukin-1. *N. Engl. J. Med.* 343: 732.
2. Bresnihan B. 2001. The safety and efficacy of interleukin-1 receptor antagonist in the treatment of rheumatoid arthritis. *Semin. Arthritis. Rheum.* 30: 17.
3. Bresnihan B. 2001. The prospect of treating rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. *BioDrugs* 15: 87.
4. Gabay C, Arend WP 1998. Treatment of rheumatoid arthritis with IL-1 inhibitors. *Springer Semin. Immunopathol.* 20: 229.
5. Jiang Y, Genant HK, Watt I, Cobby M, Bresnihan B, Aitchison R, McCabe D. 2000. A multicenter, double-blind, dose-ranging, randomized, placebo-controlled study of recombinant human interleukin-1 receptor antagonist in patients with rheumatoid arthritis: radiologic progression and correlation of Genant and Larsen scores. *Arthritis Rheum.* 43: 1001.
6. Targan SR, Hanauer SB, van Deventer SJ, Mayer L, Present DH, Braakman T, DeWoody KL, Schaible TF, Rutgeerts PJ. 1997. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. *N. Engl. J. Med.* 337: 1029.
7. Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI, Jackson CG, Lange M, Burge DJ. 1999. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N. Engl. J. Med.* 340: 253.
8. Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, Smolen JS, Weisman M, Emery P, Feldmann M, Harriman GR, Maini RN. 2000. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. *N. Engl. J. Med.* 343: 1594.
9. Maini RE, St Clair W, Breedveld F, Furst D, Kalden J, Weisman M, Smolen J, Emery P, Harriman G, Feldmann M, Lipsky P. 1999. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. Attract Study Group. *Lancet* 354: 1932.
10. Feldmann M, Maini RN. 2001. Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned? *Annu. Rev. Immunol.* 19: 163.
11. Chaudhari U, Romano P, Mulcahy LD, Dooley LT, Baker DG, Gottlieb AB. 2001. Efficacy and safety of infliximab monotherapy for plaque-type psoriasis: a randomised trial. *Lancet* 357: 1842.
12. Gorman JD, Sack KE, Davis JC. 2002. Treatment of ankylosing spondylitis by inhibition of tumor necrosis factor alpha. *N. Engl. J. Med.* 346: 1349.
13. Chen W, Havell EA, Moldawer LL, McIntyre KW, Chizzonite RA, Harmsen AG. 1992. Interleukin 1: an important mediator of host resistance against Pneumocystis carinii. *Journal of Experimental Medicine* 176: 713.
14. Kozak W, Zheng H, Conn CA, Soszynski D, van der Ploeg LH, J. Kluger M. 1995. Thermal and behavioral effects of lipopolysaccharide and influenza in interleukin-1 beta-deficient mice. *American Journal of Physiology* 269: R969.
15. Rijneveld AW, Florquin S, Branger J, Speelman P, Van Deventer SJ, van der Poll T. 2001. TNF-alpha compensates for the impaired host defense of IL-1 type I receptor-deficient mice during pneumococcal pneumonia. *J. Immunol.* 167: 5240.
16. van der Poll T, Keogh CV, Buurman WA, Lowry SF. 1997. Passive immunization against tumor necrosis factor-alpha impairs host defense during pneumococcal pneumonia in mice. *American Journal of Respiratory, Critical Care Medicine* 155: 603.
17. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, Siegel JN, Braun MM. 2001. Tuberculosis associated with infliximab, a tumor necrosis factor alpha- neutralizing agent. *N. Engl. J. Med.* 345: 1098.
18. Fleischmann RM. Addressing the safety of anakinra in patients with rheumatoid arthritis. Rheumatology (Oxford). 2003 May; 42 Suppl 2: ii 29-35.
19. Bartlett JG, Dowell SF, Mandell LA, File TM Jr, Musher DM, Fine MJ. 2000. Practice guidelines for the management of community-acquired pneumonia in adults. Infectious Diseases Society of America. *Clin. Infect. Dis.* 31: 347.
20. Horai R, Saijo S, Tanioka H, Nakae S, Sudo K, Okahara A, Ikuse T, Asano M, Iwakura Y. 2000. Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *J. Exp. Med.* 191: 313.
21. Nicklin MJ, Hughes DE, Barton JL, Ure JM, Duff GW. 2000. Arterial inflammation in mice lacking the interleukin 1 receptor antagonist gene. *J. Exp. Med.* 191: 303.
22. Bendele A, McAbee T, Woodward M, Scherrer J, Collins D, Frazier J, Chlipala E, McCabe D. 1998. Effects of interleukin-1 receptor antagonist in a slow-release hylan vehicle on rat type II collagen arthritis. *Pharm. Res.* 15: 1557.
23. Bendele AM, Chlipala ES, Scherrer J, Frazier J, Sennello G, Rich WJ, Edwards CK, 3rd 2000. Combination benefit of treatment with the cytokine inhibitors interleukin-1 receptor antagonist and PEGylated soluble tumor necrosis factor receptor type I in animal models of rheumatoid arthritis. *Arthritis Rheum.* 43: 2648.
24. Knapp S, Leemans JC, Florquin S, Branger J, Maris NA, Pater JC, van Rooijen N, van der Poll T. 2002. Alveolar Marophages have a rotective Anti-inflammatory Role during Murine Pneumococcal Pneumonia. *Am. J. Respir. Crit. Care Med.* in press.
25. Verreck FA, de Boer T, Hoeve M, van Dissel JT, Sanal O, Kurimoto M, Ottenhoff TH. 2002. Human host defense and cytokines in mycobacterial infectious diseases: interleukin-18 cannot compensate for genetic defects in the interleukin-12 system. *Clin. Infect. Dis.* 35: 210.