IL-4R α Responsiveness of Non-CD4 T Cells Contributes to Resistance in *Schistosoma mansoni* Infection in Pan-T Cell-Specific IL-4R α -Deficient Mice

Benjamin Dewals, Jennifer C. Hoving,
Mosiua Leeto, Reece G. Marillier,
Umeshree Govender, Antony J. Cutler,
William G.C. Horsnell, and Frank Brombacher

From the International Centre for Genetic Engineering and
Biotechnology and Institute of Infectious Disease and Molecular
Medicine, Division of Immunology, University of Cape Town,
Cape Town, South Africa

Interleukin (IL)-4 and IL-13 are T helper 2 cytokines whose biological functions are induced through a common IL-4 receptor α chain (IL-4R α). CD4⁺ T cell-specific IL-4R α -mediated signaling drives susceptibility to *Leishmania major* infection, but is not essential to host survival following *Schistosoma mansoni* infection. Here we generated a novel mouse model lacking IL-4R α expression specifically on all T cells (*iLck^{cre}Il4ra^{-lox}*), which was compared with CD4⁺ T cell-specific IL-4R α -deficient mice (*Lck^{cre}Il4ra^{-lox}*), to investigate the possible roles of IL-4R α responsive non-CD4⁺ T cells during either *L. major* or *S. mansoni* infection. Our results demonstrate a successful generation of transgene-bearing hemizygous *iLck^{cre}Il4ra^{-lox}* BALB/c mice that have effective deletion of IL-4R α on all T-cell populations. We show that *iLck^{cre}Il4ra^{-lox}* mice infected with *L. major* developed a healing disease phenotype as previously observed in *Lck^{cre}Il4ra^{-lox}* mice, demonstrating that absence of IL-4R α -responsive non-CD4⁺ in addition to CD4⁺ T cells does not further affect transformation of BALB/c to a healer phenotype. In acute schistosomiasis, however, *iLck^{cre}Il4ra^{-lox}* mice showed enhanced mortality compared with *Il4ra^{-lox}* and *Lck^{cre}Il4ra^{-lox}* mice. *iLck^{cre}Il4ra^{-lox}* mice died with similar kinetics to highly susceptible *Il4ra^{-/-}* mice, despite controlling gut inflammation. In addition, *iLck^{cre}Il4ra^{-lox}* mice presented increased liver granuloma sizes, as compared with *Lck^{cre}Il4ra^{-lox}* mice, with similar eosinophils, fibrosis, and liver damage. In conclusion, IL-4R α -responsive non-CD4⁺ T cells prolong survival to acute schistosomiasis and contribute to the better control of hepatic granulomatous

inflammation. (*Am J Pathol* 2009, 175:706–716; DOI: 10.2353/ajpath.2009.090137)

Interleukin (IL)-4 and IL-13 are key T helper 2 (Th2) cytokines with pleiotropic immune functions reflected by the expression of their common receptor on a wide range of cell types.¹ Both cytokines are involved in mechanisms promoting allergic pathology,² conferring resistance to parasitic helminth, like *Schistosoma sp.* infections,^{3–6} and also allowing intracellular pathogens to persist in the infected hosts, such as during *Leishmania sp.* infections.^{7–9} IL-4 and IL-13 signaling is dependent on heterodimeric receptors containing an IL-4 receptor α (IL-4R α) subunit. IL-4 alone signals through the type I receptor composed of IL-4R α and the common gamma chain (γ c) while both IL-4 and IL-13 signal through the type II receptor consisting of a heterodimer of IL-4R α and IL-13R α 1 chains.¹⁰ The cellular contributions of IL-4R α to the mechanisms conferring susceptibility or protection to the host following infection with pathogens can be dissected by using mice with IL-4R α expression disrupted specifically on certain cell types.

Experimental *Leishmania major* infection is widely used to study the mechanisms underlying the differentiation of naïve CD4⁺ T cells into Th1/Th2 in response to intracellular pathogens and the role of differentiated immune

Supported by The Wellcome Trust and Royal Society, UK (080921/Z/06/Z), South African Research Chair initiative of the Department of Science and Technology (DST), National Research Foundation (NRF), and Medical Research Council (MRC), South Africa. B.D. is a Postdoctoral Researcher of the Fonds National Belge de la Recherche Scientifique (FNRS).

B.D. and J.C.H. contributed equally to this work.

Accepted for publication May 7, 2009.

Present address of B.D.: Immunology-Vaccinology (B43b), Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liège, Belgium; of A.J.C.: Histocompatibility and Immunogenetics, NHSBT, Colindale Centre, London, UK.

Address reprint requests to Frank Brombacher, International Centre for Genetic Engineering and Biotechnology (ICGEB) University Campus, Room S1.27; Werner Beith South Wing, Observatory 7925, Cape Town, South Africa. E-mail: fbrombac@mweb.co.za.

responses in host susceptibility/resistance to infection.^{11,12} When infected subcutaneously with *L. major*, susceptible BALB/c mice develop a severe pathology. Typically, these mice manifest progressive lesion development, necrosis, and death, while resistant C57BL/6 mice are able to heal dermal lesions.¹³ Whereas *Il4*^{-/-} mice resist infection, global *Il4ra*^{-/-} mice develop progressive chronic disease after initially controlling *L. major* infection, which indicates a protective role for IL-13 signaling at least in the absence of IL-4 responsiveness.¹⁴ The generation of macrophage/neutrophil (*LysM*^{cre}*Il4ra*^{-/lox}) and CD4⁺-specific IL-4R α -deficient (*Lck*^{cre}*Il4ra*^{-/lox}) mice has recently demonstrated that absence of alternative activated macrophages (aaM ϕ) delay inflammation and parasite invasion,¹⁵ whereas IL-4R α deletion, specifically on CD4⁺ T cells, renders mice completely resistant to *L. major* infection, independently of the presence of IL-4/IL-13 responsive non-CD4⁺ T cells.¹⁶ The latter mice developed a healing disease phenotype and clinical immunity similar to genetically resistant C57BL/6 mice, with reduced lesion development, control of parasite burden, reduced IL-10 secretion, and increased Th1/type 1 responses.¹⁶

During *S. mansoni* infection, the parasite eggs induce a strong and protective Th2 response and type 2 anti-*Schistosoma* antibodies.^{17,18} In such infectious settings, IL-4R α responsiveness plays an indispensable role in protecting the host from infection, and typically from excessive inflammation induced by the parasite eggs. IL-4/IL-13-, IL-4R α -, and signal transducer and activator of transcription-6-deficient mice show impaired Th2 polarization, impaired granuloma formation, reduced fibrosis and high inflammation, which drive an increased mortality following *S. mansoni* infection.^{4,19,20} This increased susceptibility in mice unable to respond to IL-4 or IL-13 is related to liver damage and uncontrolled intestinal inflammation, which can ultimately result in endotoxemia and septic shock.⁵ Infection studies with *LysM*^{cre}*Il4ra*^{-/lox} mice (disrupted for IL-4R α specifically on macrophages/neutrophils) or *Lck*^{cre}*Il4ra*^{-/lox} (disrupted for IL-4R α specifically on CD4⁺ T cells) has demonstrated important, but conflicting roles for both cellular types in controlling schistosomiasis. Despite developing a polarized Th2 response, *LysM*^{cre}*Il4ra*^{-/lox} mice are highly susceptible to *S. mansoni* infection due to their inability to alternatively activate macrophages, leading to liver damage and intestinal inflammation.⁵ In contrast, *Lck*^{cre}*Il4ra*^{-/lox} mice showed reduced Th2 responses, increased Th1/type 1 responses and liver pathology yet did not develop increased intestinal inflammation and their survival was not affected during schistosomiasis.²¹ Studies with the latter mouse model suggest that IL-4R α -mediated signaling in CD4⁺ T cells, independently of non-CD4⁺ T cells, is not essential for host protection in schistosomiasis.

