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Differential Regulation of IL-4R α Expression by Antigen versus Cytokine Stimulation Characterizes Th2 Progression In Vivo

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Abstract

IL-4 promotes Th2 differentiation and provides immunity to helminth infections but is also associated with allergy and asthma. This suggests that precise adjustment of IL-4 responsiveness is needed to correctly balance immune responses. The IL-4R α chain is an essential component of the IL-4 receptor and signals via STAT6. In this study, we show that infection with a helminth pathogen elicited broad upregulation of IL-4R α on bystander CD4⁺ T cells in the draining lymph node, while simultaneously resulting in the loss of IL-4R α expression on activated Th2 cells. IL-4R α upregulation was restricted to the reactive lymph node, occurred within 4 d of infection, and was driven by an IL-4- and STAT6-dependent mechanism. Mice heterozygous for *Stat6* exhibited reduced IL-4R α upregulation and a correspondingly attenuated Th2 response. Indeed, the enhanced IL-4R α upregulation in BALB/c mice, compared with that in C57BL/6 mice, predicted their stronger Th2 response. The selective downregulation of IL-4R α on highly activated Th cells was triggered by antigenic stimulation, was accompanied by loss of IL-7R α , and rendered the cells unresponsive to IL-4. Together these data reveal a tightly controlled program of changing IL-4 responsiveness that characterizes the initiation, amplification, and restriction of a Th2 response in vivo.

Helminth parasites elicit Th2 responses in their hosts, defined by cytokines such as IL-4, IL-5, and IL-13 and high levels of IgE and IgG1 (1–3). IL-4 has a particular importance in orchestrating Th2 responses. Of the canonical Th2 cytokines, IL-4 alone can promote the priming of Th2 cells in vitro (4,5), is critical for the Th2 cytokine response to helminth infection (6), and instructs B cells to class switch to IgG1 and IgE (7,8). Effective Th2 polarization contributes to host survival during helminth infection (9) and is required for lasting immunity against reinfection (10). However, Th2 differentiation also is associated with allergy and asthma (11), suggesting that control of IL-4 responsiveness is key.

IL-4 signals through either the type I IL-4 receptor, which comprises IL-4R α and the common γ (γ c) chain, or the type II IL-4 receptor in which IL-4R α is paired with IL-13R α 1 (12). In both cases, signal transduction is mediated by the phosphorylation of STAT6 (12–15). Expression of each of the IL-4 receptor subunits is restricted to specific cell populations: naive T lymphocytes possess only the type I receptor, whereas nonhematopoietic cells predominantly use only the type II receptor, and monocytes and macrophages are unusual in their expression of both (12). The precise expression pattern of

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Disclosures

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the IL-4 receptors determines the contrasting cytokine sensitivities of different cell types (16).

In vitro, IL-4 stimulation of naive CD4⁺ T cells results in rapid upregulation of surface IL-4R α (17), a process that involves increased transcription of the *Il4ra* gene and de novo protein synthesis (18,19). A recent study by Leonard and colleagues (20) reported that IL-4R α expression is enhanced by IL-2 signaling, thereby facilitating Th2 differentiation in vitro. However, the in vivo significance of these observations remains unclear.

In this study, we use both in vitro cocultures of naive, responder, and bystander CD4⁺ T cells and infection with a murine helminth parasite to examine the regulation of IL-4R α expression and IL-4 responsiveness during the development of the Th2 response. We show that IL-4R α is upregulated on nonactivated, bystander CD4⁺ T cells by cytokine stimulation but is profoundly downregulated on highly activated Th cells in response to Ag encounter. Thus, changes in IL-4R α expression and the different sensitivities to IL-4 that they confer define distinct stages of initiation, amplification, and termination during Th2 progression in vivo.

Materials and Methods

Mice

4get (C.129-*Il4^{tm1Lky}/J*) (21) and KN2 (22) IL-4 reporter mice and IL-4^{-/-} (KN2 homozygotes), IL-4R α ^{-/-} (23), STAT6^{-/-} (C.129S2-*Stat6^{tm1Gru}/J*) (24), TCR α ^{-/-} (25), and Yeti IFN- γ reporter mice (26) were on a BALB/c CD90.2 or CD90.1 genetic background. 4get mice on a C57BL/6 CD90.2 background were backcrossed for >10 generations. TCR transgenic mice specific for the *Salmonella typhimurium* flagellin₄₂₇₋₄₄₁ epitope (SM1) (27) were used on a C57BL/6 RAG2^{-/-}, CD90.1⁺ background; TCR transgenic mice specific for I-A^d-restricted epitopes of OVA (DO11.10) (28) and influenza virus hemagglutinin (HNT) (29) were used on BALB/c CD90.2⁺ and BALB/c CD90.1⁺ backgrounds, respectively. Animals were kept under specific pathogen-free conditions in filter-top cages at the animal facility of the Trudeau Institute and were used at 8–12 wk old. All of the experimental procedures involving mice were approved by the Institutional Animal Care and Use Committee.

Infections and immunizations

Mice were inoculated by gavage with 200 *Heligmosomoides polygyrus* infective third-stage larvae, as described (22). Two hundred *H. polygyrus* larvae were used for s.c. immunization. In *Toxoplasma gondii* infections, mice were given 10 cysts ME49 by gavage as described (30).

Flow cytometry and cell sorting

The following mAbs against mouse Ags were used as biotin, FITC, PE, allophycocyanin, Alexa Fluor 750, or Pacific Blue conjugates: CD4 (RM4-5), CD25 (7D4), CD44 (IM7), CD90.1 (CD90.1; OX-7), CD124 (IL-4R α , M1), CD127 (IL-7R α ; A7R34), and CD132 (IL-2R γ c; 4G3). Additional reagents included anti-human CD2 (RPA-2.10), streptavidin-PE, and streptavidin-allophycocyanin. Dead cells were discriminated by the addition of propidium iodide (0.5 μ g/ml; Sigma-Aldrich, St. Louis, MO) and excluded from the analyses. Surface staining with mAb was performed as described (22). Phosphorylation of STAT6 at tyrosine 641 (pY641) and total STAT6 were detected by intracellular staining with the Phosflow kit (BD Biosciences, San Jose, CA) according to the manufacturer's instructions. Samples were acquired on a FACSCalibur or Canto II flow cytometer (BD Biosciences) and analyzed using FlowJo (Tree Star, Ashland, OR) software. Cell sorting

was performed on a FACSVantage flow cytometer (BD Biosciences) equipped with DiVa electronics.

