A Gene-Dosage Effect for Interleukin-4 Receptor \( \alpha \)-Chain Expression Has an Impact on Th2-Mediated Allergic Inflammation during Bronchopulmonary Mycosis

Uwe Müller,1 Werner Stenzel,2,3 Gabriele Köhler,4 Tobias Polte,2 Manfred Blessing,1 Amrit Mann,1 Daniel Piehler,1 Frank Brombacher,5 and Gottfried Alber1

1Institute of Immunology, College of Veterinary Medicine, and 2Helmholtz Centre for Environmental Research and Medical Faculty, University of Leipzig, 3Institute of Neuropathology, Medical Faculty, University of Cologne, Cologne, and 4Gerhard Domagk Institute for Pathology, University of Münster, Münster, Germany; 5Division of Immunology, Institute of Infectious Disease and Molecular Medicine, Health Sciences Faculty, University of Cape Town, and International Centre for Genetic Engineering and Biotechnology, Cape Town, South Africa

Interleukin (IL)–4 and IL-13 are key factors in the pathogenesis of bronchopulmonary mycosis induced in mice by infection with Cryptococcus neoformans. Both cytokines use the IL-4 receptor \( \beta \)-chain (IL-4R \( \beta \)). In this study, we investigated the role played by IL-4R \( \beta \) expression in susceptibility to pulmonary C. neoformans infection. IL-4R \( \beta \)/mice were extremely resistant. To characterize the effect of IL-4R \( \beta \) expression level on disease outcome, we generated IL-4R \( \beta \)/first-generation (F1) mice. IL-4R \( \beta \)/mice showed intermediate levels of IL-4R \( \beta \) expression, in contrast to higher levels in wild-type mice and no expression in IL-4R \( \beta \)/mice, indicating biallelic expression of the gene for IL-4R \( \beta \) (Il4ra). Concomitant with intermediate IL-4R \( \beta \) expression, F1 mice showed intermediate susceptibility associated with altered Th2/Th17 cytokine production, decreased immunoglobulin E levels, and reduced allergic inflammation. This indicates a gene-dosage effect of IL-4R \( \beta \) expression on susceptibility to bronchopulmonary mycosis. These data provide the basis for novel therapies antagonizing IL-4R \( \beta \) in Th2-related pulmonary infection and possibly also in asthma.

The interleukin (IL)–4 receptor \( \alpha \)-chain (IL-4R\( \alpha \)) is ubiquitously expressed and is part of the receptor for the Th2 cytokines IL-4 and IL-13 [1], which have been shown to be involved in the fatal outcome of experimental murine cryptococcosis [2–6]. The regulation of Th2 responses is controlled by early IL-4 production, which induces the differentiation of naive T helper cells to Th2 cells [7]. Th2 cells, along with basophils, eosinophils, mast cells, NKT, and \( \gamma \delta \) T cells, are the main producers of IL-4 and IL-13. IL-4 acts as an important factor affecting B cells, causing isotype switching that leads to the production of IgG1 or IgE, antibody classes that are important for antiparasitic defense mechanisms.

However, these mechanisms are ineffective or even disease promoting against a facultative intracellular pathogen such as Cryptococcus neoformans. IL-13, on the other hand, can act on smooth muscle cells and goblet cells to cause bronchoconstriction and mucus production, respectively [8–10]. It is of interest that C. neoformans infection can contribute to asthma development, as shown in a rat model [11]. The IL-4– and IL-13–dependent mechanisms lead to the pathogenesis of pulmonary cryptococcosis, especially by induction of alternatively activated macrophages (aaMphs) [3, 5, 12]. Thus, the IL-4 receptor is likely a key regulator in the pathogenicity of cryptococcosis, but this has not yet been proved experimentally. Downstream of the IL-4
receptor, the signal transduction cascade is controlled by signal transducer and activator of transcription (STAT) 6 [13, 14].