We investigated possible roles of IL-4R α -responsive non-CD4⁺ T cells during *L. major* or *S. mansoni* infection. Our results demonstrate a successful generation of transgene-bearing hemizygous *iLck*^{cre}*Il4ra*^{-/lox} BALB/c mice that have effective impairment of IL-4R α on all T cell populations, in contrast to previously described *Lck*^{cre}*Il4ra*^{-/lox} mice showing a CD4⁺ T cell-restricted

deletion of the receptor. We show that *iLck*^{cre}*Il4ra*^{-/lox} mice infected with *L. major* developed a healing disease phenotype as previously observed in *Lck*^{cre}*Il4ra*^{-/lox} mice, demonstrating that absence of IL-4R α -responsive non-CD4⁺ T cells in addition to CD4⁺ T cells does not affect the observed healer phenotype. In contrast, we show that specific IL-4R α responses by non-CD4⁺ T cells contribute to prolonging survival to acute schistosomiasis, as *iLck*^{cre}*Il4ra*^{-/lox} mice quickly succumbed to acute *S. mansoni* infection and developed a liver restricted pathology with increased granuloma sizes.

Materials and Methods

Generation of *iLck*^{cre}*Il4ra*^{-/lox} BALB/c Mice

Transgenic *iLck*^{cre} mice (kindly provided by Dr C.B. Wilson, University of Washington)²² back-crossed to BALB/c mice for nine generations were intercrossed with *Il4ra*^{-/-} and *Il4ra*^{lox/lox} mice to generate *iLck*^{cre}*Il4ra*^{-/lox} mice. The *iLck*^{cre} construct was engineered by insertion of a nuclear localization signal and optimum eukaryotic translation start site at the 5' end of the *Cre*-recombinase and inserting it downstream of the *Lck* proximal promoter.²³ Hemizygous *Il4ra*^{-/lox} littermates were used as controls to *iLck*^{cre}*Il4ra*^{-/lox} mice. The genotype of *iLck*^{cre}*Il4ra*^{-/lox} mice was confirmed using the following specific primer pairs: IL-4R α : 5'-TGACCTACAAGGAACCCAGGC-3' and 5'-CTCGGCGCACTGACCCATCT-3'; deletion: 5'-GGCTGCTGACCTGGAATAACC-3' and 5'-CCTTTGAGAACTGCGGGCT-3'; *loxP*: 5'-CCCTTCCTGGCCCTGAATTT-3' and 5'-GTTTCCTCTACCGCTGATT-3'; and *Cre*: 5'-GAGGGTGAATGAAACTCTCGGT-3' and 5'-CAGGTATGCTCAGAAAACGCCTGG-3'. PCR conditions were as follows: 94°C/1 minute, 94°C/30 seconds, 55°C to 60°C/20 seconds, 72°C/1 minute for 40 cycles, and 72°C/5 minutes using a MJ thermocycler (Biozym, Germany). Mice were housed in specific pathogen-free barrier conditions at the University of Cape Town, South Africa, and used in accordance with University ethical committee guidelines. All experimental mice were sex-matched and used between 8 to 12 weeks of age with appropriate littermate controls of the same generation.

Analysis of *Il4ra* Deletion Efficiency

DNA was prepared from CD3⁺CD4⁺ and CD3⁺CD4⁻ sorted spleen cells from *Il4ra*^{-/lox}, *iLck*^{cre}*Il4ra*^{-/lox}, *Lck*^{cre}*Il4ra*^{-/lox}, and *Il4ra*^{-/-} naive mice using a FACSVantage cell sorter (BD Biosciences) to $\geq 99\%$ purity. A standard curve was prepared from serial 10-fold dilutions of cloned *Il4ra* exon 5 and floxed region DNA. Primers: exon 5 forward 5'-AACCTGGGAAGTTGTG-3', exon 5 reverse 5'-CACAGTTCCATCTGGTAT-3'; floxed forward 5'-CTTTGGAGTAGGGTGCTTGG-3', floxed reverse 5'-CCTACCGCTGATTTTCTCG-3'.

Parasite Infections and Antigen Preparation

L. major LV 39 (MRHO/Sv/59/P) strain was maintained by continuous passage in susceptible BALB/c mice as previously described.²⁴ Infections were performed subcutaneously in one hind footpad with 2×10^6 stationary phase metacyclic promastigote parasites/50 μ l. Footpad swelling was monitored weekly using a pocket thickness gauge (Mitutoyo, Japan). Parasite burden was determined from infected organs by the limiting dilution method.²⁴ Two-fold serial dilutions were prepared in Schneider's culture medium (Sigma) with 20% fetal calf serum and the cultures were examined for parasites after 7 days at room temperature. For *S. mansoni* infection studies mice were infected percutaneously with different doses of cercariae of a Puerto Rican strain of *S. mansoni* obtained from infected *Biomphalaria glabrata* snails (generous gift from Dr A.P. Mountford, University of York, UK), weighted weekly and mortality was recorded. Soluble egg antigen was prepared from purified eggs as described.²⁵

Delayed Type Hypersensitivity Reaction

Mice were inoculated subcutaneously with 5 μ g soluble *Leishmania* antigen in the right hind footpad. Footpad swelling was measured at 24 hours intervals for 4 days.

Liver Granuloma-Associated Leukocyte Purification

Granulomatous livers were finely cut in small pieces and digested in culture media containing 50 μ g/ml collagenase type IV (Sigma) at 37°C for 1.5 hours. Single cell suspensions were further passed through a 100- μ m nylon mesh before leukocytes were isolated on a 30% Percoll cushion at 600 $\times$ g during 15 minutes. Liver leukocytes were treated for 2 minutes with NH₄Cl lysis buffer to lyse erythrocytes. Purified granuloma cells were used for ex vivo restimulation or flow cytometry analysis.

Cell Preparation and ex Vivo Restimulation

Single-cell suspensions were prepared from mesenteric lymph node, spleen, or liver and cultured at 2×10^6 cells/ml with or without plate-bound anti-CD3 (20 μ g/ml). In some experiments, popliteal lymph node single cell suspensions were pooled and CD4⁺ cells were enriched (>95%) by depletion of non-CD4⁺ cells using rat anti-mouse monoclonal antibodies (mAb) to CD8 α (53-6.7), B220 (RA3-6B2), CD11b (M1/70), and Gr-1 (RB6-8C5), followed by goat anti-rat IgG Biomag beads (Qiagen). antigen-presenting cells (APC) were T cell depleted by fluorescence-activated cell sorting of non-Thy1.2 labeled splenocytes and fixed with Mitomycin C (50 μ g/ml, 20 minutes at 37°C) and washed three times in complete Iscove's Modified Dulbecco's Medium (IMDM). SLA (50 μ g/ml) was added to 2×10^5 purified CD4⁺ T cells and 1×10^5 APCs. Culture supernatants were harvested at 72 hours for cytokines analysis. mAbs were purified from hybridoma supernatants.

