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Role of Innate and Adaptive Immunity in the Outcome of Primary Infection with *Chlamydia pneumoniae*, as Analyzed in Genetically Modified Mice

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Infection with *Chlamydia pneumoniae* is a common cause of acute respiratory disease in man and is also associated with atherosclerotic cardiovascular disorder. Herein, we have compared bacterial load and immune parameters of *C. pneumoniae*-infected mice genetically lacking T cell coreceptors, cytokine receptors, or cytotoxic effector molecules. A protective role for CD8*+* cells is shown by the enhanced severity of infection of CD8*−/−* or TAP-1*−/−*/β2-microglobulin*−/−* mice. CD8*+* cells hindered a parasite growth-promoting role of CD4*+* T cells, as indicated by the higher sensitivity to early infection of CD8*−/−* than CD4*−/−*/CD8*−/−* mice, which was further confirmed in experiments in which SCID mice were reconstituted with either CD4*+* or CD4*+* plus CD8*+* T cells. Interestingly, CD4*+* T cells played a dual role, detrimental early (14 and 24 days) after infection but protective at later time points (60 days after infection). The CD8*+* T cell protection was perforn independent. The early deleterious role of CD4*+* in the absence of CD8*+* T cells was associated with enhanced IL-4 and IL-10 mRNA levels and delayed IFN-γ mRNA accumulation in lungs. In line with this, IFN-γR*−/−* (but not TNFRp55*−/−*) mice showed dramatically increased susceptibility to *C. pneumoniae*, linked to reduced inducible nitric oxide synthase (iNOS) mRNA accumulation, but not to diminished levels of specific Abs. The increased susceptibility of iNOS*−/−* mice indicates a protective role for iNOS activity during infection with *C. pneumoniae*. The higher sensitivity of IFN-γR*−/−* mice to *C. pneumoniae* compared with that of SCID or recombination-activating gene-1*−/−* mice suggested a relevant protective role of IFN-γ-dependent innate mechanisms of protection. *The Journal of Immunology*, 1999, 162: 2829–2836.

Infection with the obligate intracellular Gram-negative bacterium *Chlamydia pneumoniae* is recognized as a major cause of sinusitis, pharyngitis, bronchitis, and pneumonia (1, 2). Seroepidemiological studies indicate that it is by far the most common chlamydial infection, affecting at least 50% of the population worldwide. It is probably the third common cause of community-acquired pneumonia, accounting for 6–12% of pneumonias in young adults and children. Moreover, coronary heart disease and myocardial infarction have been associated with *C. pneumoniae* infection by seroprevalence studies and by the direct detection of the organism within atheromatous plaques (1–4). This link became more persuasive when prospective trials with anti-chlamydial antibiotics successfully diminished the morbidity and mortality of patients with coronary artery disease (5).

Multiple arms of the immune system are activated in response to infection with microorganisms. Although the primary immune response to infection is designed to clear the primary infection from the body and to provide protection against reinfection with the same pathogen, generation of tissue injury may also ensue, as exemplified by the sensitization to a more severe disease in individuals vaccinated against *Chlamydia trachomatis* (6). Protective or adverse effects depend not only on the specific Ag(s) but also on the immune effector mechanisms that are mobilized. Furthermore, diverse innate immune mechanisms not only constitute a first barrier against pathogens, but also dictate the quality of the clonally dependent mechanisms elicited. Thus, knowledge of these mechanisms is central in vaccine design. In this context, a mouse model of *C. pneumoniae* pneumonitis showing extensive similarities to human disease has been recently developed (7, 8). However, the arms of the immune response that participate in the outcome of the primary infection with *C. pneumoniae* have not been investigated.

In the present study we have compared the consequence of infection with *C. pneumoniae* in mice genetically lacking T cell coreceptors, cytokine receptors, and cytotoxic effector molecules. We propose different involvement(s) for these molecules in the outcome of *C. pneumoniae* pneumonitis.