In the present study, we sought to determine whether antagonism of Th2 development during pulmonary cryptococcosis by IL-4Rα ablation (i.e., using IL-4Rα−/− mice) has an additive or synergistic effect compared with antagonism of individual ligands (i.e., using IL-4−/− or IL-13−/− mice). Moreover, we wished to clarify whether the gene for IL-4Rα (Il4ra) is expressed biallelically and whether 1 allele containing functional IL-4Rα (i.e., using IL-4Rα+/− mice) is sufficient to induce a full or only a gradual Th2 response in cryptococcosis. Our findings unambiguously demonstrate that Il4ra is indeed expressed biallelically and that its expression level is critical for susceptibility to experimentally induced bronchopulmonary mycosis. Depending on the IL-4Rα expression level, there is a gradual appearance of Th2-dependent mechanisms, such as IgE production, allergic inflammation with eosinophilia, goblet cell metaplasia, mucus hyperproduction, and alternative macrophage activation after pulmonary C. neoformans infection.

METHODS

Mice. Female mice (6–10 weeks old) included 3 groups: wild-type (WT; IL-4Rα+/+), IL-4Rα−/− mice on a BALB/c background [15], and the first generation of WT by IL-4Rα−/− intercrosses (IL-4Rα+/−). They were maintained in an individually ventilated caging system under specific pathogen–free conditions and in accordance with the guidelines approved by the Animal Care and Usage Committee of the Regierungspräsidium Leipzig. Sterile food and water were given ad libitum. The mice were tested periodically for pathogens, in accordance with the recommendations for health monitoring of mice provided by the Federation of European Laboratory Animal Science Associations accreditation board.

C. neoformans and infection of mice. Encapsulated C. neoformans (strain 1841, serotype D) was kept as frozen stock in skim milk and was grown in Sabouraud dextrose medium (2% glucose and 1% peptone; Sigma) overnight on a shaker at 30°C. The mice were infected intranasally, and the acapsular C. neoformans serotype D strain CAP67 was used as an in vitro stimulus, both as described elsewhere [5].

Determination of survival rate and organ burden. Infected mice were monitored daily for survival and morbidity. Organ burden was determined as described elsewhere [5].

Restimulation of spleen. Splenocytes were stimulated as described elsewhere [5].

Histopathological analysis. On day 70 after infection, C. neoformans–infected WT, IL-4Rα−/−, and IL-4Rα+/− mice as well as uninfected mice of the same genotypes were perfused intracardially with 0.9% saline while under deep CO2 asphyxia. The lungs of the animals were removed, mounted on thick filter paper with Tissue-Tek OTC compound (Miles Scientific), snap-frozen in isopentane (Fluka) precooled on dry ice, and stored at −80°C.

For immunohistochemical analysis, 10-µm frozen sections were prepared in a serial fashion (15 transversal sections per lung on 4 consecutive levels). The YM1 (ECF-L) goat anti-mouse antibody was used to detect aaMphs (R&D Systems). Arginase-1 (BD Biosciences) immunostaining was performed using the Dako ARK peroxidase kit, in accordance with the manufacturer’s protocol. Immunohistochemical analysis was performed as described elsewhere [16]. Negative controls, without application of the primary antibody, confirmed the specificity of the reactions.

Other parts of the lungs and of the other organs were fixed in neutral-buffered formalin and embedded in paraffin. Sections were stained with hematoxylin–eosin (H&E) to estimate the extent of granulomatous lesion formation in the various organs or with periodic acid–Schiff reagent to study the distribution of cryptococci and mucus production by goblet cells in lungs, liver, spleen, and kidneys. Analyses of collagen deposition in the organs were done by elastica–van Gieson staining. To study the recruitment of granulocytes, the tissue sections were stained with naphthol AS-D-chloracetate esterase. Histopathological alterations were microscopically evaluated on H&E–stained and immunostained lung sections.