Enzyme-Linked Immunosorbent Assay and Serum Analysis

Cytokines in supernatants and antigen-specific antibody isotypes in sera were determined as described.⁹ Serum aspartate transaminase was determined as described.⁵ Levels of serum lipopolysaccharide (LPS) were determined by using the QCL-1000 kit for endotoxin determination (Lonza) in combination with an Endotoxin standard (Sigma).

T Cell Proliferation

CD4⁺ T cells purified from pooled lymph nodes were enriched by positive selection as described above (>95% pure) and treated as described.¹⁴

Determination of Macrophage-Specific Arginase Activity

Arginase activity was determined as described.²⁶ Briefly, adherent peritoneal macrophages were incubated for 16 hours with medium, IL-4 or IL-13 at 1,000U/ml (R&D Systems). Cells were subsequently stimulated with LPS (15 ng/ml; Sigma) and interferon (IFN)- γ (100 U/ml) and production of urea from L-arginine was measured after 48 hours.

Induction of IgE Response

Mice were sensitized by intraperitoneal inoculation of 50 μ g ovalbumin (Sigma) in 1.5% aluminum hydroxide adjuvant (Sigma) and boosted at 7 and 14 days. After 21 days serum was collected and analyzed by enzyme-linked immunosorbent assay (ELISA) as described above.

Ex Vivo Th2 Differentiation

Positively selected CD4⁺ lymph node cells were enriched (>95%) as described above and used for ex vivo Th1/Th2 differentiation as previously described.¹⁶

Antibodies and Flow Cytometry

mAbs targeting the following cell surface markers were used: CD4 (GK1.5, RM4-5), CD3 (145-2C11), CD8 (53-6.7), TCR β (H57-597), TCR $\gamma\delta$ (GL3), CD19 (1D3), pan-natural killer (DX5), CD11c (HL3), F4/80 (A3-1, Caltag), CD11b (M1/70), I-A/I-E (MHC-II, M5/114), CD204 (2F8, Serotec), and CD124 (IL-4R α , M-1). Dead cells were stained with 7-AAD and excluded from analysis. Acquisition was performed using FACSCalibur (BD), and data were analyzed by Cellquest (BD) or Flowjo software (Treestar). Antibodies were from BD Pharmingen except where noted otherwise.

Figure 1. Generation of *iLck^{cre}IL4ra^{-/-lox}* mice. **A:** Mouse breeding strategy to generate *iLck^{cre}IL4ra^{-/-lox}* and *Lck^{cre}IL4ra^{-/-lox}* mice. Deleted allele (black arrows) and loxP site (gray arrows) are shown. **B:** Genotyping of transgene bearing T cell-specific IL-4R α -deficient mice. The deleted IL-4R α PCR yields a product of 471 bp, lox-P, 188 bp (floxed), or 94 bp (wild-type) and Cre-specific PCR a product of 450 bp. **C:** Flow cytometry of IL-4R α surface expression on T or non-T cell subpopulations. Spleen or peritoneal cells from naive mice were co-stained using mAbs against IL-4R α and various cell surface markers on *IL4ra^{-/-lox}* (dotted line), *IL4ra^{-/-}* (greyscale), and *iLck^{cre}IL4ra^{-/-lox}* (left columns, bold line) or *Lck^{cre}IL4ra^{-/-lox}* (right columns, bold line) mice. The geometric means are given for each histogram. T cells were CD4⁺/CD3⁺ or CD8⁺/CD3⁺, $\gamma\delta$ T cells were TCR $\gamma\delta$ ⁺/CD3⁺, natural killer T cells were DX5⁺/CD3⁺, B cells were CD19⁺/CD3⁺, natural killer cells were DX5⁺/CD3⁻, DCs were CD11c⁺/MHC-II⁺, and macrophages were CD11b⁺/F4/80⁺. Data are representative of three independent experiments ($n = 4$). **D:** Efficiency of *IL4ra* deletion. Floxed allele was determined and normalized to exon 5 alleles, present in all strains by real-time PCR from genomic DNA isolated from fluorescence activated cell-sorted ($\geq 99\%$ purity) CD3⁺CD4⁺ or CD3⁺CD4⁻ splenocytes. Data are representative of two independent experiments with triplicate values $\pm$ SD.

Histology, Fibrosis, and Immunohistochemistry

Five-micron sections were stained with H&E or with chromotrope 2R and aniline blue solution and counterstained with Wegert's hematoxylin for collagen staining. Hepatic collagen was measured as hydroxyproline by a modified technique.⁵ Fifteen to 30 granulomas per mouse were included in the analyses. The diameters of each granuloma containing a single egg were measured with the ImageJ 1.34 software (<http://rsb.info.nih.gov/ij/docs/intro.html>). Numbers of eosinophils in granulomas were also evaluated in the same sections used for granulomas measurement. Micrographs of liver granulomas were captured using a Nikon 5.0 mega pixels color digital camera (Digital SIGHT DS-SMc).

Statistical Analysis

Values are given as mean $\pm$ SEM or SD and significant differences were determined using two-tailed *t*-test (Prism software, Irvine, CA).

Results

Pan-T Cell-Specific IL-4R α Deletion in *iLck^{cre}IL4ra^{-/-lox}* Mice

To impair IL-4R α expression on T cells we used a previously described breeding strategy.¹⁶ We intercrossed *IL4ra^{lox/lox}* BALB/c mice with transgenic *iLck^{cre}* mice or *Lck^{cre}* mice and *IL4ra^{-/-}* BALB/c mice to generate *iLck^{cre}IL4ra^{-/-lox}* or *Lck^{cre}IL4ra^{-/-lox}* mice (Figure 1A), which were identified by PCR genotyping (Figure 1B).^{5,9,22,27} *iLck^{cre}* mice were engineered to improve Cre-recombinase expression under the control of the T cell-specific *Lck* promoter.²² Flow cytometry analysis of IL-4R α surface expression in the different T cell and non-T cell populations demonstrated that *iLck^{cre}IL4ra^{-/-lox}* mice showed effective deletion of the IL-4R α on CD4⁺, CD8⁺, natural killer T, and $\gamma\delta$ ⁺ T cells when compared with *IL4ra^{-/-}* and *IL4ra^{-/-lox}* control mice (Figure 1C). In contrast, previously published *Lck^{cre}IL4ra^{-/-lox}* mice¹⁶ showed effective deletion of the IL-4R α only on CD4⁺ T cells

Figure 2. Functional analysis of *iLck*^{cre}*Il4ra*^{-/-lox} mice. Lymph node CD4⁺ T cells from *Il4ra*^{-/-lox}, *iLck*^{cre}*Il4ra*^{-/-lox}, *Lck*^{cre}*Il4ra*^{-/-lox}, and *Il4ra*^{-/-} mice were stimulated with serial dilutions of IL-4 (A) or IL-2 (B). Cell proliferation was measured by [³H] thymidine incorporation and mean values ± SD are shown. C: Th1 and Th2 differentiation of noncommitted CD4⁺ T cells was achieved *ex vivo* by treatment with either IL-12/anti-IL-4 (Th1) or IL-4/anti-IFN-γ (Th2), respectively. IL-4 and IFN-γ secretion was measured by ELISA. Mean ± SEM from triplicate cultures. Data are representative of two independent experiments (n = 3). **P < 0.01, ***P < 0.001 compared with *Il4ra*^{-/-lox} mice. D: Mice were immunized and boosted with ovalbumin. Serum was harvested 3 weeks after immunization and total IgE levels were analyzed by ELISA. Data are representative of two independent experiments (n = 3), mean values ± SEM and samples not detected (ND) are indicated. ***P < 0.001 *vs.* *Il4ra*^{-/-lox} mice. E: IL-4 and IL-13 compete with LPS/IFN-γ stimulation to induce arginase activity in *Il4ra*^{-/-lox}, *iLck*^{cre}*Il4ra*^{-/-lox}, and *Lck*^{cre}*Il4ra*^{-/-lox} mice. Peritoneal macrophages were incubated with IL-4 or IL-13 in combination with LPS/IFN-γ or LPS/IFN-γ alone. Metabolism of L-arginine into urea was measured and data are expressed as fold difference of urea compared with nonstimulated macrophages.