Cell cultures

For 24-h activation, naive (CD44^{lo}) CD4⁺ T cells were sorted from pooled lymph node cells from either DO11.10 or HNT TCR transgenic mice and cocultured (5×10^5 cells per milliliter) with irradiated APCs (5×10^6 cells per milliliter), using either TCR $\alpha\beta$ ^{-/-} splenocytes or IL-4^{-/-} splenocytes depleted of CD4⁺ and CD8⁺ cells by MACS. Recombinant IL-4 (0.5 ng/ml), OVA₃₂₃₋₃₃₉ peptide (2 μ M), and neutralizing Abs against IL-2 (S4B6, 20 μ g/ml) or IL-2R α (PC61, 20 μ g/ml) were included as indicated. For 8-d cultures, naive CD4⁺ T cells were likewise sorted from 4get mice and stimulated with plate-bound anti-CD3 (145-2C11, 10 μ g/ml) and anti-CD28 (37.51, 5 μ g/ml) in the presence of IL-2 alone (10 U/ml; “neutral” conditions); IL-2, IL-4, and anti-IFN γ (10 U/ml, 50 ng/ml, and 20 μ g/ml, clone XMG1.2; “Th2”); or IL-2, IL-12, and anti-IL-4 (10 U/ml, 5 ng/ml, and 20 μ g/ml, clone 11B11; “Th1”). Cells were recovered on day 3, rested in IL-2 alone, and restimulated with anti-CD3 on day 7. Flow cytometric analysis was performed 24 h later. To test the IL-4 responsiveness of CD4⁺ T cells, mesenteric lymph node (mesLN) cells from 14-d *H. polygyrus* infected 4get mice were first incubated with anti-IL-4 (11B11, 20 μ g/ml) for 1 h, washed extensively, and then cultured in the absence or presence of IL-4 (50 ng/ml) for 15 min. Subsequent flow cytometric analysis was gated on CD4⁺ T cells.

Quantitative RT-PCR

RNA was extracted from sorted cell populations using the RNAqueous-4PCR kit (Ambion, Austin, TX) and reverse transcribed with Superscript II RNase H (Invitrogen, Carlsbad, CA) with oligo(dT)₁₈ priming. Quantitative real-time RT-PCR with specific primers and probes (22) was performed using an ABI Prism 7700 Sequence BioDetector according to the manufacturer’s instructions (TaqMan; PE Applied Biosystems, Foster City, CA). Additionally, we designed primers and probes to discriminate the alternative splice variants of the IL-4R α mRNA (31,32):

membrane IL-4R (mIL-4R) forward, 5'-CTACTATACGGCGCGTGTGAT-3' (exon 7);

mIL-4R reverse, 5'-GCAGCTGGAAGTGGTTGTACCA-3' (exon 9);

mIL-4R probe, 5'-TCCCAGATACTCACTGGCACCTGGA-3';

soluble IL-4R (sIL-4R), forward 5'-CTACTATACGGCGCGTGTGAT-3' (exon 7);

sIL-4R reverse, 5'-CCGTGAGCTCTCCTCACC-3' (exon 8);

sIL-4R probe, 5'-TCCCAGATACTCACTGGCACCTGGA-3'.

Control experiments established a >95% efficiency of serial 10-fold dilutions, and no amplification was detected within 40 cycles with mRNA isolated from IL-4R α ^{-/-} lymphocytes. Cycle threshold values for GAPDH were routinely between 17 and 20 cycles.

Adoptive transfers

To assess the Ag specificity of IL-4R α upregulation, 1×10^7 lymphocytes from pooled spleen and lymph nodes of naive SM1 \times RAG2^{-/-} \times CD90.1⁺ TCR transgenic mice were transferred i.v. into naive or 14d *H. polygyrus* infected C57BL/6 CD90.2⁺ 4get mice, and recipient mesLNs were taken 18 h later. To assess the longevity of IL-4R α upregulation, 2×10^7 mesLN cells from 4get CD90.1⁺ mice infected with *H. polygyrus* 14 d earlier were transferred i.v. into naive or 14d *H. polygyrus* infected 4get CD90.2⁺ recipients. Twenty-four hours later, mesLN cells were harvested from the recipient mice and analyzed by flow cytometry.

Mixed bone marrow chimeras

To compare wild-type (WT) and gene-deficient cells in the same animal, 4get CD90.1⁺ cells were lethally irradiated (950 rad) and reconstituted with a total of 1×10^7 donor bone marrow (BM) cells from WT 4get CD90.1⁺ mice mixed in equal parts with either STAT6^{-/-} 4get CD90.2⁺ or IL-4^{-/-} CD90.2⁺ BM cells.

To generate chimeras with a defined population of TCR transgenic cells, C57BL/6 4get CD90.2⁺ mice were lethally irradiated and reconstituted with 10% SM1 \times RAG2^{-/-} \times CD90.1⁺-derived and 90% WT C57BL/6 4get CD90.2⁺ BM. Mice were allowed to reconstitute for 6–8 wk before infection.

Statistical analysis

The differences between two data sets were assessed using Student *t* test. Significant values are denoted by the following symbols: **p* \leq 0.05; ***p* \leq 0.01; ****p* \leq 0.001.