Digestion of lung tissue and analysis of lung leukocytes and blood cells. For IL-4Rα expression experiments, lungs were perfused through the right ventricle with PBS. The lungs were treated as described elsewhere [5]. IL-4Rα expression in cells was analyzed by flow cytometry (FACSCalibur; BD). The cells were stained for IL-4R (CD124; clone mIL4R-M1; BD; phycoerythrin conjugate for staining of lung cells; biotinylated M1 antibody was used to detect aaMphs (R&D Systems). Arginase-1 antibody was used to detect aaMphs (R&D Systems). Arginase-1 (BD Biosciences) immunostaining was performed using the Dako ARK peroxidase kit, in accordance with the manufacturer’s protocol. Immunohistochemical analysis was performed as described elsewhere [16]. Negative controls, without application of the primary antibody, confirmed the specificity of the reactions.

Cytokine and antibody analysis. Cytokine concentrations were determined by sandwich ELISA systems using unlabeled capture antibodies and labeled detection antibodies, followed by incubation with peroxidase-labeled streptavidin if not otherwise indicated, as described elsewhere [5]. The total serum IgE, IgG1, and IgG2a concentrations in naive and infected mice were measured as detailed elsewhere [5].

Measurement of respiratory lung function. The respiratory lung function of intranasally infected mice was measured in a plethysmographic chamber (model PLT UNR MS; Emka Technologies) for freely moving animals. The pressure inside the chamber was measured by a differential pressure transducer connected to an amplifier (model AMP-B01) and continuously
monitored with IOX software (version 22.17.19). Airway hyperreactivity was examined as described elsewhere [17].

**RESULTS**

**Susceptibility to pulmonary C. neoformans infection determined by IL-4Rα expression level.** We and others have shown that each of the Th2 cytokines IL-4 and IL-13 is associated with susceptibility to *C. neoformans* infection [2–6]. To analyze the effect of simultaneous abrogation of IL-4 and IL-13 on *C. neoformans* infection, we used IL-4Rα−/− mice, which were deficient in the common receptor chain of the IL-4 and IL-13 receptor [18]. The survival of *C. neoformans*-infected IL-4− or IL-13− deficient mice was compared with that of IL-4Rα−deficient mice. In these experiments, the IL-4Rα−deficient (IL-4Rα−/−) mice were more resistant than the ligand-deficient mice, which were significantly more resistant than the WT (IL-4Rα+/+) mice (proportion of surviving mice in total from 3 independent experiments: for IL-4Rα−/− mice, 26/26; for IL-4−/− mice, 24/25; for IL-13−/− mice, 24/28). Although 100% of the IL-4Rα−/− mice died of the infection, the IL-4Rα−/− mice were completely resistant during an observation period of >200 days after infection (up to 275 days after infection) (figure 1A). Examination of the organ burdens in lung and brain at day 70 after infection showed significantly reduced numbers of cryptococci in the lungs of the IL-4Rα−/− mice (figure 1B). In the absence of IL-4Rα expression, dissemination of *C. neoformans* to the brain is prevented (figure 1C). Long-term examination for >200 days after infection (up to 275 days after infection) revealed that stable control of the fungal burden in the lung is achieved in IL-4Rα−/− mice, but not sterile elimination (figure 1B).