Materials and Methods

**Mice and bacteria**

Mutant mouse strains without CD4 (9), CD8 (10), IFN-γR (11), TNF receptor p55 (TNFrp55) (12), perforin (13), recombination-activating gene-1 (RAG-1)*−/−* (14), TAP-1 (15), β2-microglobulin (βm) (16), AB (H2b) MHc II (17), and inducible nitric oxide synthase (iNOS) (18) were generated by homologous recombination in embryonic stem cells. Mice (H2b) underwent several backcrosses (five to nine) with C57BL/6 mice, which were used as controls. Unless otherwise indicated, mice possess the B6 background. Mice heterozygous for both the CD4 and the CD8 mutation (CD4*+/CD8*+*) were mated, and the offspring were selected for the absence of both loci (19). TAP-1*−/−*/βm*−/−* were similarly obtained (20). SCID and CD8*−/−* animals were backcrossed with BALB/c mice. Mice

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2 Address correspondence and reprint requests to Dr. Martín Rottenberg, Microbiology and Tumor Biology Center, Karolinska Institute, S-171 77 Stockholm, Sweden. E-mail address: martin.rottenberg@mtc.ki.se
3 Abbreviations used in this paper: RAG-1, recombination-activating gene-1; iNOS, inducible nitric oxide synthase; IFU, inclusion-forming units; OMP2, outer membrane protein-2; Trx, thioredoxin; ABP, albumin binding protein; βm, β2-microglobulin.
were bred and kept under specific pathogen-free conditions and were main-
tained in isolation under negative pressure during experiments. They were used between 6 and 10 wk of age. B6-Aβ (H2b) mice were purchased from Tachonica (Germantown, NY).

Mycoplasma-free C. pneumoniae isolate Kajaani (21) was propagated in HL cells. Infected cells were sonicated, cell remnants were removed by centrifugation, and the bacteria were stored in dimethyl sulfoxide at −70°C until used. The infectivity, as mea-
sured by inclusion-forming units (IFU), of the bacterial preparation was determined in HL cell culture. Mice were mildly sedated with metofane and inoculated intranasally with 10^6 IFU diluted in 40 μl of PBS.

**Infectivity assay**

Mice were sacrificed, and right lungs were removed, minced, and mechani-
cally homogenized in 2 ml of sucrose-phosphate-gluatamate solution. Ho-
mogenates were centrifuged for 10 min at 12,000 rpm to remove coarse tissue. Lysates were then diluted 10- and 100-fold in DMEM containing 5% FCS and streptomycin (DMEM). The infectious titer was assayed by culturing 500 μl of duplicate dilutions of the lysates on confluent Vero cells grown on round 13-mm coverslides in a shell vial. Inoculated cells were centrifuged for 1 h at 1600 rpm. Thereafter, supernatant was removed, and DMEM containing cycloheximide and streptomycin was added. Cells were incubated at 35°C for 72 h, fixed with methanol, and stained with an FITC-conjugated *Chlamydia* genus-specific mAb (Pathfinder Chlamydia Confirmation System, Kallestad Diagnostics, Chaska, MN). Inclusion bod-
ies were counted by fluorescence microscopy. The infectivity was ex-
pressed as IFU per lung.