Results

Opposite effects of cytokine and antigenic stimulation on IL-4R α expression

To investigate changes in the surface expression of the IL-4R α chain during Th2 development, we first used a highly defined in vitro system. To mimic the presence of Ag-specific and nonspecific populations in a reactive lymph node, we sort purified naive (CD44^{lo}) CD4⁺ T cells from DO11.10 and HNT TCR transgenic mice, which have distinct Ag specificities, and cocultured them in the presence of irradiated APCs (Fig. 1A). In the absence of stimulation, DO11.10 (CD90.1⁻) and HNT (CD90.1⁺) T cells expressed identical levels of IL-4R α . As predicted (12,17), the addition of IL-4 resulted in the upregulation of IL-4R α on both populations within 24 h. When DO11.10 cells (CD90.1⁻) were selectively stimulated with OVA peptide, IL-4R α expression was substantially reduced on the Ag-specific population but increased on the bystander HNT cells (CD90.1⁺) present in the same culture. Reciprocal results were obtained when the HNT population was selectively stimulated with the hemagglutinin peptide: Ag-responsive cells lost receptor expression, whereas cocultured and nonspecific cells instead upregulated IL-4R α . Although only ~50% of the HNT donor CD4⁺ T cells reacted to the hemagglutinin peptide, as described (29), costaining confirmed that the loss of IL-4R α was restricted to activated, CD25⁺ cells, whereas the nonactivated, CD25⁻ cells upregulated IL-4R α like the defined bystander DO11.10 cells (Fig. 1B). Of note, the increase in IL-4R α expression on bystander cells elicited by either recombinant IL-4 or the activation of specific T cells occurred despite the absence of CD25, the high-affinity IL-2R α chain, on these cells (Fig. 1). Moreover, neither the addition of a neutralizing anti-IL-2 nor a blocking IL-2RmAb caused any inhibition of the upregulation of IL-4R α (data not shown), and activated DO11.10 cells downregulated IL-4R α despite robust CD25 expression (Fig. 1A). IL-2 signals therefore appear to be dispensable for the induction of IL-4R α on naive CD4⁺ T cells in vitro.

Together these data indicate that IL-4R α expression on CD4⁺ T cells can be differentially regulated by antigenic stimulation and IL-4 signals and that the primary activation of naive CD4⁺ T cells by their cognate Ag is sufficient to alter receptor expression on bystander CD4⁺ T cells.

Regulation of IL-4R α expression during helminth infection

To assess whether a similar modulation of IL-4R α also occurs during Th2 development in vivo, we infected WT and IL-4R α ^{-/-} 4get IL-4 reporter mice with the enteric helminth parasite *H. polygyrus* and analyzed CD4⁺ T cells in the draining mesLNs 2 wk later. In 4get mice, GFP fluorescence marks IL-4 expression and indicates prior Ag encounter (21,33).

Consistent with our *in vitro* observations (Fig. 1), IL-4R α expression was increased on the majority of CD4⁺ T cells in infected animals (Fig. 2A). These cells appeared to represent pathogen nonspecific bystanders, as denoted by their high frequency, their lack of GFP expression, and their predominantly CD44^{lo} CD25⁻ phenotype (Fig. 2B). A small subset of CD4⁺ T cells, with a GFP⁺ and CD44^{hi} phenotype suggestive of Ag experience, instead displayed a profound downregulation of the IL-4R α to a level equivalent to that of IL-4R α ^{-/-} cells (Fig. 2A, 2B). Expression of the γ c chain, which pairs with IL-4R α to form the type I IL-4R, was not changed in response to infection (data not shown and Fig. 6A).

To formally confirm that the enhanced IL-4R α expression seen in the bulk population of mesLN CD4⁺ T cells after *H. polygyrus* inoculation (Fig. 2A) was a feature of cells with TCR specificities irrelevant to the current infection, we transferred CD4⁺ T cells from SM1 TCR transgenic mice into naive or *H. polygyrus* infected C57BL/6 hosts and analyzed IL-4R α expression 18 h later (Fig. 2C). SM1 cells recognize the flagellin₄₂₇₋₄₄₁ epitope of *S. typhimurium* (27), a bacterium phylogenetically distant from the helminth parasite *H. polygyrus*. We used SM1 mice on a RAG2^{-/-} background to exclude the possibility of confounding endogenous TCR rearrangements. As shown in Fig. 2C, *Salmonella*-specific SM1 CD4⁺ T cells upregulated IL-4R α expression within 18 h to a level equivalent to that of the endogenous cells of the infected host.

Our *in vitro* data suggested that this heightened IL-4R α expression on bystander cells could be triggered as a consequence of antigenic stimulation of naive CD4⁺ T cells. To test whether the activation of Ag-specific T cells is also required for increased IL-4R expression on CD4⁺ T cells *in vivo*, we infected intact DO11.10TCR transgenic mice and WT controls with *H. polygyrus* and analyzed IL-4R α expression 2 wk later (Fig. 2D). In DO11.10 animals, all of the cells, including CD4⁺ T cells, are competent to make IL-4. However, their TCR specificity is restricted to the OVA₃₂₃₋₃₃₉ epitope, and consequently, very little or no Ag-mediated activation of CD4⁺ cells can occur during *H. polygyrus* infection. Basal IL-4R α expression was identical on CD4⁺ T cells from naive mice of both groups, but those of DO11.10 animals failed to increase IL-4R α expression upon *H. polygyrus* infection. This suggests that antigenic stimulation of CD4⁺ T cells is required for IL-4R upregulation *in vivo*.

IL-4R α upregulation is restricted to the draining lymph node

To determine whether the IL-4R α upregulation in *H. polygyrus* infected animals is driven by local signals or represents a systemic event, CD4⁺ T cells in the draining mesLNs and nondraining lymph nodes were analyzed in WT mice that were infected orally with *H. polygyrus* 2 wk earlier. In naive mice, CD4⁺ cells from mesLNs and peripheral lymph nodes displayed equivalent basal levels of IL-4R α . However, 2 wk after *H. polygyrus* infection, IL-4R α expression was higher in the mesLNs than in nondraining lymph nodes (Fig. 3A). Similarly, the s.c. injection of live *H. polygyrus* larvae, which are known to elicit local Th2 responses in the absence of patient infection [(34) and data not shown], into one footpad triggered IL-4R α upregulation in the draining popliteal lymph node but not in the contralateral popliteal lymph node, the mesLN, or any other nondraining lymph node (Fig. 3B and data not shown).