In light of the contrasting phenotypes observed in infected IL-4Rα+/+ versus IL-4Rα−/− mice, we wanted to learn whether an intermediate level of expression of IL-4Rα would have an effect on the resistance or susceptibility to pulmonary *C. neoformans* infection. Therefore, IL-4Rα heterozygous (IL-4Rα+/− mice) were generated. To determine the relative expression of IL-4Rα on leukocytes of naive and *C. neoformans*-infected IL-4Rα+/+ and IL-4Rα−/− mice, we determined the median fluorescence intensity by flow cytometry, using a monoclonal antibody specific for IL-4Rα. Expression of IL-4Rα was characterized for leukocytes in lungs, blood (table 1), brain, lymph nodes, and spleen (data not shown). Interestingly, IL-4Rα expression in all examined tissues of naive IL-4Rα+/− mice showed an intermediate level between the high levels found in IL-4Rα+/+ mice and minimal expression in IL-4Rα−/− mice (figure 1D). Long-term examination of these mice revealed that IL-4Rα heterozygous (IL-4Rα+/− mice) were intranasally infected with 500 cfu of *C. neoformans* (A). The survival time of mice was recorded for 150 days after infection. Although no IL-4Rα−/− mice died of the infection, IL-4Rα+/− and IL-4Rα−/− mice had median survival times of 75 and 92.5 days, respectively; 26.7% of IL-4Rα−/− mice survived for >150 days after infection. In one experiment the mice were examined for 272 days after infection, and 1 of 12 survived the whole period. The survival graph represents data from 3 independent experiments; the log-rank test was used for statistical analysis. Organ burdens in lungs (B) and brain (C) were analyzed on day 70 after infection and, for IL-4Rα−/− mice, on days 202, 246, 272, and 275 after infection as well (pooled as >200 days after infection). IL-4Rα−/− mice had significantly lower organ burdens in lungs and brain than did IL-4Rα+/− mice. IL-4Rα−/− mice tended to have lower organ burdens than the WT mice. IL-4Rα−/− mice did not completely eliminate the cryptococci but had low organ burdens even beyond 200 days after infection. There were no differences in the lung burdens of the IL-4Rα−/− mice between 70 and >200 days after infection. Data from 3 independent experiments were pooled. Significance was calculated with the 1-tailed Mann-Whitney U test, dpi, days post infection.
Intermediate levels of IL-4Rα expression in blood leukocytes and lung cells of naive and Cryptococcus neoformans–infected mice 70 days after infection.

Table 1. Interleukin (IL)–4 receptor α-chain (IL-4Rα) expression in blood leukocytes and lung cells of naive and Cryptococcus neoformans–infected mice 70 days after infection.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Median fluorescence intensity</th>
<th>IL-4Rα&lt;sup&gt;+/+&lt;/sup&gt; mice</th>
<th>IL-4Rα&lt;sup&gt;+&lt;/sup&gt;/— mice</th>
<th>IL-4Rα&lt;sup&gt;−/−&lt;/sup&gt; mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive PBLs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T helper cells</td>
<td></td>
<td>53.61</td>
<td>32.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.05&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cytotoxic T cells</td>
<td></td>
<td>56.20</td>
<td>41.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.67&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B cells</td>
<td></td>
<td>57.38</td>
<td>27.14</td>
<td>16.25</td>
</tr>
<tr>
<td>Macrophages</td>
<td></td>
<td>47.92</td>
<td>27.79</td>
<td>20.28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Granulocytes</td>
<td></td>
<td>33.50</td>
<td>24.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td></td>
<td>29.25</td>
<td>16.70</td>
<td>16.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NK cells</td>
<td></td>
<td>31.10</td>
<td>24.95</td>
<td>15.94</td>
</tr>
<tr>
<td>Lung cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All lung cells</td>
<td></td>
<td>5.75</td>
<td>4.16</td>
<td>2.88</td>
</tr>
<tr>
<td>CD3&lt;sup&gt;+&lt;/sup&gt; lung cells</td>
<td>6.58</td>
<td>3.57</td>
<td>1.86</td>
<td></td>
</tr>
<tr>
<td>CD11b&lt;sup&gt;+&lt;/sup&gt; lung cells</td>
<td>2.94</td>
<td>2.56</td>
<td>2.32</td>
<td></td>
</tr>
<tr>
<td>70 days after infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All lung cells</td>
<td></td>
<td>4.54</td>
<td>3.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD3&lt;sup&gt;+&lt;/sup&gt; lung cells</td>
<td>4.28</td>
<td>3.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CD11b&lt;sup&gt;+&lt;/sup&gt; lung cells</td>
<td>3.19</td>
<td>2.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.81</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Heterozygous IL-4Rα-deficient mice have an intermediate level of IL-4Rα expression in blood and lung leukocytes. Although the fluorescence intensity of IL-4Rα in IL-4Rα<sup>+/−</sup> mice marks the minimum median fluorescence intensity and IL-4Rα<sup>−/−</sup> leukocytes show the maximum value of IL-4Rα expression, intermediate expression levels of IL-4Rα were found in IL-4Rα<sup>+/−</sup> leukocytes. For peripheral blood leukocytes (PBLs), each value represents blood analyses for 5–9 naive mice from 4 independent experiments. For lung cells, data are shown from 1 of 2 independent experiments with lung cells from naive mice and pooled data from 2 independent experiments 70 days after infection.