**Competitive PCR assay**

The accumulation of iNOS, IFN-γ, IL-10, and β-actin mRNA in freshly extracted lungs from infected mice was measured by competitive PCR assays as previously described (22). Competitor fragments with a different length but using the same primers as the target DNA were constructed using composite primers (23) and an exogenous DNA fragment or by sub-
cloning of mutated (deleted or ligated) cytokine cDNA. Competitors were amplified by PCR, purified (Qiagen, Studio City, CA), and quantified in a spectrophotometer. The primer sequences for the amplification of the cDNA were: sense iNOS, 5′-CCC TTC CGA GTT TGG CAG CAG CAG C-3′; antisense iNOS, 5′-GGG TCT CAG ATC TGC GTC GGT TTG G-3′; sense IFN-γ, 5′-AAC GCT ACA CAC TGC ATG G-3′; antisense IFN-γ, 5′-GAC TAC AAA GAG TCT GAG G-3′; sense IL-10, 5′-AGG AGC TCT GTC GAT GTC-3′; antisense IL-10, 5′-CCG GAA GAC AAT AAC TG-3′; sense β-actin, 5′-ATG GGC GCC CTC TCT AGG CAG CAC A-3′; and antisense β-actin, 5′-CTC TTT GAT GTC ACG CAC GAT TCT-3′.

Ten- or threefold serial dilutions of the competitor were amplified in the presence of a constant amount of cDNA. Reactions were conducted for 28–45 cycles in a thermal cycler (Perkin-Elmer/Cetus, Norwalk, CT) using an annealing step at 60°C (except 58°C for IL-10).

**Southern blot of RT-PCR-amplified IL-4 transcripts**

IL-4 transcripts were amplified from lung cDNA from individual mice containing similar titers of β-actin transcripts as measured in a competitive PCR assay. The primer sequences for amplification of IL-4 cDNA were: sense IL-4, 5′-ATG GGT CTC AAC CCC CAG CTA-3′; and antisense IL-4, 5′-GCT TTA GGT TCT CAC GAA GAT-3′. After amplification (45 cycles, annealing step at 60°C), PCR products were subjected to electrophoresis in 1.5% agarose gels. The gels were denatured, neutralized, and a semi-dry transfer onto nylon membrane (Hybond-N, Amersham, Aylesbury, U.K.) was performed overnight at room temperature as previously described (24). After baking and prehy-
bridization in a buffer containing 2× SSPE, 5× Denhart’s solution, and 0.5% SDS for 2 h at 62°C, membranes were hybridized with 5 × 10^6 cpm/membrane of [32P]ATP labeled DNA fragments (25). The labeling of the probe with [γ-32P]ATP was catalyzed by T4 polynu-
cleotide kinase. After hybridization, the membranes were washed twice with 0.2× SSPE and 0.5% SDS for 15 min at 62°C. The signal intensity of Southern blots was quantitated using a PhosphorImager (Molecular Dy-
namics, Sunnyvale, CA).

**Isolation, purity, and passive transfer of T cells**

CD4+ and CD8+ T cell and CD4+ T cell suspensions were prepared from spleens from uninfected BALB/c or BALB/c-CD8−/− mice. Briefly, cell suspensions were obtained and washed once with RPMI 1640 medium containing 5% heat-inactivated FCS, 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 5 × 10−5 M 2-ME (RPMI-5% FCS). RBC were re-
moved by hypotonic shock, and the remaining cells were washed twice in cold RPMI-5% FCS. Cells were filtered through a nylon wool column, and B cells were depleted by using anti-B220-coated magnetic beads (Dyna-
beads, Dynal, Oslo, Norway). The resulting population contained >95% of T cells and <5% B cells (anti-Ab5) as analyzed by flow cytometry. Recombinant FITC-labeled anti-mouse IgG or FITC-labeled anti-mouse IgM (PharMingen, San Diego, CA) or FITC-labeled anti-mouse IgG (Dakopatts, Glostrup, Denmark) were used for this purpose. More than 95% of such purified cells from BALB/c CD8−/− mice were stained with anti-CD4 Abs (PharMingen). SCID mice were reconstituted with T cells in 0.2 ml of PBS 24 h before intranasal inoculation of *C. pneumoniae*. Mononuclear infiltrates were detected in infected SCID reconstituted mice but not in unreconstituted controls.