The presence of IL-4R α ^{hi} cells in only the draining lymph nodes suggested that IL-4R α expression is increased in response to local stimuli. To test this, we transferred IL-4R α ^{hi} CD4⁺ T cells from the mesLNs of 2-wk *H. polygyrus* infected mice into either naive or *H. polygyrus* infected congenic hosts. The IL-4R α expression on donor cells adjusted within 24 h to a level equivalent to that of CD4⁺ host T cells in the local environment: transferred cells recovered from the mesLNs of naive hosts had downregulated IL-4R α expression to basal

levels, whereas those isolated from infected hosts maintained an IL-4R α^{hi} phenotype (Fig. 3C).

Increased IL-4R α expression is dependent on IL-4 and direct STAT6 signaling

On the basis of our in vitro data (Fig. 1) and published observations (14,17,23), we hypothesized that the local signal driving the upregulation of IL-4R α in vivo is IL-4. To examine this, we infected WT, IL-4 $^{-/-}$, and STAT6 $^{-/-}$ mice with *H. polygyrus* and analyzed IL-4R α expression 2 wk later. Indeed, both IL-4 $^{-/-}$ and STAT6 $^{-/-}$ animals failed to increase IL-4R α expression on CD4 $^{+}$ cells upon infection (Fig. 4A).

To determine whether the upregulation of IL-4R α expression in vivo requires direct IL-4 and STAT6 signaling, we generated mixed BM chimeras by reconstituting lethally irradiated, WT recipients (CD90.1) with BM from WT mice (CD90.1) mixed 1:1 with either CD90.2 IL-4 $^{-/-}$ or CD90.2STAT6 $^{-/-}$ BM. This approach allowed us to compare the gene-deficient populations with WT control cells in the same animal. As shown in Fig. 4B, WT and STAT6 $^{-/-}$ or IL-4 $^{-/-}$ cells in naive BM chimeras expressed identical levels of IL-4R α . However, 2 wk after infection with *H. polygyrus*, STAT6 $^{-/-}$ cells failed to upregulate the IL-4R α , whereas IL-4 $^{-/-}$ cells increased expression equivalently with their WT counterparts. These data show that CD4 $^{+}$ T cell-intrinsic STAT6 signals are required for IL-4R α upregulation, whereas paracrine sources of IL-4 are sufficient.

Stat6 heterozygous animals have been reported to exhibit reduced IL-4 responsiveness compared with that of WT littermates (35). Because STAT6 is required for IL-4R α upregulation in *H. polygyrus* infected mice, we reasoned that the phenotype observed in *Stat6* heterozygous animals might be at least in part due to impaired IL-4R α upregulation. As shown in Fig. 4C, in *H. polygyrus* infected *Stat6* $^{+/-}$ animals, the haplosufficiency translated directly into decreased STAT6 protein abundance and, importantly, into reduced upregulation of IL-4R α surface expression on the bystander population of GFP $^{-}$ CD4 $^{+}$ cells in the mesLNs. Using *Stat6* $^{+/-}$ and *Stat6* $^{-/-}$ mice crossed onto the 4get IL-4 reporter background, we analyzed the magnitude of the Th2 response. Infection of *Stat6* heterozygous animals with *H. polygyrus* elicited a frequency of GFP $^{+}$ Th2 cells that was intermediate between those of WT and STAT6 $^{-/-}$ controls (Fig. 4C). The total number of GFP $^{+}$ cells followed the same trend but did not reach statistical significance (Fig. 4C). Together these data show that IL-4R α expression is increased on CD4 $^{+}$ T cells during helminth infection by an IL-4- and STAT6-dependent mechanism. Paracrine sources of IL-4 are sufficient, but cell-intrinsic STAT6 signals are indispensable.

Early IL-4R α expression predicts the size of the ensuing Th2 response

IL-4 drives both IL-4R α upregulation and Th2 differentiation, which suggests that the two effects might comprise a positive feedback amplification loop. To investigate this, we performed kinetic studies in WT 4get IL-4 reporter mice to identify the temporal relation between IL-4R α induction and Th2 differentiation during the onset of an *H. polygyrus* infection. Surface expression of IL-4R α on bystander, GFP $^{-}$ CD4 $^{+}$ T cells increased markedly between 3 and 4 d after infection, a time point coincident with the earliest indication of Th2 development (Fig. 5A). Indeed, the first IL-4 production by T cells, revealed by huCD2 expression in 4get/KN2 IL-4 dual-reporter mice (22), also was detected at this time (Fig. 5A). Together with previous data (Fig. 4), this suggested that IL-4R α upregulation occurs rapidly, initiated by early IL-4 expression.

To explore whether the extent of early IL-4R upregulation influences the magnitude of the subsequent Th2 response, we compared 4get IL-4 reporter mice on BALB/c and C57BL/6 genetic backgrounds. BALB/c mice characteristically show sustained and enhanced Th2

development, relative to that of C57BL/6 animals (36). Although basal IL-4R α expression was similar on mesLN CD4⁺ T cells from both strains, by 4 d after *H. polygyrus* infection, cells from BALB/c mice expressed markedly higher levels of IL-4R α than those from C57BL/6 animals (Fig. 5B). This was consistent with an enhanced, earliest detectable IL-4 expression in BALB/c versus C57BL/6 mice (data not shown). Importantly, subsequent Th2 expansion was correspondingly stronger in BALB/c mice than that in C57BL/6 mice (Fig. 5B).

Loss of IL-4R α expression occurs in highly activated Th2 cells

Although IL-4R α expression was generally increased on CD4⁺ cells in the mesLNs of *H. polygyrus* infected animals, GFP^{hi} cells downregulated IL-4R α to levels equivalent to those of IL-4R α ^{-/-} cells (Fig. 2). To analyze the loss of IL-4R α expression in relation to Th2 differentiation, we infected 4get IL-4 reporter mice with *H. polygyrus* and examined mesLN cells 14 d later. Downregulation of IL-4R was specific for the IL-4R α component of the type I IL-4R complex because expression of its partner subunit, the γ c chain, was not altered (Fig. 6A). Interestingly, the IL-7R α chain, which also depends on the γ c chain for signal transduction, was also markedly downregulated in GFP^{hi} cells (Fig. 6A).