<sup>a</sup> P < .05 for the comparison with IL-4Rα<sup>+/+</sup> mice (1-tailed Mann-Whitney U test).

<sup>b</sup> P < .05 for the comparison with IL-4Rα<sup>−/−</sup> mice (1-tailed Mann-Whitney U test).

Role of IL-4 Receptor in Cryptococcosis

The absent expression in IL-4Rα<sup>−/−</sup> mice. This argues for biallelic expression of IL4ra. The levels of IL-4Rα expression were especially high in lymphocytes compared with innate immune cells (table 1). No difference could be found between the IL-4Rα expression levels of IL-4Rα<sup>+/+/</sup> and IL-4Rα<sup>+/−</sup> phagocytes and dendritic cells. This may be related to the detection limit of the FACS method (owing to the lower IL-4Rα expression in innate immune cells) or to monoallelic expression of IL-4Rα in phagocytes and dendritic cells in contrast to lymphocytes (table 1).

Importantly, intranasal infection of mice with the highly virulent C. neoformans strain 1841 did not modulate IL-4Rα expression in lung cells (table 1) or blood leukocytes (data not shown).

To define the susceptibility of IL-4Rα<sup>+/−</sup> mice expressing intermediate levels of IL-4Rα against C. neoformans, we infected IL-4Rα<sup>+/−</sup> mice intranasally, together with IL-4Rα<sup>+/+</sup> and IL-4Rα<sup>−/−</sup> mice. Strikingly, C. neoformans–infected IL-4Rα<sup>+/−</sup> mice exhibited a significantly prolonged median survival time compared with IL-4Rα<sup>+/+</sup> mice (92.5 vs. 75 days after infection) (figure 1A). In accordance with the lower susceptibility found in infected IL-4Rα<sup>+/−</sup> mice, the lung and brain fungal burden of the IL-4Rα<sup>+/−</sup> mice was reduced (figure 1B). This finding indicates a gene-dosage effect of IL-4Rα expression on resistance to pulmonary infection with C. neoformans.

**Degree of fatal Th2 induction induced by C. neoformans infection determined by IL-4Rα expression level.** To define the mechanism(s) responsible for the partial resistance of the heterozygous IL-4Rα<sup>+/−</sup> mice, immune parameters were investigated. It has been shown elsewhere that development of protective Th1 versus pathological Th2 responses is critical for resistance rather than susceptibility to C. neoformans infection [19–22]. Interestingly, the absence of a Th2 response appears to be even more important than the magnitude of the Th1 response for the course of cryptococcosis [3, 5]. The Th2-dependent isoforms IgE and IgG1 were chosen as in vivo markers of Th2 development. Although serum IgE was significantly reduced after C. neoformans infection of IL-4Rα<sup>+/−</sup> mice compared with IL-4Rα<sup>+/+</sup> mice, the former group showed comparably elevated levels of IgG1 (table 2). Both IgE and IgG1 levels were significantly reduced in IL-4Rα<sup>−/−</sup> mice; IgE was not even detectable in infected IL-4Rα<sup>−/−</sup> mice. Naive mice of the IL-4Rα<sup>+/+</sup> and IL-4Rα<sup>+/−</sup> genotypes showed comparably low IgE levels (table 2). Thus, induction of IgE is strictly IL-4Rα dependent (table 2) and correlates with the IL-4Rα expression level in leukocytes, as shown above (table 1). Furthermore, with IgE as an important marker of fatal Th2 development during cryptococcosis, the data showed that IL-4Rα expression levels critically determine the degree of Th2-mediated susceptibility. For the Th1-related isotype IgG2a, no differences were found between the 3 genotypes. Compared with naive mice, all 3 groups showed comparably

Table 2. Serum IgE and IgG1 levels in naive and Cryptococcus neoformans–infected mice 70 days after infection.