**Recombinant outer membrane protein-2 (OMP-2) production**

The *C. pneumoniae* OMP-2 gene (GenBank accession no. X5311) was amplified by PCR using pKTH339 (a gift from M. Sarvas, National Public Health Institute, Helsinki, Finland) as a template. The signal sequence was deleted from the template, and the OMP-2 gene was cloned into a pTrx-
ABP expression vector (J. Nilsson, unpublished observations). The DNA sequence was confirmed. The pTrx-ABP encodes a 26-kDa affinity fusion partner consisting of *Escherichia coli* thioredoxin (Trx) protein (25) and an albumin binding protein (ABP) derived from streptococcal protein G. *E. coli* BL-21 (DE3, Novagen, Madison, WI) harboring pTrx-ABP-
OMP sequences were grown overnight at 37°C in 100 ml Luria broth supplemented with 100 μg/ml ampicillin. Thereafter, culture was diluted 1/100 in Luria broth and grown for 3–5 h. Expression of the recombinant fusion protein was induced by adding of isopropyl-β-D-thiogalactosidase. Protein production continued for 4–5 h at room temperature until bacteria reached an OD600 nm of 1.8–3. Cells were then centrifuged, and pellets were frozen at −20°C; thawed; resuspended in 50 mM Tris-HCl, 0.2 mM NaCl, and 0.05% Tween (pH 7.5); and sonicated. The sonicated cells were then centrifuged, and the supernatant was collected and applied to a human serum albumin-Sepharose column (Pharmacia). The size and purity of Trx-
ABP-OMP were verified by SDS-PAGE and were further confirmed by Western blot.

**Ab determinations**

The contents of anti-OMP-2 Abs in the sera from infected mice were mea-
ured by ELISA. The plates were coated overnight with 0.7 μg/ml of Trx-
ABP-OMP fusion protein. After blocking, sera from individual mice were then added at 1/100 or 1/400 dilutions. The plates were subsequently developed with horseradish peroxidase-conjugated rabbit anti-mouse IgG (γ-chain specific; Sigma, St. Louis, MO) or anti-mouse IgM (μ-chain specific; Sigma). The assay was standardized between plates by including the titration of pooled sera from *C. pneumoniae*-infected IFN-γ−/− mice.

**Results**

Initial experiments were designed to examine the relative roles of CD4+ and CD8+ T cells in *C. pneumoniae* infection. The lungs of C57BL/6/CD8−/− mice showed 10-fold higher numbers of IFU than those of control C57BL/6 (B6) mice at 14 and 24 days after infection, whereas no significant differences were observed in CD4−/− mice at the same time points (Fig. 1). Similar levels of bacteria were found in lungs from MHC II−/− and wild-type mice, corroborating data from CD4 mice (Fig. 2). Furthermore, a higher number of IFU was found in CD8−/− than in CD4−/− or CD8−/− SCID (which lack mature T and B cells) mice on days 14 and 24 after infection (Fig. 1). However, both T cell populations seem to play a protective role during late infection (60 days after infection; Fig. 1A) or reinfection (Fig. 1B) as indicated by the higher IFU in lungs of CD4−/−/CD8−/− mice compared with the level in single mutants. Thus, our data indicate that early after infection (14 and 24 days) CD4+ T cells, in fact, promote bacterial growth in the absence of CD8+ T cells. Also, BALB/c CD8−/− mice showed a 10-fold higher bacterial load than controls, indi-
cating that CD8+ T cell-mediated protection against *C. pneu-
moniae* infection is not restricted to the B6 strain (Fig. 3). To confirm data obtained from CD8−/− mice, we infected TAP-1/β-deficient mice, lacking both MHC class I-restricted CD8+ T cells.
and CD1-restricted T cells. These mice also showed enhanced susceptibility compared with wild-type mice (Fig. 4).

To finally confirm the detrimental role for the host of CD4$^+$ T cells early after infection with C. pneumoniae, we performed reconstitution experiments with SCID mice. An increased number of bacteria was recovered from SCID mice reconstituted with $2 \times 10^8$ naive CD4$^+$ T cells 24 h before infection with C. pneumoniae compared with that in nonreconstituted SCID mice. This was not observed when SCID mice instead were passively transferred with a mixture of $2 \times 10^8$ naive CD4$^+$ and CD8$^+$ T cells (Fig. 5).