(Figure 1C), but retained receptor expression on non-CD4⁺ T cells. Maintenance of IL-4Rα expression on B cells (CD19⁺), natural killer cells (DX-5⁺), dendritic cells (CD11c⁺), and macrophages (F4/80⁺) of *iLck*^{cre}*Il4ra*^{-/-lox} and *Lck*^{cre}*Il4ra*^{-/-lox} mice confirmed the specificity of the deletion in both mouse models (Figure 1C).

Efficiency of deletion of *Il4ra* in CD3⁺CD4⁺ and CD3⁺CD4⁻ cells was further analyzed at the genomic level by PCR. The floxed allele (one copy in ^{-/-lox} and absent in ^{-/-} mice) of *Il4ra* were determined in cells sorted to high purity, and normalized on exon 5 alleles (present in all cells of each mouse strain). As expected, the floxed allele was efficiently deleted in both T cell populations from *Il4ra*^{-/-} mice (Figure 1D). Confirming flow cytometry analysis, efficient deletion of loxP-flanked *Il4ra* region was observed in CD3⁺CD4⁺ from both *iLck*^{cre}*Il4ra*^{-/-lox} and *Lck*^{cre}*Il4ra*^{-/-lox} mice (2.06 ± 0.14% and 4.15 ± 0.95% of floxed allele in *Il4ra*^{-/-lox} control mice, respectively). However, efficient deletion of loxP-flanked *Il4ra* region in CD3⁺CD4⁻ cells was only observed in *iLck*^{cre}*Il4ra*^{-/-lox} mice (3.86 ± 0.26% of floxed allele in *Il4ra*^{-/-lox} control mice). In contrast, PCR detected the floxed region in CD3⁺CD4⁻ cells from *Lck*^{cre}*Il4ra*^{-/-lox} mice (32.24 ±

11.21% of floxed allele in *Il4ra*^{-/-lox} control mice) (Figure 1D). These data provide evidence of efficient deletion of *Il4ra* restricted to CD4⁺ T cells from *Lck*^{cre}*Il4ra*^{-/-lox} mice and to all T cell populations from *iLck*^{cre}*Il4ra*^{-/-lox} mice.

Specific Impairment of IL-4Rα Function in T Cells

IL-4 promotes proliferation of naive CD4⁺ T cells *ex vivo*.²⁸ Functional impairment of IL-4Rα on T cells from *iLck*^{cre}*Il4ra*^{-/-lox} mice was compared with previously described *Lck*^{cre}*Il4ra*^{-/-lox} mice.¹⁶ Naive CD4⁺ T cells were stimulated with IL-4, and proliferation measured by [³H] thymidine incorporation (Figure 2A). CD4⁺ T cells isolated from naive *iLck*^{cre}*Il4ra*^{-/-lox} and *Lck*^{cre}*Il4ra*^{-/-lox} mice were unable to proliferate in response to IL-4, as also observed in *Il4ra*^{-/-} mice. In contrast, *Il4ra*^{-/-lox} control CD4⁺ T cells proliferated in response to IL-4 in a dose-dependent manner. Impairment of IL-4 signaling was IL-4Rα specific as proliferative responses to IL-2, which shares a γ_c-chain with the type I IL-4R, were unaffected in all four strains (Figure 2B). Impairment of CD4⁺ T cell IL-4 responsiveness was further verified by T helper cell dif-

Figure 3. T cell-specific IL-4R α -deficient mice control acute *L. major* infection. **A:** C57BL/6, *Il4ra*^{-lox}, *iLck^{cre}Il4ra*^{-lox}, *Lck^{cre}Il4ra*^{-lox}, and *Il4ra*^{-/-} mice (5 to 10 per group) were infected with 2×10^6 *L. major* LV39 parasites in the left hind footpad. Footpad swelling indicates disease progression. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, all groups versus C57BL/6. **B:** Parasite number was determined using limiting dilution of single cell suspensions from homogenized footpads (left) and popliteal lymph nodes (right) 6 weeks p.i. *** $P < 0.001$, vs. *Il4ra*^{-lox}. **C:** Resistant mice demonstrate a protective delayed type hypersensitivity response to 10 μ g SLA in the right hind footpad. Swelling was monitored every 24 hours for 4 days. * $P < 0.05$, ** or *** $P < 0.01$, *** $P < 0.001$ *Lck^{cre}Il4ra*^{-lox} and *iLck^{cre}Il4ra*^{-lox} vs. *Il4ra*^{-lox}, respectively. **D:** Serum was obtained from 4 to 5 mice per group at 6 weeks p.i. SLA-specific IgG1 titers were determined by ELISA. * $P < 0.05$ all groups versus C57BL/6. **E:** Draining lymph node cells from 4 to 5 mice per group were pooled and CD4⁺ cells isolated at week 6. Cells were cultured in duplicate with APCs pulsed with 50 μ g/ml SLA and supernatants were collected after 72 hours to measure IFN- γ by ELISA. Representative results of two individual experiments for week 6 shown with mean values $\pm$ SEM. * $P < 0.05$ *Lck^{cre}Il4ra*^{-lox} and *iLck^{cre}Il4ra*^{-lox} vs. *Il4ra*^{-lox}.

ferentiation assays. Th1 versus Th2 differentiation of non-committed CD4⁺ T cells may be achieved *ex vivo* by treatment with either IL-12/anti-IL-4 or IL-4/anti-IFN- γ , respectively.²⁸ Naive CD4⁺ T cells stimulated with anti-CD3/CD28 and polarized with cytokine/neutralizing mAb treatment demonstrated that Th2 polarization, indicated by IL-4 production, was impaired in *iLck^{cre}Il4ra*^{-lox}, *Lck^{cre}Il4ra*^{-lox}, and *Il4ra*^{-/-} but not *Il4ra*^{-lox} mice (Figure 2C). As expected, Th1 polarization was achieved in all four strains. Production of IgE antibodies is strictly dependent on IL-4 signaling.²⁹ IL-4R α responsiveness of B cells in *iLck^{cre}Il4ra*^{-lox} mice was demonstrated in Figure 2D. OVA-induced IgE were present at similar levels in OVA-immunized *iLck^{cre}Il4ra*^{-lox} mice when compared with those of *Lck^{cre}Il4ra*^{-lox} and *Il4ra*^{-lox} mice, while IgE production was completely abrogated in *Il4ra*^{-/-} mice (Figure 2D). IL-4R α -dependent macrophage function was demonstrated in Figure 2E. Arginase activity was induced by IL-4 and IL-13 in macrophages from *iLck^{cre}Il4ra*^{-lox}, *Lck^{cre}Il4ra*^{-lox}, and *Il4ra*^{-lox} mice, but not *Il4ra*^{-/-} macrophages (Figure 2E). Together, these data provide evidence for effective impairment of IL-4R α -mediated functions in *iLck^{cre}Il4ra*^{-lox} CD4⁺ T cells, but not in other lymphocyte subpopulations such as B cells and macrophages.