<table>
<thead>
<tr>
<th>Immunoglobulin</th>
<th>Serum concentration, µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-4Rα&lt;sup&gt;+/+&lt;/sup&gt; mice</td>
</tr>
<tr>
<td>IgE</td>
<td></td>
</tr>
<tr>
<td>Naive</td>
<td>0.97</td>
</tr>
<tr>
<td>70 days after infection</td>
<td>24.50</td>
</tr>
<tr>
<td>IgG1</td>
<td></td>
</tr>
<tr>
<td>Naive</td>
<td>58.04</td>
</tr>
<tr>
<td>70 days after infection</td>
<td>701.08</td>
</tr>
</tbody>
</table>

**NOTE.** Intermediate interleukin (IL–4) receptor α-chain (IL-4Rα) expression leads to reduced IgE but comparable IgG1 levels after infection with C. neoformans. In infected mice, both serum levels of IgE differ significantly between these genotypes, whereas naive IL-4Rα<sup>+/+</sup> and IL-4Rα<sup>+/−</sup> mice show comparable levels of IgE and IgG1. Values shown are medians from 3–4 experiments with 3 mice per experiment.

<sup>a</sup> P < .01 for the comparison with IL-4Rα<sup>+/+</sup> mice (2-tailed Mann-Whitney U test).

<sup>b</sup> P < .001 for the comparison with IL-4Rα<sup>−/−</sup> mice (2-tailed Mann-Whitney U test).
Elevated serum IgG2a levels on day 70 after C. neoformans infection (data not shown).

To assess Th2 responses more directly in IL-4Rα+/+, IL-4Rα+/−, and IL-4Rα−/− mice infected intranasally with C. neoformans, we analyzed ex vivo cytokine production by splenocytes. Because IL-4 was consumed by cultured WT (IL-4Rα+/+) splenocytes [23] but to a lesser degree by IL-4Rα+/− splenocytes and not at all by IL-4Rα−/− splenocytes, it was difficult to interpret antigen-specific production of IL-4. Therefore, we studied IL-5, another Th2 cytokine that has been characterized as being associated with the immunopathology of cryptococcosis [3, 5]. Antigen-specific stimulation of splenocytes with heat-killed cryptococci led to high levels of IL-5 production by splenocytes derived from susceptible IL-4Rα+/+ mice, whereas splenocytes from partially resistant IL-4Rα+/− mice and totally resistant IL-4Rα−/− mice showed gradually reduced IL-5 production (figure 2A). In addition, splenecytic IL-17 production, recently shown by us to be associated with protection in C. neoformans infection [24], was found to be gradually elevated in partially resistant IL-4Rα+/− mice and totally resistant IL-4Rα−/− mice, compared with susceptible IL-4Rα+/+ mice (figure 2B).

Nature of the pulmonary inflammatory response to C. neoformans infection determined by IL-4Rα expression level, which affects lung function. The pulmonary inflammatory response to C. neoformans infection crucially depends on the nature of the T cell response, that is, a protective Th1/Th17 versus a nonprotective Th2 response [6, 19, 24–26]. Th2 responses are associated with the appearance of eosinophils, the modulation of goblet cells, and alternative macrophage activation. In accordance with lower IL-5 production (figure 2A), fewer eosinophils were detected in the lungs of partially resistant IL-4Rα+/− mice compared with IL-4Rα+/+ mice with pronounced cryptococcosis (figure 3A and 3B). The goblet cell metaplasia and mucous hyperproduction found in susceptible Cryptococcus-infected IL-4Rα+/+ mice was strongly reduced in IL-4Rα+/− mice (figure 3D and 3E). No eosinophils and no alteration of goblet cells could be found in the highly resistant IL-4Rα−/− mice (figure 3C and 3F). Therefore, the allergic inflammatory response of the lungs reflects the IL-4Rα expression levels responsible for susceptibility against C. neoformans infection (figure 1 and table 1).