We have previously shown that in 4get mice enhanced GFP fluorescence correlates with increased IL-4 transcript levels and the production of IL-4 protein in GFP^{hi} cells (22). To test whether the loss of IL-4R α expression was associated with IL-4 production in vivo, we analyzed CD4⁺ T cells in the mesLNs of *H. polygyrus* infected 4get/KN2 IL-4 dual-reporter mice (Fig. 6B). Indeed, although IL-4R α expression on GFP^{lo}huCD2⁻ cells was reduced compared with that on the GFP⁻ population, essentially all of the GFP^{hi}huCD2⁺ cells had lost IL-4R α expression [Fig. 6B and (22)].

The absence of surface IL-4R α on IL-4-producing Th2 cells could indicate either globally reduced expression of the full-length mL-4R α mRNA or alternative splicing to sIL-4R α RNA (31,32). To distinguish these possibilities, CD4⁺ T cells with GFP⁻huCD2⁻, GFP^{lo}huCD2⁻, and GFP^{hi}huCD2⁺ phenotypes were sorted from the mesLNs of *H. polygyrus* infected 4get/KN2 mice and analyzed by quantitative PCR for the expression of the respective IL-4R α mRNA species. The abundance of both IL-4R α mRNA species was substantially decreased in all of the GFP⁺ cells as compared with that of the GFP⁻ cells, and there was no apparent skewing toward the alternatively spliced sIL-4R α mRNA in either GFP⁺ population (Fig. 6C). The sort purity was internally confirmed by the direct correlation between the abundance of IL-4 transcripts and the intensity of GFP fluorescence (Fig. 6C) (22).

IL-4R α downregulation in vivo is induced by TCR engagement and renders the cell unresponsive to IL-4

The data above indicated that the loss of IL-4R α expression in GFP^{hi} CD4⁺ T cells is associated with highly polarized Th2 cells. From our in vitro data (Fig. 1) and because acute TCR activation results in increased IL-4 mRNA expression, the production of IL-4 (22), and a substantial downregulation of IL-7R α expression (37), we reasoned that antigenic stimulation could be responsible for the IL-4R α downregulation that occurs in vivo. To test this, we sought an experimental approach in which an identifiable population of CD4⁺ T cells is present throughout the course of a *H. polygyrus* infection but cannot receive TCR stimulation by *H. polygyrus* Ags. Because it is difficult to track adoptively transferred naive CD4⁺ T cells for extended periods of time, particularly in the presence of an ongoing, irrelevant immune response, we devised a BM chimeric approach. Lethally irradiated CD90.2 C57BL/6 4get mice were reconstituted with BM comprising 10% SM1 \times RAG2^{-/-} \times CD90.1 (27) and 90% WT cells. The TCR transgenic BM provided an internal source of

CD4⁺ T cells unable to recognize *H. polygyrus* derived Ags. Eight weeks after reconstitution, the CD4⁺ T cell compartment contained a stable population of 6–14% SM1 TCR transgenic cells that expressed IL-4R α levels identical to those of the polyclonal nontransgenic population. As expected from Fig. 3, *H. polygyrus* infection caused increased IL-4R α expression on both the polyclonal WT and the *Salmonella*-specific SM1 CD4⁺ T cells (Fig. 7A). However, unlike cells within the WT population, none of the SM1 cells displayed reduced IL-4R α expression, despite their presence throughout the 2-wk course of infection. These data show that Ag nonspecific cells do not downregulate IL-4R α expression despite their presence in the reactive lymph node, suggesting that TCR engagement is required for this process.

To assess the functional consequence of the loss of IL-4R α on activated Th2 cells, we tested their ability to phosphorylate STAT6 in response to IL-4 stimulation. Lymphocytes from mesLNs of *H. polygyrus* infected 4get or 4get \times IL-4R α ^{-/-} mice were cultured for 15 min in the absence or presence of IL-4, and CD4⁺ T cells were analyzed for the presence of phosphorylated STAT6 (Fig. 7B). In contrast to GFP⁻ and GFP^{lo} cells, the GFP^{hi} cells, which show pronounced IL-4R α downregulation (Fig. 6B), did not phosphorylate STAT6 in response to IL-4 stimulation; instead, they behaved equivalently to the IL-4R α ^{-/-} cells that served as negative controls. These findings were corroborated in similarly infected and analyzed 4get/KN2 dual-reporter mice in which IL-4-producing GFP^{hi} huCD2⁺ cells that have lost surface expression of IL-4R α [Fig. 6B and (22)] failed to phosphorylate STAT6 upon IL-4 stimulation (Fig. 7C). Importantly, the absence of STAT6 phosphorylation in GFP^{hi} cells was not due to the loss of STAT6 protein per se (Fig. 7D). These experiments illustrate that the selective downregulation of IL-4R α on IL-4-producing GFP^{hi} Th2 cells renders them unresponsive to IL-4.

To explore whether the loss of IL-4R α and IL-4 responsiveness is limited to activated Th2 cells or is elicited by TCR signals regardless of the Th subset, we first polarized naive, 4get CD4⁺ cells in vitro. The cultures were rested and restimulated by TCR ligation. All of the cells, whether polarized under Th2, Th0, or Th1 conditions, displayed pronounced downregulation of IL-4R α upon TCR stimulation (Fig. 7E). To confirm this observation in vivo, we analyzed IL-4R α expression on highly polarized Th1 cells induced by acute infection with the protozoan parasite *T. gondii*. Using both Yeti IFN- γ reporter (26) and 4get IL-4 reporter mice, we assessed IL-4R α expression on CD4⁺ T cells 7 d after *T. gondii* infection (Fig. 7F). Indeed, CD4⁺ T cells that expressed high levels of the yellow fluorescent protein (YFP) IFN- γ reporter selectively lost IL-4R α expression. As expected, *T. gondii* did not induce any expression of IL-4, illustrated by the absence of GFP in infected 4get mice, and consequently, IL-4R α expression was not increased on YFP⁻ or GFP⁻ bystander cells (Fig. 7F). These data suggest that TCR engagement triggers a reduction in IL-4R α expression on CD4⁺ T cells irrespective of their Th polarization.