iLck^{cre}Il4ra^{-lox} and *Lck^{cre}Il4ra*^{-lox} Mice Are Resistant to *L. Major* Infection

The results described above demonstrate a specific deletion of the IL-4R α in CD4⁺ and non-CD4⁺ T cells in *iLck^{cre}Il4ra*^{-lox} mice whereas the deletion is restricted to CD4⁺ cells in *Lck^{cre}Il4ra*^{-lox} mice. Specific deletion of IL-4R α on CD4⁺ T cells (*Lck^{cre}Il4ra*^{-lox}) enables BALB/c mice to control *L. major* infection,¹⁶ however global deletion of the IL-4R α in *Il4ra*^{-/-} mice progressively leads to chronic disease, suggesting a protective role of IL-4R α -responsive cells other than CD4⁺ T cells. To determine the role of IL-4R α -responsive non-CD4⁺ T cell subpopulations in *L. major* infection, we injected 2×10^6 LV 39 (MRHO/Sv/59/P-strain) metacyclic promastigotes subcutaneously in the hind footpad of *iLck^{cre}Il4ra*^{-lox} and *Lck^{cre}Il4ra*^{-lox} mice. *iLck^{cre}Il4ra*^{-lox} mice were able to resolve lesion development similarly to previously described *Lck^{cre}Il4ra*^{-lox} and C57BL/6 mice (Figure 3A), while the nonhealing BALB/c control *Il4ra*^{-lox} mice developed severe footpad swelling by 6 weeks postinfection (p.i.). As described, *Il4ra*^{-/-} mice initially controlled infection but progressively increased footpad swelling.^{14,16} Both pan-T and CD4⁺ T cell-specific IL-4R α -

Figure 4. IL-4R α -dependent non-CD4⁺ T cell responses contribute to host protection after *S. mansoni* infection. **A:** $Il4ra^{-/-}$, $Il4ra^{-/-}$, $Lck^{cre}Il4ra^{-/-}$, and $iLck^{cre}Il4ra^{-/-}$ mice were infected with 75, 85, or 100 *S. mansoni* cercariae and survival rates were recorded at 12 weeks p.i. Each value represents the mean percentage of living mice per group of two independent experiments ($n = 8$). **B:** Survival kinetics of representative experiment with mice infected percutaneously with 100 cercariae. Data represent three independent experiments ($n = 8$). Survival curves of infected $iLck^{cre}Il4ra^{-/-}$ and $Lck^{cre}Il4ra^{-/-}$ mice were compared with the curves of $Il4ra^{-/-}$ mice using the Mantel-Haenszel test. **Arrow** indicates time of analysis in **A**. **C:** Soluble egg antigen-specific immunoglobulin production of IgG1, IgG2a, and IgG2b, or total IgE production (mean $\pm$ SD). **D:** Cytokine production after 72 hours anti-CD3 restimulation of mesenteric lymph node (mLN) cells and splenocytes (mean $\pm$ SEM). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs. $Il4ra^{-/-}$ mice.

deficient mice had significantly reduced parasite numbers in footpad and draining popliteal lymph node compared with $Il4ra^{-/-}$ mice (Figure 3B). Similarly both mice developed a protective delayed type hypersensitivity response to SLA comparable with the C57BL/6 healer strain (Figure 3C). We observed in $Lck^{cre}Il4ra^{-/-}$ and $iLck^{cre}Il4ra^{-/-}$ mice reduced SLA-specific IgG1 production comparable with the C57BL/6 healer strain (Figure 3D). In comparison with control littermate mice, $Lck^{cre}Il4ra^{-/-}$ and $iLck^{cre}Il4ra^{-/-}$ mice also had increased IFN- γ production suggesting a shift toward Th1 polarization (Figure 3E). Together these data demonstrated that both strains were able to control disease progression and parasite numbers comparably with C57BL/6 control mice.

IL-4R α Responsive Non-CD4 T Cells Contribute to Survival during Schistosomiasis

Th2/type 2 responses are essential for host protection against schistosomiasis.^{17,18} We previously showed in

mice deficient for IL-4R α responsiveness in CD4⁺ T cells only ($Lck^{cre}Il4ra^{-/-}$) that a Th1-dominant granulomatous pathology does not inhibit fibrosis or increase lethality during murine schistosomiasis.²¹ To investigate possible roles of IL-4R α responsive non-CD4⁺ T cells during acute schistosomiasis, $iLck^{cre}Il4ra^{-/-}$ or $Lck^{cre}Il4ra^{-/-}$ mice were infected with increasing doses of *S. mansoni* cercariae (75-85-100 cercariae) and survival rates were recorded up to 12 weeks p.i. (Figure 4A). Comparative dose-dependent mortality studies resulted in reduced survival in $iLck^{cre}Il4ra^{-/-}$ mice compared with $Lck^{cre}Il4ra^{-/-}$ mice at all infection doses used (Figure 4A). At the highest infection dose $iLck^{cre}Il4ra^{-/-}$ mice significantly lost body weight (data not shown) and died at similar times compared with highly susceptible global $Il4ra^{-/-}$ mice (Figure 4B). No significant difference in survival was observed between $Lck^{cre}Il4ra^{-/-}$ and littermate control $Il4ra^{-/-}$ mice. Antigen-specific antibody type 2 (Figure 4C) and Th2 cytokine responses (Figure 4D) were impaired in *S.*

Figure 5. Intestinal pathology during acute schistosomiasis is not affected by IL-4R α -dependent T cell responses. *Il4ra*^{-/-lox}, *iLck*^{cre}*Il4ra*^{-/-lox}, *Lck*^{cre}*Il4ra*^{-/-lox}, and *Il4ra*^{-/-} mice were infected with 100 *S. mansoni* cercariae and were sacrificed at 8 weeks p.i. ****P* < 0.001 vs. *Il4ra*^{-/-lox} mice. Data are representative of two independent experiments. **A:** Serum levels of endotoxin (mean $\pm$ SD, *n* = 4). **B:** Fecal egg counts (mean $\pm$ SD, *n* = 8). **C:** Representative micrographs of 5- μ m sections from formalin-fixed ileums and H&E stained. Arrowheads, eggs; g, goblet cell; L, lacteal spaces; Lp, lamina propria; m, muscularis layer. Original magnification, $\times$ 100.

mansoni infected *iLck*^{cre}*Il4ra*^{-/-lox} mice, similarly to *Lck*^{cre}*Il4ra*^{-/-lox} with increased Th1/type 1 responses, compared with littermate controls. These results demonstrate that IL-4R α -dependent T cell responses are required for resistance to schistosomiasis and indicate that IL-4R α responsiveness of non-CD4⁺ T cells contribute to survival in acute schistosomiasis.