Alternative activation of lung macrophages is associated with a fatal course of cryptococcosis [3, 5, 12]. The immunostaining of chitinase-like YM1 and arginase-1, both markers of aaMphs, revealed only marginal expression of YM1 and arginase-1 in the lungs of infected IL-4Rα−/− mice but strongly enhanced expression of both in IL-4Rα+/+ mice (figure 3G, 3I, 3J, and 3L). The number of aaMphs was found to be reduced in the heterozygous mice compared with IL-4Rα+/+ mice (figure 3G, 3H, 3J, and 3K), again pointing to an intermediate phenotype of IL-4Rα+/− mice. YM1+ cells appeared voluminous and stained strongly positive for macrophage markers, such as F4/80 and CD11b (data not shown). The lungs of IL-4Rα+/+ mice showed massive focal accumulations of YM1+ macrophages. In contrast, the distribution of YM1+ macrophages in IL-4Rα+/− mice was more diffuse, and these cells appeared smaller and more compact. These results provide evidence for IL-4Rα−/−dependent development of aaMphs in C. neoformans infection.

To see whether the IL-4Rα−/−dependent pulmonary inflammatory response had a functional consequence, we studied airway hyperreactivity in the 3 mouse groups. After receipt of high doses of the asthma-inducing agent methacholine, C. neoformans–infected IL-4Rα+/+ mice displayed significantly elevated airway hyperreactivity compared with IL-4Rα−/− mice (figure 3M). IL-4Rα+/− mice showed a phenotype between those of WT and IL-4Rα−/− mice, an important hint for a critical effect of the IL-4Rα expression level on lung function.

DISCUSSION

Susceptibility to C. neoformans infection is critically correlated with Th2 development [2–4, 6]. In the present study, we chose to target IL-4Rα to interfere simultaneously with the fatal action of IL-4 and IL-13. Interestingly, IL-4Rα−/− mice were found to acquire a particularly high degree of protective immunity associated with complete control of brain infection and long-term survival. As expected, targeting the shared receptor IL-4Rα was more effective than targeting the individual ligands (especially IL-13) in protecting against C. neoformans infection [5]. Interestingly, IL-4Rα−/− mice were unable to eliminate C. neoformans in the lungs during a prolonged period (up to 275 days after infection) but were very well able to control the pulmonary fungal load at a stable level between 70 and 275 days after infection. This shows the impressive efficacy of antagonizing Th2 activity to prevent reactivation of C. neoformans infection from the
lungs. Moreover, our data indicate, for the first time, a gene-dosage effect of IL-4R in anti-infective immunity.

The data from *C. neoformans*–infected IL-4R mice do not allow us to draw conclusions on the individual contribution of IL-4 versus IL-13. Certainly, the survival rates of IL-4 mice (96%) and IL-13 mice (86% and [5]) suggest that the action of IL-4 is somewhat more important than that of IL-13 for susceptibility during cryptococcosis. However, as we have shown
recently, goblet cell metaplasia and mucus production during pulmonary cryptococcosis are IL-13 dependent [5]; this may even contribute to enhanced airway hyperreactivity (figure 3M and [5]). Owing to the reduced goblet cell metaplasia and mucus production found in infected IL-4Rα−/− mice (figure 3D), IL-13 definitely plays a role in susceptibility different from that played by IL-4.

The strikingly contrasting phenotypes of WT versus IL-4Rα−/− mice prompted us to determine whether an intermediate level of expression of IL-4Rα would also result in intermediate susceptibility. Indeed, our study describing IL-4Rα expression level–dependent immunity to C. neoformans infection is, to the best of our knowledge, the first example of a gene-dosage effect of IL-4Rα in antimicrobial immunity. Infection with C. neoformans appears to be different from a number of other infections studied, for which the level of IL-4Rα expression has not been found to be critical for IL-4/IL-13 responsiveness.