**FIGURE 1.** A, Course of C. pneumoniae infection in CD4$^{-/-}$, CD8$^{-/-}$, CD4$^{-/-}$/CD8$^{-/-}$, and C57BL/6 mice (9–17 mice/time point and group). Mice were sacrificed at the indicated time points after intranasal (i.n.) infection with $10^6$ C. pneumoniae. Mean IFU titers per lung are depicted. Bars indicate SEs. *, Differences vs B6, CD4$^{-/-}$, and CD4$^{-/-}$/CD8$^{-/-}$ mice are significant ($p < 0.05$, by Mann-Whitney and Wilcoxon U tests). Death occurred in 2 of 18 CD8$^{-/-}$ mice, which otherwise were sacrificed 60–67 days after infection. B, Role of CD4$^+$ and CD8$^+$ cells in protection against reinfection. B6, CD4, and or CD8 deficient mice (7–15/group) were infected with $10^6$ C. pneumoniae. Groups of B6 and CD4$^{-/-}$/CD8$^{-/-}$ mice were also left untreated as controls. Sixty days after, all mice were challenged with $10^6$ C. pneumoniae. Mice were sacrificed 7 days later. Mean IFU titers per lung ± SEM are depicted. # Differences vs B6 after primary infection are significant ($p < 0.05$, by Mann-Whitney and Wilcoxon U tests). *

**FIGURE 2.** Individual, mean, and SEM IFU per lung of B6 MHC class II$^+$ and B6 mice (eight individuals per group) obtained 14 days after intranasal (i.n.) infection with C. pneumoniae are depicted. Differences between the groups are not significant.

**FIGURE 3.** Individual, mean, and SEM IFU per lung of BALB/c CD8$^{-/-}$ and BALB/c mice (seven individuals per group) obtained 14 days after intranasal (i.n.) infection with C. pneumoniae are depicted. Differences between CD8$^{-/-}$ and B6 mice are significant ($p < 0.01$, by Mann-Whitney and Wilcoxon U tests). Similar results are observed on groups at 24 days after infection.

FIGURE 3.
enhanced susceptibility to *C. pneumoniae* rely on their major cytolytic mechanism, since perforin levels of IL-4 mRNA in lungs from CD8 mice were infected intranasally (i.n.) with 10⁶ *C. pneumoniae* were significant (*p* < 0.001, by Mann-Whitney and Wilcoxon U tests). Differences between CD8 and TAP1/β₂m−/− mice are not significant.

Although different Th cell responses might occur after the transfer of different numbers of cells (26), our data obtained after inoculation of a constant number of different cell populations support the conclusions obtained with genomically deficient mice.

The protective effect of CD8 T cells against infection did not rely on their major cytolytic mechanism, since perforin−/− mice showed kinetics of infection similar to those of wild-type mice (Fig. 6).

The enhanced early susceptibility of CD8−/− mice was associated with an altered cytokine balance; IL-10 mRNA accumulation was increased in lungs of CD8−/− mice compared with that in B6, CD4−/−, or CD4−/−/CD8−/− mice (Fig. 7). We likewise detected higher levels of IL-4 mRNA in lungs from CD8−/− mice than in B6 mice at 14 days after infection (Fig. 8). On the contrary, levels of IFN-γ mRNA were lower in CD8−/− mice compared with those in B6 mice at 7, but not 14, days after infection, suggesting a delayed kinetics of production (Fig. 7). More direct evidence that IFN-γ made a significant contribution to the clearance of bacteria was obtained in IFN-γR−/− mice lacking IFN-γ signaling. IFN-γR−/− mice showed 100-fold increased bacterial load compared with B6 counterparts throughout the infection (Fig. 9). The susceptibility of IFN-γR−/− mice largely exceeded that of B and T cell-deficient RAG−1−/− mice, suggesting an important role for non-T cell-mediated IFN-γ-producing cells in the resistance against *C. pneumoniae* (Fig. 9). Resistance to infection was not associated with increased titers of specific Abs, as higher titers of anti-OMP.2 IgG were detected in sera from susceptible IFN-γR−/− mice than in those from B6 controls (Fig. 10). Moreover, TNFRp55−/− mice displayed similar susceptibility to infection as controls but showed diminished levels of specific IgG, further suggesting that Abs play no major role in control of *C. pneumoniae* (Fig. 10).