IL-4R α Responsive Non-CD4 T Cells Contribute to Control Hepatic Granulomatous Inflammation

We previously showed that global *Il4ra*^{-/-} mice die of septic shock with high LPS levels (Figure 5A) due to infiltrating bacteria caused by damaging gut integrity by egg retention (Figure 5B) and subsequent high egg-induced intestinal inflammation (Figure 5C). Interestingly, 8 weeks p.i., *iLck*^{cre}*Il4ra*^{-/-lox} mice showed a similar phenotype compared with *Lck*^{cre}*Il4ra*^{-/-lox} and littermate *Il4ra*^{-/-lox} control mice with low LPS blood levels (Figure 5A), unimpaired egg expulsion (Figure 5B) and intact gut (Figure 5C). In contrast, liver inflammatory pathology was increased in both *iLck*^{cre}*Il4ra*^{-/-lox} and *Lck*^{cre}*Il4ra*^{-/-lox} mice compared with littermate controls. This was demonstrated by increased serum aspartate transaminase, a marker for hepatocellular damage (Figure 6A), reduced eosinophilia (Figure 6B), increased granuloma size (Figure 6C), presence of collagen-induced fibrosis (Figure 6D) and less organized granuloma structure (Figure 6F). Increased pathology in the liver was associated with reduced IL-5 and increased IFN- γ and transforming growth factor- β cytokine responses during anti-CD3 stimulation of liver granuloma-associated cells (Figure 6E). Of note, *iLck*^{cre}*Il4ra*^{-/-lox} mice showed highly significantly increased granuloma size compared with *Lck*^{cre}*Il4ra*^{-/-lox} mice (Figure 6C), as well as reduced IL-4 secretion during restimulation compared with *Lck*^{cre}*Il4ra*^{-/-lox} mice. Flow cytometry analysis of IL-4R α surface expression on liver granuloma-associated cell subpopulations confirmed specific deletion of the receptor on CD4⁺ and non

CD4⁺ T cells in *iLck*^{cre}*Il4ra*^{-/-lox} but restricted to CD4⁺ T cells in *Lck*^{cre}*Il4ra*^{-/-lox} mice (Figure 6G). In summary, *iLck*^{cre}*Il4ra*^{-/-lox} mice developed a liver restricted inflammatory pathology during schistosomiasis with increased granuloma sizes compared with *Lck*^{cre}*Il4ra*^{-/-lox} mice.

Discussion

Our understanding of how cell-specific IL-4R α signaling coordinates Th2-type immunity is relatively poor, in comparison with our knowledge of Th1-, Th17-, or Treg-mediated immunity. Transgenic mouse models with IL-4R α expression disrupted in defined cell populations are now an invaluable tool redressing this paucity of understanding and have highlighted key cellular players in the control of infections by pathogens and pathologies where Th2 effector responses are involved. Studies using cell-specific IL-4R α -deficient mice demonstrated aaM ϕ and CD4⁺ Th2 cells to drive nonhealing response in BALB/c mice infected by *L. major*.^{15,16} In schistosomiasis, aaM ϕ mediate host protection and CD4⁺ Th2 responses control liver pathology without affecting host survival.^{5,21} In the present study we investigated possible roles of IL-4R α responsive T cells other than CD4⁺ T cells during two different infectious settings where Th2 effector responses are involved. We generated a new mouse specifically impaired for IL-4R α expression in all T cell populations (*iLck*^{cre}*Il4ra*^{-/-lox}). These mice were compared with CD4⁺-specific IL-4R α -deficient *Lck*^{cre}*Il4ra*^{-/-lox} mice after either *L. major* or *S. mansoni* infections. During subcutaneous leishmaniasis, *iLck*^{cre}*Il4ra*^{-/-lox} mice developed a healing phenotype, similarly to *Lck*^{cre}*Il4ra*^{-/-lox} mice. Interestingly, *iLck*^{cre}*Il4ra*^{-/-lox} quickly succumbed during the acute phase of *S. mansoni* infection whereas CD4⁺-specific IL-4R α -deficient mice survived similar to wild-type mice. These results demonstrate that IL-4R α -responsive non-CD4⁺ T cells contribute to host protection in acute schistosomiasis but not in leishmaniasis.

Figure 6. *iLck^{cre}Il4ra^{-/-lox}* mice develop a liver restricted inflammatory pathology during schistosomiasis. *Il4ra^{-/-lox}*, *iLck^{cre}Il4ra^{-/-lox}*, *Lck^{cre}Il4ra^{-/-lox}*, and *Il4ra^{-/-}* mice were infected with 100 *S. mansoni* cercariae and were sacrificed at 8 weeks p.i. **A:** Serum levels of aspartate transaminase (mean ± SD, *n* = 4). **B:** Numbers of eosinophils per granuloma assessed by microscopic analysis on H&E stained sections. Each dot represents individual measurement. **C:** Granuloma volumes around eggs measured by microscopy of H&E-stained liver sections (mean ± SEM, *n* = 6). **D:** Fibrosis, measured as liver hydroxyproline content normalized to egg numbers (mean ± SD, *n* = 4). **E:** Cytokine production after 72 hours anti-CD3 restimulation of liver granuloma-associated cells (mean ± SEM, *n* = 4). **F:** Representative micrographs of 5-μm sections from formalin-fixed liver sections stained with H&E for morphological analysis (panels **a-d**) of granulomas or chromotrope 2R and aniline blue solution for collagen content (panels **e-h**). Granuloma areas are indicated with dotted lines. **Black arrowheads**, eggs; Hp, hepatocytes. Original magnification, ×200 (H&E) and ×40 (chromotrope 2R and aniline blue solution). **G:** Flow cytometry of IL-4Rα surface expression on liver granuloma-associated leukocyte populations. Cells were co-stained using mAbs against IL-4Rα and various cell surface markers on *Il4ra^{-/-lox}* (dotted line), *Il4ra^{-/-}* (greyscale), and *iLck^{cre}Il4ra^{-/-lox}* (top row, bold line) or *Lck^{cre}Il4ra^{-/-lox}* (bottom row, bold line) mice. T cells were defined as CD4⁺/CD3⁺ or CD4⁻/CD3⁺, B cells as CD19⁺/SSC^{low}, granulocytes as CD11b⁺/CD204⁻/FSC^{low}/SSC^{high} and macrophages as CD11b⁺/CD204⁺/MHC-II⁺. The geometric means are given for each histogram. Data are representative of two independent experiments (*n* = 4). ***P* < 0.01 and ****P* < 0.001 vs. *Il4ra^{-/-lox}* mice. ****P* < 0.001 *iLck^{cre}Il4ra^{-/-lox}* vs. *Lck^{cre}Il4ra^{-/-lox}* mice.