Discussion

IL-4 plays a key role in governing Th2 development (4–6,8). IL-4 expression is tightly regulated (21,22,38,39), but its impact also depends on the ability of target cells to bind the cytokine and transmit IL-4 signals. In this study, we demonstrate that the Th2 response to a murine helminth pathogen involves a series of precise adjustments in IL-4R expression. Infection triggers both a rapid upregulation of the IL-4R α chain on the bystander majority of CD4⁺ T cells in the draining mesLN and a profound downregulation on the Ag-activated population. Increased IL-4R α expression is elicited by IL-4, and its loss occurs in response to TCR engagement. We propose that the different effects of cytokine and Ag on IL-4R expression result in a series of changes in IL-4 sensitivity that guide the initiation, expansion, and restriction of Th2 development.

Many studies have examined the sources and kinetics of IL-4 production during infection (21,22,38,40), but only recently has attention focused on the regulation of IL-4 responsiveness (16,41,42). There is precedence for the differential expression of cytokine receptors rather than cytokine production playing a decisive role *in vivo*. In acute lymphocytic choriomeningitis virus infection, the transition from an effector phenotype into a long-lasting memory CD8⁺ cell correlates with persistent IL-7R α expression (43); cells lacking a functional receptor unit are eliminated during immune contraction (44). In a Th2 context, myeloid cells determine their response to IL-3, IL-5, and GM-CSF, which signal through a common receptor β -chain, by selective expression of the cytokine-specific α -subunits (45). Indeed, it has recently been proposed that the contrasting regulatory and effector functions of IL-4 and IL-13, respectively, are the consequence of differential receptor expression on specific target cells, conferring distinct patterns of cytokine sensitivity (16).

In this study, we show that a single cell type, the CD4⁺ T lymphocyte, modulates its expression of the IL-4 receptor during Th2 differentiation *in vivo*. STAT6 signaling mediates IL-4 function, and although IL-4 expression can be STAT6-independent (39), STAT6^{-/-} cells, like IL-4R α ^{-/-} cells, do not respond to IL-4 stimulation (12–15,23,46). Targeted deletions of both *Il4ra* and *Stat6* have revealed gene dosage effects in heterozygous mice, indicating that a 2-fold alteration in IL-4R α /STAT6 signaling has functional significance: heterozygous *Il4ra* animals display an intermediate disease phenotype in situations of Th2-mediated pulmonary and pancreatic inflammation compared with those of WT and IL-4R α -deficient controls (47,48). Similarly, the reduced IL-4R signaling capacity of *Stat6* heterozygotes causes sufficiently impaired Th2 development such that, in contrast to WT littermates, these mice are as resistant to *Leishmania major* infection as STAT6^{-/-} animals (35).

Our observation that IL-4 alone is sufficient for the upregulation of IL-4R α is in contrast to a recent study that proposed a critical role for IL-2 signals in the same process *in vitro* (20). The discrepancy may reflect the different culture regimes employed in the two studies: we analyzed naive TCR transgenic CD4⁺ T cells within the first 24 h of peptide stimulation, whereas Liao et al. (20) examined cells that were preactivated with anti-CD3 and anti-CD28 for 2 d and then rested overnight prior to their exposure to IL-2. We and others have previously shown that naive CD4⁺ T cells undergo substantial cell division within the first 3 d of stimulation and are then compromised in the plasticity of their cytokine repertoire (49–52). Importantly, we extend our studies *in vivo* and show that in *H. polygyrus* infected mice, IL-4R α upregulation on CD4⁺ T cells is strictly dependent on direct IL-4 signals and occurs independently of CD25 expression, the high-affinity IL-2R (Figs. 2,4). In mixed BM chimeras, IL-4R α induction is selectively abrogated in STAT6^{-/-} CD4⁺ cells compared with that in WT equivalents in the same animal, despite the equal availability of IL-2 for both populations (Fig. 4). Nonetheless, IL-2 has been shown to support the Th2 response to helminth infections (53,54), and its influence may occur later in Th2 development, stabilizing the open *Il4* locus in previously activated cells (55) rather than enhancing IL-4R α expression early during Th differentiation.

The ability of IL-4 alone to elicit IL-4R α upregulation implies that the effect is not restricted to T cells of a particular Ag specificity. Indeed, with the exception of activated Th2 cells, all of the CD4⁺ T cells within the draining lymph node show an increase in IL-4R α expression during *H. polygyrus* infection (Figs. 2, 3). Heightened IL-4R α expression might be expected to predispose naive CD4⁺ T cells toward Th2 differentiation. Bottomly and colleagues (56,57) have reported a similar effect, termed “collateral priming,” in which IL-4 promotes Th2 development by conditioning both dendritic cells and T cells to circumvent the need for TLR signals to activate the APCs. In the context of helminth infection, during which the

parasite undergoes a series of developmental molts that likely result in a succession of distinct Ags, the ability of IL-4 to facilitate Th2 polarization in bystander cells could be beneficial, ensuring that Ag presentation occurring after the initiation of the Th2 response will also result in a supportive Th2 bias in newly activated T cells. Conversely, it may also have deleterious consequences: patients with allergies of a single specificity are more likely to become allergic to other, unrelated Ags than are healthy controls (58,59).

The loss of IL-4R α expression on acutely activated Th cells renders them unable to respond to IL-4 (Fig. 7). The absence of IL-4R α on activated Th cells is unusual among lymphocytes, because the IL-4R α chain is generally believed to be ubiquitously expressed on hematopoietic cells (12). IL-4 is a growth factor for T cells (60,61), and consequently the downregulation of IL-4R α may serve to limit the extent of a Th2 response by depriving activated effector cells from the survival signals provided by IL-4. Such a mechanism of immune regulation would mirror that of IL-7R α , which is downregulated on activated Th cells and thus disconnects them from essential survival signals, curtailing their response (62). This hypothesis would predict that a failure to extinguish IL-4R α expression would result in uncontrolled Th2 proliferation. Intriguingly, Tanaka and colleagues (41) recently demonstrated that Dock2^{-/-} mice, which show impaired downregulation of IL-4R α on CD4⁺ T cells, develop exaggerated Th2 responses and consequent allergic disease. The disease phenotype was prevented when Dock2^{-/-} mice were either treated with anti-CD4 or bred to the IL-4R α ^{-/-} background, which demonstrates a direct role for CD4⁺ T cells and IL-4R α (41). In the context of a Th1 response, the same consequence of IL-4R α downregulation on highly activated cells may not serve to curtail Th2 differentiation but instead to reinforce correct Th1 polarization.