To analyze whether increased susceptibility of IFN-γR−/− mice was due to an altered differentiation of Th cells, IL-4 and IL-10 transcripts were measured. Similar levels of IL-10 mRNA were found in lungs from IFN-γR−/− mice (54 ± 26 mol of IL-10/10 mol of β-actin mRNA × 10⁻³; *n* = 4) and wild-type controls (33 ± 23 mol of IL-10/10 mol of β-actin mRNA × 10⁻³ ± SEM; *n* = 4) 14 days after infection with *C. pneumoniae*. Levels of IL-4 in both strains were not different from those in noninfected controls, suggesting that these mice do not mount a polarized Th2 response. The susceptibility of IFN-γR−/− mice was associated with diminished levels of iNOS mRNA accumulation in lungs (Fig. 11). To determine whether the iNOS-mediated high output of NO activity plays a role in resistance to *C. pneumoniae*, iNOS−/− mice were infected. iNOS-deficient mice showed higher sensitivity to infection with *C. pneumoniae* than B6 controls, but were not as susceptible as IFN-γR−/− mice (Fig. 11).

**Discussion**

In the studies reported above, we have demonstrated that CD4⁺ T cells in the absence of CD8⁺ T cells initially facilitate bacterial growth as demonstrated by 1) the enhanced susceptibility of CD8−/− mice compared with B6, CD4−/−, and CD4−/−/CD8−/− mice; and 2) reconstitution experiments in which SCID mice were rendered more sensitive to *C. pneumoniae* by transfer of CD4⁺ T cells. CD4⁺ T cell-mediated enhancement was not haplotype specific and was further confirmed by the enhanced susceptibility to *C. pneumoniae* of mice lacking TAP1-1−/−/β₂m−/−. Mice lacking class II molecules or depleted of CD4⁺ T cells by the administration of anti-CD4 Abs (J. Penttilä et al., unpublished observations) showed similar numbers of bacteria in lungs than wild-type controls, corroborating results obtained with CD4⁺ mice. Interestingly, susceptibility as measured by bacterial load is related to

![FIGURE 4](image)

![FIGURE 5](image)

![FIGURE 6](image)
disease activity, since CD8<sup>−/−</sup> mice showed more severe pneumonia than B6 or CD4<sup>−/−</sup>/CD8<sup>−/−</sup> mice (V. Puurula et al., manuscript in preparation).

CD8<sup>+</sup> T cells are particularly prominent in mucosal tissues, where they may provide a first line of defense against potential pathogens, fulfilling an immunological gatekeeper function. The conventional view of CD8<sup>+</sup> T cells has primarily been as killers of infected cells by direct cytolysis or actors via secretion of cytokines such as IFN-γ and TNF-α. The primary cytolytic mechanism of lymphocytes involves the release of perforin-containing granules upon contact with target cells (13, 27). We showed that such a mechanism is not necessary for CD8-mediated protection against C. pneumoniae infection, since perforin<sup>−/−</sup> mice show a normal course of infection. In line with this, infection with another intracellular microorganism, Mycobacterium tuberculosis, is also normal in perforin- or granzyme-deficient mice (28). Thus, it seems more likely that the ability of CD8<sup>+</sup> T cells to release certain cytokines can down-modulate disease activity during C. pneumoniae infection, as also suggested for infection with C. trachomatis (29). Different functional subsets of CD4<sup>+</sup> T cells have been described based on the cytokines produced by these T cells, Th1 CD4<sup>+</sup> T cells that secrete IL-2 and IFN-γ, and Th2 T cells that secrete IL-4, IL-5, and IL-10. We found that the early higher susceptibility of CD8-deficient mice correlates with a delayed accumulation of IFN-γ mRNA in lungs from infected mice, characterized by high IL-10 and IL-4 and a delayed accumulation of IFN-γ mRNA in lungs from infected mice ex vivo. The increased resistance to early infection with C. pneumoniae of CD4<sup>−/−</sup>/CD8<sup>−/−</sup> compared with CD8<sup>−/−</sup> mice coupled with lower levels of IL-10 mRNA suggest that CD8<sup>+</sup> T cells normally