While IL-4 mediates multiple effects on T cells, murine T and B cells do not respond to IL-13.¹⁴ Generation of pan-T cell-specific IL-4Rα-deficient (*iLck^{cre}Il4ra^{-/-lox}*) mice therefore allows investigation into the role of IL-4 signaling specifically on CD4⁺ and non-CD4⁺ T cells in comparison with mice specifically disrupted for the IL-4Rα chain on CD4⁺ T cells, but maintaining IL-4/IL-13-mediated functions in non-CD4⁺ T cells (*Lck^{cre}Il4ra^{-/-lox}*). Both *iLck^{cre}Il4ra^{-/-lox}* and *Lck^{cre}Il4ra^{-/-lox}* mice were generated using the *Cre/loxP* recombination system in BALB/c embryonic stem cells. Previous studies have shown efficiency of cell-specific *Cre* mediated gene disruption may vary between 38% to 85% depending on recombinase efficiency and promoter activity.³⁰ Efficiency of IL-4Rα disruption was increased by using hemizygous *Il4ra^{-/-lox}* mice instead of *Il4ra^{lox/lox}* as mating partners for trans-

genic *Lck^{cre}* mice, thereby reducing the *loxP* substrate for *Cre*-recombinase by 50%. Flow cytometry analysis comparing the efficiency of disruption of IL-4Rα gene expression in *iLck^{cre}Il4ra^{-/-lox}* and *Lck^{cre}Il4ra^{-/-lox}* mice between non-T cells and T cell subpopulations demonstrated efficient impairment of the receptor in CD4⁺ T cells for both mice with complete deletion in CD8⁺, γδ, and natural killer T cells in *iLck^{cre}Il4ra^{-/-lox}* mice only, whereas *Lck^{cre}Il4ra^{-/-lox}* mice showed incomplete deletion in CD8⁺ and natural killer T cells and unaltered receptor expression in γδ-T cells as previously described.¹⁶ Non-T cells retained unaltered receptor expression in both cell-specific IL-4Rα-deficient mouse strains. These data demonstrate that the use of the improved promoter *iLck* to drive *Cre* recombination allows deletion of *loxP*-flanked genomic DNA sequences in all T cell subsets, in contrast

to *Lck^{cre}//4ra^{-lox}* mice retaining receptor expression, at least partially in non-CD4⁺ T cells. Functional analysis further confirmed effective and specific impairment of the IL-4 responsiveness of CD4⁺ T cells, while B cells and macrophages retained IL-4- and IL-13-mediated functions. Thus, *iLck^{cre}//4ra^{-lox}* mice are pan-T cell-specific IL-4R α knockout mice, whereas *Lck^{cre}//4ra^{-lox}* mice are CD4⁺ T cell-specific IL-4R α knockout mice, with all other cell types remaining responsive to IL-4/IL-13.

Absence of IL-4R α -mediated signaling in CD4⁺ T cells renders BALB/c mice resistant to *L. major* infection.¹⁶ Global *Il4ra^{-/-}* mice however still develop progressive chronic disease, suggesting the existence of IL-4R α -independent responses that drive susceptibility to infection.⁹ We therefore infected pan-T cell-specific IL-4R α deficient *iLck^{cre}//4ra^{-lox}* mice with *L. major* and compared the progression of the infection with *Lck^{cre}//4ra^{-lox}* mice. Following infection both mice developed similar kinetics of lesion development and resolution, controlled parasite invasion, and developed increased antigen-specific IFN- γ production, as well as similar delayed type hypersensitivity responses and type 1 antibody responses as those observed in C57BL/6 mice genetically resistant to *L. major*. In contrast, control *Il4ra^{-lox}* and *Il4ra^{-/-}* BALB/c mice developed progressive lesion swelling leading to necrosis during the acute and chronic phases of disease as expected. Together, our studies demonstrate that absence of IL-4R α -mediated signaling in non-CD4⁺ T cells do not interfere with resistance to *L. major* infection in absence of IL-4R α -responsive CD4⁺ T cells.

Impaired IL-4R α responsiveness by T cells resulted in higher mortality after *S. mansoni* infections, as compared with IL-4R α responsive *Il4ra^{-lox}* controls or CD4⁺ T cell-specific IL-4R α deficient *Lck^{cre}//4ra^{-lox}* mice. Increasing the infection dose in *iLck^{cre}//4ra^{-lox}* mice resulted in rapid mortality similarly to global *Il4ra^{-/-}* mice, or mice with impaired aaM ϕ (*LysM^{cre}//4ra^{-lox}*) (data not shown). In contrast, *Lck^{cre}//4ra^{-lox}* showed a trend to increased mortality at high dose of infection but survived similarly to *Il4ra^{-lox}* control mice. These results strongly suggest a role for IL-4 responsive non-CD4⁺ T cells in prolonging survival during schistosomiasis, at least in the presence of IL-4R α -mediated signaling in CD4⁺ T cells. In previous studies we demonstrated that global *Il4ra^{-/-}* and *LysM^{cre}//4ra^{-lox}* mice strains succumb after *S. mansoni* infection due to septic shock caused by severe intestinal inflammation and damage with subsequent bacterial invasion, due to the absence of aaM ϕ .⁵ Neither infected *Lck^{cre}//4ra^{-lox}* mice nor *iLck^{cre}//4ra^{-lox}* mice showed signs of augmented intestinal inflammation with integrity of the gut and absence of bacterial invasion during acute schistosomiasis.²¹ Though Th1 biased responses dominate in both *Lck^{cre}//4ra^{-lox}* and *iLck^{cre}//4ra^{-lox}* mice, control of gut inflammation could be explained as IL-4R α -responsive aaM ϕ remain present in the gut and able to control gut inflammation through regulatory functions.^{31–33}

Both mouse strains developed increased hepatic pathology, which is probably due to a shift toward Th1/type 1 responses with increased IFN- γ -mediated classical macrophage activation and toxic nitric oxide production

in liver granulomas.²¹ Also increased levels of transforming growth factor- β production were observed in the liver of *Lck^{cre}//4ra^{-lox}* mice and *iLck^{cre}//4ra^{-lox}* mice. This is in agreement with recent published results where transforming growth factor- β has been shown to play host-protective roles and decrease liver pathology.³⁴ Of importance, infected *iLck^{cre}//4ra^{-lox}* mice presented further and highly significant increased liver granuloma sizes compared with *Lck^{cre}//4ra^{-lox}* mice. These data indicate that possible enhanced liver damage may have caused increased mortality. Possible non-CD4⁺ IL-4R α responsive T cell candidates are CD8⁺ T cells, shown to down-regulate CD4 effector function in chronic *S. mansoni* infections, apparently independently of influencing hepatic granulomatous responses.^{35,36,37} Future studies, developing appropriate cell type-specific IL-4R α deficient mouse models and transfer studies may allow to further define the role of IL-4R α -responsive non-CD4⁺ T cells in schistosomiasis.

In conclusion, our studies demonstrate that IL-4R α -mediated signaling in non-CD4⁺ T cells prolongs survival to acute schistosomiasis and contributes to a better control of hepatic granulomatous inflammation induced by the parasite eggs.

Acknowledgments

For technical assistance we thank Mrs. Lizette Fick, Mrs. Wendy Green, Mrs. Zenaria Abbas, and Mr. Reagan Peterson.

References

1. Nelms K, Keegan AD, Zamorano J, Ryan JJ, Paul WE: The IL-4 receptor: signaling mechanisms and biologic functions. *Annu Rev Immunol* 1999, 17:701–738
2. Brombacher F: The role of interleukin-13 in infectious diseases and allergy. *Bioessays* 2000, 22:646–656
3. Anthony RM, Rutitzky LI, Urban JF, Jr., Stadecker MJ, Gause WC: Protective immune mechanisms in helminth infection. *Nat Rev Immunol* 2007, 7:975–987
4. Fallon PG, Richardson EJ, McKenzie GJ, McKenzie AN: Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: iL-13 is a profibrotic agent. *J Immunol* 2000, 164:2585–2591
5. Herbert DR, Holscher C, Mohrs M, Arendse B, Schwegmann A, Radwanska M, Leeto M, Kirsch R, Hall P, Mossman H, Claussen B, Forster I, Brombacher F: Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. *Immunity* 2004, 20:623–635
6. Hoffmann KF, Cheever AW, Wynn TA: IL-10 and the dangers of immune polarization: excessive type 1 and type 2 cytokine responses induce distinct forms of lethal immunopathology in murine schistosomiasis. *J Immunol* 2000, 164:6406–6416
7. Kopf M, Brombacher F, Kohler G, Kienzle G, Widmann KH, Lefrang K, Humborg C, Ledermann B, Solbach W: IL-4-deficient Balb/c mice resist infection with *Leishmania major*. *J Exp Med* 1996, 184:1127–1136
8. Noben-Trauth N, Kopf P, Muller I: Susceptibility to *Leishmania major* infection in interleukin-4-deficient mice. *Science* 1996, 271:987–990
9. Mohrs M, Ledermann B, Kohler G, Dorfmueller A, Gessner A, Brombacher F: Differences between IL-4- and IL-4 receptor alpha-deficient mice in chronic Leishmaniasis reveal a protective role for IL-13 receptor signaling. *J Immunol* 1999, 162:7302–7308
10. LaPorte SL, Juo ZS, Vaclavikova J, Colf LA, Qi X, Heller NM, Keegan