Another hypothesis, not exclusive to that above, is that the loss of IL-4R α expression on acutely activated T cells enables them to provide effective help to B cells. In the lymph node, activated, IL-4-producing CD4⁺ T cells are restricted to B cell areas and display the phenotype of follicular Th helper cells (63–65). The dialogue between these cells and the B cells that they support is mediated in part by CD40–CD40L interaction, yet IL-4 signaling attenuates CD40L expression on activated CD4⁺ T cells (66). Downregulation of IL-4R α could allow IL-4-producing Th cells to maintain CD40L expression, thus promoting the B cell response. Moreover, because IL-4 has been proposed to be a limiting factor for B cell maturation (63), the absence of IL-4R α on IL-4-producing Th2 cells may also serve an altruistic purpose, preventing the consumption of IL-4 by follicular Th cells and thereby maximizing the local concentration available to B cells. A similar mechanism of regulating cytokine availability by modulating receptor expression on T cells has been suggested for IL-7 (67).

We have shown that, during helminth infection, IL-4 stimulation alone drives IL-4R α upregulation on the bulk population of CD4⁺ T cells in the lymph node and Ag encounter causes its profound downregulation on highly activated Th cells. These changes in IL-4R α expression, and the different sensitivities to IL-4 that they confer, characterize distinct stages of Th2 progression in vivo.

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Abbreviations used in this paper

γc	common γ
BM	bone marrow
HNT, TCR	transgenic mice specific for influenza virus hemagglutinin
mesLN	mesenteric lymph node
mIL-4R	membrane IL-4R
sIL-4R	soluble IL-4R
SM1	TCR transgenic mice specific for the <i>Salmonella typhimurium</i> flagellin ₄₂₇₋₄₄₁ epitope
YFP	yellow fluorescent protein
WT	wild-type

References

1. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J. Helminth infections: the great neglected tropical diseases. *J. Clin. Invest.* 2008; 118:1311–1321. [PubMed: 18382743]
2. Finkelman FD, Shea-Donohue T, Goldhill J, Sullivan CA, Morris SC, Madden KB, Gause WC, Urban JF Jr. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. *Annu. Rev. Immunol.* 1997; 15:505–533. [PubMed: 9143698]
3. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 1986; 136:2348–2357. [PubMed: 2419430]
4. Le Gros G, Ben-Sasson SZ, Seder R, Finkelman FD, Paul WE. Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing cells. *J. Exp. Med.* 1990; 172:921–929. [PubMed: 2117636]
5. Swain SL, Weinberg AD, English M, Huston G. IL-4 directs the development of Th2-like helper effectors. *J. Immunol.* 1990; 145:3796–3806. [PubMed: 2147202]
6. Kopf M, Le Gros G, Bachmann M, Lamers MC, Bluethmann H, Köhler G. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. *Nature.* 1993; 362:245–248. [PubMed: 8384701]
7. Snapper CM, Finkelman FD, Paul WE. Regulation of IgG1 and IgE production by interleukin 4. *Immunol. Rev.* 1988; 102:51–75. [PubMed: 3284816]
8. Kühn R, Rajewsky K, Müller W. Generation and analysis of interleukin-4 deficient mice. *Science.* 1991; 254:707–710. [PubMed: 1948049]
9. Brunet LR, Finkelman FD, Cheever AW, Kopf MA, Pearce EJ. IL-4 protects against TNF- α -mediated cachexia and death during acute schistosomiasis. *J. Immunol.* 1997; 159:777–785. [PubMed: 9218595]
10. Urban JF Jr, Katona IM, Paul WE, Finkelman FD. Interleukin 4 is important in protective immunity to a gastrointestinal nematode infection in mice. *Proc. Natl. Acad. Sci. USA.* 1991; 88:5513–5517. [PubMed: 2062833]
11. Taube C, Dakhama A, Gelfand EW. Insights into the pathogenesis of asthma utilizing murine models. *Int. Arch. Allergy Immunol.* 2004; 135:173–186. [PubMed: 15375327]
12. 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. [PubMed: 10358772]
13. Shimoda K, van Deursen J, Sangster MY, Sarawar SR, Carson RT, Tripp RA, Chu C, Quelle FW, Nosaka T, Vignali DA, et al. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. *Nature.* 1996; 380:630–633. [PubMed: 8602264]
14. Kaplan MH, Schindler U, Smiley ST, Grusby MJ. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. *Immunity.* 1996; 4:313–319. [PubMed: 8624821]