FIGURE 7. Altered cytokine mRNA accumulation in lungs of CD8<sup>−/−</sup> mice after infection with C. pneumoniae. Total RNA was obtained from lungs of individual mice after 0, 7 (A and C), or 14 days (B and D) and transcribed into cDNA. Equal aliquots of cDNA from four individual mice were pooled and then amplified with IL-10, IFN-γ, or β-actin primers in the presence of threefold serial dilutions of the respective competitors. The mean moles of IL-10 mRNA (A and B) or IFN-γ (C and D) per mole of β-actin mRNA from two independent pools are depicted. Levels of IFN-γ and IL-10 mRNA in samples from individual CD8<sup>−/−</sup> or B6 mice (n = 5/group) after 14 days of infection with C. pneumoniae were significantly different (p < 0.05, by Mann-Whitney and Wilcoxon U test).

FIGURE 8. Southern blot analysis of RT-PCR-amplified IL-4 transcript levels in lungs from individual CD8<sup>−/−</sup> and B6 mice at 0 or 14 days after intranasal (i.n.) infection with 10<sup>6</sup> C. pneumoniae. Samples contained similar levels of β-actin cDNA as quantified by competitive PCR.
from individual B6, TNFRp55 rats and mice suppress IgE production and thereby regulate a CD4-mediated immune response from a Th2- to a protective pattern (30–32). Moreover, CD8+ T cells have been shown to prevent Th2 responses in murine Schistosoma infection (33), and depletion of CD8+ T cells in young nonobese diabetic mice prevents the onset of insulitis (34).

Bacterial load in lungs from SCID or RAG-1−/− mice show no difference compared with wild-type counterparts early (7 and 14 days) after infection. Both SCID and RAG-1−/− mice show higher levels of IFN-γ mRNA than wild-type infected controls, indicating a role for compensatory innate mechanisms in mice lacking T and B cells and suggesting the participation of acquired immune mechanisms early after infection (data not shown). Thus, we propose that CD4+ in the absence of CD8+ T cells would inhibit immune mechanisms of chlamydial control early after infection. Concordantly, Th2-derived cytokines such as IL-4, IL-10, and TGF-β have been shown to inhibit IFN-γ-dependent microbialic mechanisms of macrophages (35). In line with this, a major involvement of IFN-γ-dependent innate mechanisms in the control of infection with C. pneumoniae is suggested by the higher susceptibility of IFN-γR−/− mice compared with SCID or RAG-1−/− infected mice. Identification of non-T cell, IFN-γ-producing cells in these mice remains to be elucidated.

Interestingly, CD4+ T cells seem to play a dual role, promoting bacterial growth and disease early after infection, but participating in the control of bacterial growth at later time points as well as in protection against reinfection. Moreover, the role of CD4+ T cells might have been underestimated (involved to necessary in late control of infection), since CD4−/− mice possess MHC class II restricted T cells incapable of mounting a Th2 response (9, 36, 37) that might be protective. Also, CD8+ T cells seem to suffer qualitative changes; they inhibit a deleterious role of CD4+ T cells early (14–24 days) after infection but seem to participate participate in direct protection later (60 days) after infection or during reinfection.