- AD, Garcia KC: Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. *Cell* 2008, 132:259–272
11. Sacks D, Noben-Trauth N: The immunology of susceptibility and resistance to *Leishmania major* in mice. *Nat Rev Immunol* 2002, 2:845–858
12. Locksley RM, Scott P: Helper T-cell subsets in mouse *Leishmaniasis*: induction, expansion and effector function. *Immunol Today* 1991, 12:A58–61
13. Reiner SL, Locksley RM: The regulation of immunity to *Leishmania major*. *Annu Rev Immunol* 1995, 13:151–177
14. Mohrs M, Holscher C, Brombacher F: Interleukin-4 receptor alpha-deficient BALB/c mice show an unimpaired T helper 2 polarization in response to *Leishmania major* infection. *Infect Immun* 2000, 68:1773–1780
15. Holscher C, Arendse B, Schwegmann A, Myburgh E, Brombacher F: Impairment of alternative macrophage activation delays cutaneous *Leishmaniasis* in nonhealing BALB/c mice. *J Immunol* 2006, 176:1115–1121
16. Radwanska M, Cutler AJ, Hoving JC, Magez S, Holscher C, Bohms A, Arendse B, Kirsch R, Hunig T, Alexander J, Kaye P, Brombacher F: Deletion of IL-4R alpha on CD4 T cells renders BALB/c mice resistant to *Leishmania major* infection. *PLoS Pathog* 2007, 3:e68
17. Pearce EJ, MacDonald AS: The immunobiology of schistosomiasis. *Nat Rev Immunol* 2002, 2:499–511
18. Wynn TA: Fibrotic disease and the T(H)1/T(H)2 paradigm. *Nat Rev Immunol* 2004, 4:583–594
19. Jankovic D, Kullberg MC, Noben-Trauth N, Caspar P, Ward JM, Cheever AW, Paul WE, Sher A: Schistosome-infected IL-4 receptor knockout (KO) mice, in contrast to IL-4 KO mice, fail to develop granulomatous pathology while maintaining the same lymphokine expression profile. *J Immunol* 1999, 163:337–342
20. Kaplan MH, Whitfield JR, Boros DL, Grusby MJ: Th2 cells are required for the *Schistosoma mansoni* egg-induced granulomatous response. *J Immunol* 1998, 160:1850–1856
21. Leeto M, Herbert DR, Marillier R, Schwegmann A, Fick L, Brombacher F: TH1-dominant granulomatous pathology does not inhibit fibrosis or cause lethality during murine schistosomiasis. *Am J Pathol* 2006, 169:1701–1712
22. Lee PP, Fitzpatrick DR, Beard C, Jessup HK, Lehar S, Makar KW, Perez-Melgosa M, Sweetser MT, Schlissel MS, Nguyen S, Cherry SR, Tsai JH, Tucker SM, Weaver WM, Kelso A, Jaenisch R, Wilson CB: A critical role for Dnmt1 and DNA methylation in T cell development, function, and survival. *Immunity* 2001, 15:763–774
23. Garvin AM, Abraham KM, Forbush KA, Farr AG, Davison BL, Perlmutter RM: Disruption of thymocyte development and lymphomagenesis induced by SV40 T-antigen. *Int Immunol* 1990, 2:173–180
24. Mattner F, Magram J, Ferrante J, Launois P, Di Padova K, Behin R, Gately MK, Louis JA, Alber G: Genetically resistant mice lacking interleukin-12 are susceptible to infection with *Leishmania major* and mount a polarized Th2 cell response. *Eur J Immunol* 1996, 26:1553–1559
25. Boros DL, Warren KS: Delayed hypersensitivity-type granuloma formation and dermal reaction induced and elicited by a soluble factor isolated from *Schistosoma mansoni* eggs. *J Exp Med* 1970, 132:488–507
26. Corraliza IM, Campo ML, Soler G, Modolell M: Determination of arginase activity in macrophages: a micromethod. *J Immunol Methods* 1994, 174:231–235
27. Gu H, Marth JD, Orban PC, Mossmann H, Rajewsky K: Deletion of a DNA polymerase beta gene segment in T cells using cell type-specific gene targeting. *Science* 1994, 265:103–106
28. Seder RA, Paul WE: Acquisition of lymphokine-producing phenotype by CD4+ T cells. *Annu Rev Immunol* 1994, 12:635–673
29. Shimoda K, van Deursen J, Sangster MY, Sarawar SR, Carson RT, Tripp RA, Chu C, Quelle FW, Nosaka T, Vignali DA, Doherty PC, Grosveld G, Paul WE, Ihle JN: Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. *Nature* 1996, 380:630–633
30. Rajewsky K, Gu H, Kuhn R, Betz UA, Muller W, Roes J, Schwenk F: Conditional gene targeting. *J Clin Invest* 1996, 98:600–603
31. Horsnell WG, Brombacher F: Alternatively activated macrophages in experimental parasitic infections. Edited by LI Terrazas. Kerala, Research Signpost, 2007, pp 1–19
32. Gallina G, Dolcetti L, Serafini P, De Santo C, Marigo I, Colombo MP, Basso G, Brombacher F, Borrello I, Zanovello P, Biccato S, Bronte V: Tumors induce a subset of inflammatory monocytes with immunosuppressive activity on CD8+ T cells. *J Clin Invest* 2006, 116:2777–2790
33. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, Eagle AR, Vats D, Brombacher F, Ferrante AW, Chawla A: Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. *Nature* 2007, 447:1116–1120
34. Herbert DR, Orekov T, Perkins C, Finkelman FD: IL-10 and TGF-beta redundantly protect against severe liver injury and mortality during acute schistosomiasis. *J Immunol* 2008, 181:7214–7220
35. Chensue SW, Warmington KS, Hershey SD, Terebuh PD, Othman M, Kunkel SL: Evolving T cell responses in murine schistosomiasis. Th2 cells mediate secondary granulomatous hypersensitivity and are regulated by CD8+ T cells in vivo. *J Immunol* 1993, 151:1391–1400
36. Ragheb S, Boros DL: Characterization of granuloma T lymphocyte function from *Schistosoma mansoni*-infected mice. *J Immunol* 1989, 142:3239–3246
37. Yap G, Cheever A, Caspar P, Jankovic D, Sher A: Unimpaired down-modulation of the hepatic granulomatous response in CD8 T-cell- and gamma interferon-deficient mice chronically infected with *Schistosoma mansoni*. *Infect Immun* 1997, 65:2583–2586