In Leishmania and Schistosoma infection models, heterozygous IL-4Rα mice were found to have phenotypic and immunologic responses similar to those in WT mice [27, 28]. It is intriguing to speculate that, in these models, higher IL-4 levels are generated, making it impossible to distinguish between IL-4Rα+/+ and IL-4Rα−/− mice. Presumably, both the level of IL-4 production and the level of IL-4Rα expression together regulate the intensity of Th2-driven disease. Small changes in the level of IL-4Rα expression may be particularly limiting with low-level IL-4 production (as may be the case for C. neoformans infection).

As shown earlier, IL-4Rα up-regulation is dependent on the concentration of IL-4 [29]. With higher IL-4 levels produced, low or intermediate levels of IL-4Rα may be up-regulated and less limiting. It is noteworthy that we could not find modulation or even up-regulation of IL-4Rα due to C. neoformans infection (table 1), arguing for a low-level IL-4 system in our model. In vitro experiments need to be designed to model quantitatively the IL-4/IL-13/IL-4Rα–dependent pulmonary allergic immune response driven by C. neoformans.

The data from this bronchopulmonary mycosis model may have relevant implications for future therapeutic strategies against asthma and atopy. IL-4Rα polymorphism has been shown elsewhere to affect asthma development and prevalence [30–37]. It has been demonstrated that strong IL-4Rα–dependent signaling in newborns’ monocytes and Th lymphocytes could contribute to Th1/Th2 imbalance [38]. These authors concluded that IL-4R overexpression in newborns’ monocytes and lymphocytes could be an early risk marker of allergy development. In line with these findings, significantly reduced expression of IL-4Rα associated with reduced IL-4–induced signaling was found in neonatal B cells [39].

In another study, combined extended haplotypes involving IL-4, IL-13, IL-4Rα, and STAT6 were analyzed to assess the combined effect of single-nucleotide polymorphisms in the IL-4/IL-13 signaling pathway [35]. When polymorphisms in all 4 major pathway genes were combined in a stepwise procedure, the risk of high serum IgE levels increased 10.8-fold and the risk of the development of asthma increased 16.8-fold, compared with the maximum effect of any single polymorphism. Interestingly, in Leishmania–infected BALB/c STAT6−/− mice, Burgis and Gessner [40] found evidence of distinct STAT6 dosage requirements for different IL-4 functions. Their finding underscores the fact that the IL-4/IL-13 pathway is tightly regulated at different levels of the cascade. Earlier it was shown that different IL-4Rα allotypes exist in inbred mouse strains associated with different levels of IL-4–neutralizing activity [41]. Different degrees of IL-4 responsiveness may be involved in the specific phenotypes of inbred mouse strains in IL-4–dependent infections.

For therapeutic strategies against Th2-related diseases, such as asthma and atopy, our findings, together with other epidemiological data, reveal IL-4Rα to be an attractive target. It is obvious that a complete blockade would not be feasible and, as we have shown, is not necessary. Even a partial blockade could be effective for ameliorating IL-4/IL-13–driven diseases.

**Acknowledgments**

We thank J. Richter and M. Brenkmann for their excellent technical assistance. Also, we thank N. Kirchoff, U. Zirkler, and R. Voigtlander in particular for their excellent work in breeding the mice. We are grateful to A. Hoelscher and Dr. C. Hoelscher (Research Center Borstel, Germany) for helpful advice on lung digestion and immunohistochemistry. We thank Dr. M. Mohrs for valuable discussions and Dr. A. Gessner for critical reading of the manuscript.

**References**

25. Hoag KA, Lipscomb MF, Izzo AA, Street NE. IL-12 and IFN-gamma are required for initiating the protective Th1 response to pulmonary cryptococcosis in resistant C.B-17 mice. Am J Respir Cell Mol Biol 1997; 17:733–9.