Our data demonstrate a major contribution of IFN-γ in the control of infection with C. pneumoniae, as also shown during infections with other species of Chlamydia (38, 39). Accordingly, mice treated intranasally with eukaryotic expression plasmids encoding IFN-γ or IL-12, but not the backbone plasmid alone, showed increased resistance against infection with C. pneumoniae (data not shown). Interestingly, the presence of IFN-γ did not result in a major manner change in the cytokine pattern, since lung tissues from IFN-γR−/− and control animals showed similar IL-4 and IL-10 mRNA levels. However, reduced levels of iNOS transcripts were noticed in lungs of infected IFN-γR−/− mice. Moreover, iNOS−/− mice showed increased susceptibility to C. pneumoniae. Nitric oxide produced after cell activation by cytokines is important for killing or inhibiting the growth of various microorganisms (40). However, a protective role of iNOS during infection with other chlamydial species is controversial, being found by some (41) but disregarded by others (42). The presence of both iNOS-dependent and -independent IFN-γ-mediated effector mechanisms is suggested by the fact that although iNOS−/− mice are more susceptible than B6 controls, IFN-γR−/− mice show diminished resistance to C. pneumoniae compared with iNOS−/− mutants. The identification of iNOS-independent chlamydotoxic mechanisms remains to be elucidated. To our mind, induction of indoleamine-2,3-dioxygenase, a tryptophan-decyclizing enzyme, may account for the IFN-γ-mediated protection, as it is responsible for inhibition of chlamydial growth in IFN-γ-treated human cells (43).

TNF activity is regulated by two homodimeric receptors with molecular masses of 55 and 75 kDa. TNFRp75 has been implicated in thymocyte proliferation and apoptosis, whereas TNFRp55 participates in creating resistance to parasites,
fungi, and intracellular bacteria, as shown by the increased susceptibility of TNFRp55−/− mice to infections with *Leishmania, Listeria, Candida, Trypanosoma,* and *Mycobacterium* (12, 45–47). It was therefore surprising that infection of mice with *C. pneumoniae* was largely unaffected by TNFRp55 deficiency. TNFRp55−/− mice show a lack of primary follicles and aberrant germinal center formation and are thereby defective in Ig− switch and affinity maturation (48, 49). We confirmed such Ig switch deficiency, also suggesting a subordinate role for specific Abs in the resistance to *C. pneumoniae.*

To our knowledge, this manuscript constitutes the first immunogenetic analysis of the roles of acquired and innate components of the immune response in an experimental infection with *C. pneumoniae.* Extensive differences are apparent when comparing our data with those obtained in experimental infections with *C. trachomatis.* In infections with the later microorganism, a predominant role for CD4+ class II-restricted cells in protection has been shown, whereas a protective role for CD8+ T cells is controversial (29, 50, 51). Such differences are not surprising, as the two organisms display only 5 and 10% homology at the DNA and protein levels, respectively, as also reflected in the different pathobiologies of these infections (52).

In conclusion, our data illustrate the relevance of CD4+ T cells in advocating bacterial growth and pathology early after infection. Such deleterious activity is transient, as CD4+ T cells play a protective role later during infection. CD8+ T cells inhibit this CD4+ T cell early activity. CD8+ T cell protection is perforin independent and associated with an altered cytokine balance as indicated by increased IL-4 and IL-10 and delayed accumulation of IFN-γ mRNA in CD8−/− mice. Accordingly, IFN-γR and thereby IFN-γ (but not TNF-αRp55) molecules play a relevant role in protection against *C. pneumoniae.* IFN-γR-requiring resistance was associated with iNOS induction, but not with the induction of specific Abs. iNOS activity played a protective role during infection with *C. pneumoniae,* although it accounts only partially for IFN-γ-mediated protection.

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