15. Takeda K, Tanaka T, Shi W, Matsumoto M, Minami M, Kashiwamura S, Nakanishi K, Yoshida N, Kishimoto T, Akira S. Essential role of Stat6 in IL-4 signalling. *Nature*. 1996; 380:627–630. [PubMed: 8602263]
16. Junttila IS, Mizukami K, Dickensheets H, Meier-Schellersheim M, Yamane H, Donnelly RP, Paul WE. Tuning sensitivity to IL-4 and IL-13: differential expression of IL-4R α , IL-13R α 1, and γ c regulates relative cytokine sensitivity. *J. Exp. Med.* 2008; 205:2595–2608. [PubMed: 18852293]
17. Ohara J, Paul WE. Up-regulation of interleukin 4/B-cell stimulatory factor 1 receptor expression. *Proc. Natl. Acad. Sci. USA*. 1988; 85:8221–8225. [PubMed: 3263648]
18. Dokter WH, Borger P, Hendriks D, van der Horst I, Halie MR, Vellenga E. Interleukin-4 (IL-4) receptor expression on human T cells is affected by different intracellular signaling pathways and by IL-4 at transcriptional and posttranscriptional level. *Blood*. 1992; 80:2721–2728. [PubMed: 1450403]
19. Renz H, Domenico J, Gelfand EW. IL-4-dependent up-regulation of IL-4 receptor expression in murine T and B cells. *J. Immunol.* 1991; 146:3049–3055. [PubMed: 2016538]
20. Liao W, Schones DE, Oh J, Cui Y, Cui K, Roh TY, Zhao K, Leonard WJ. Priming for T helper type 2 differentiation by interleukin 2-mediated induction of interleukin 4 receptor α -chain expression. *Nat. Immunol.* 2008; 9:1288–1296. [PubMed: 18820682]
21. Mohrs M, Shinkai K, Mohrs K, Locksley RM. Analysis of type 2 immunity in vivo with a bicistronic IL-4 reporter. *Immunity*. 2001; 15:303–311. [PubMed: 11520464]
22. Mohrs K, Wakil AE, Killeen N, Locksley RM, Mohrs M. A two-step process for cytokine production revealed by IL-4 dual-reporter mice. *Immunity*. 2005; 23:419–429. [PubMed: 16226507]
23. Mohrs M, Ledermann B, Köhler G, Dorfmueller A, Gessner A, Brombacher F. Differences between IL-4- and IL-4 receptor α -deficient mice in chronic leishmaniasis reveal a protective role for IL-13 receptor signaling. *J. Immunol.* 1999; 162:7302–7308. [PubMed: 10358179]
24. Kaplan MH, Wurster AL, Smiley ST, Grusby MJ. Stat6-dependent and -independent pathways for IL-4 production. *J. Immunol.* 1999; 163:6536–6540. [PubMed: 10586046]
25. Philpott KL, Viney JL, Kay G, Rastan S, Gardiner EM, Chae S, Hayday AC, Owen MJ. Lymphoid development in mice congenitally lacking T cell receptor $\alpha\beta$ -expressing cells. *Science*. 1992; 256:1448–1452. [PubMed: 1604321]
26. Stetson DB, Mohrs M, Reinhardt RL, Baron JL, Wang ZE, Gapin L, Kronenberg M, Locksley RM. Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function. *J. Exp. Med.* 2003; 198:1069–1076. [PubMed: 14530376]
27. McSorley SJ, Asch S, Costalonga M, Reinhardt RL, Jenkins MK. Tracking *Salmonella*-specific CD4 T cells in vivo reveals a local mucosal response to a disseminated infection. *Immunity*. 2002; 16:365–377. [PubMed: 11911822]
28. Murphy KM, Heimberger AB, Loh DY. Induction by antigen of intrathymic apoptosis of CD4⁺CD8⁺TCR^{lo} thymocytes in vivo. *Science*. 1990; 250:1720–1723. [PubMed: 2125367]
29. Scott B, Liblau R, Degermann S, Marconi LA, Ogata L, Caton AJ, McDevitt HO, Lo D. A role for non-MHC genetic polymorphism in susceptibility to spontaneous autoimmunity. *Immunity*. 1994; 1:73–83. [PubMed: 7889402]
30. Johnson LL, Berggren KN, Szaba FM, Chen W, Smiley ST. Fibrin-mediated protection against infection-stimulated immunopathology. *J. Exp. Med.* 2003; 197:801–806. [PubMed: 12629066]
31. Blum H, Wolf M, Enssle K, Röllinghoff M, Gessner A. Two distinct stimulus-dependent pathways lead to production of soluble murine interleukin-4 receptor. *J. Immunol.* 1996; 157:1846–1853. [PubMed: 8757301]
32. Mosley B, Beckmann MP, March CJ, Idzerda RL, Gimpel SD, VandenBos T, Friend D, Alpert A, Anderson D, Jackson J, et al. The murine interleukin-4 receptor: molecular cloning and characterization of secreted and membrane bound forms. *Cell*. 1989; 59:335–348. [PubMed: 2805066]
33. Mohrs K, Harris DP, Lund FE, Mohrs M. Systemic dissemination and persistence of Th2 and type 2 cells in response to infection with a strictly enteric nematode parasite. *J. Immunol.* 2005; 175:5306–5313. [PubMed: 16210636]

34. Ekkens MJ, Liu Z, Liu Q, Whitmire J, Xiao S, Foster A, Pesce J, VanNov J, Sharpe AH, Urban JF, Gause WC. The role of OX40 ligand interactions in the development of the Th2 response to the gastrointestinal nematode parasite *Heligmosomoides polygyrus*. *J. Immunol.* 2003; 170:384–393. [PubMed: 12496423]
35. Burgis S, Gessner A. Unexpected phenotype of STAT6 heterozygous mice implies distinct STAT6 dosage requirements for different IL-4 functions. *Int. Arch. Allergy Immunol.* 2007; 143:263–268. [PubMed: 17347574]
36. Sacks D, Noben-Trauth N. The immunology of susceptibility and resistance to *Leishmania major* in mice. *Nat. Rev. Immunol.* 2002; 2:845–858. [PubMed: 12415308]
37. Dooms H, Wolslegel K, Lin P, Abbas AK. Interleukin-2 enhances CD4⁺ T cell memory by promoting the generation of IL-7R α -expressing cells. *J. Exp. Med.* 2007; 204:547–557. [PubMed: 17312008]
38. Min B, Prout M, Hu-Li J, Zhu J, Jankovic D, Morgan ES, Urban JF Jr, Dvorak AM, Finkelman FD, LeGros G, et al. Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. *J. Exp. Med.* 2004; 200:507–517. [PubMed: 15314076]
39. Finkelman FD, Morris SC, Orekhova T, Mori M, Donaldson D, Reiner SL, Reilly NL, Schopf L, Urban JF Jr. Stat6 regulation of in vivo IL-4 responses. *J. Immunol.* 2000; 164:2303–2310. [PubMed: 10679064]
40. Finkelman FD, Morris SC. Development of an assay to measure in vivo cytokine production in the mouse. *Int. Immunol.* 1999; 11:1811–1818. [PubMed: 10545485]
41. Tanaka Y, Hamano S, Gotoh K, Murata Y, Kunisaki Y, Nishikimi A, Takii R, Kawaguchi M, Inayoshi A, Masuko S, et al. T helper type 2 differentiation and intracellular trafficking of the interleukin 4 receptor- α subunit controlled by the Rac activator Dock2. *Nat. Immunol.* 2007; 8:1067–1075. [PubMed: 17767160]
42. Maldonado RA, Soriano MA, Perdomo LC, Sigrist K, Irvine DJ, Decker T, Glimcher LH. Control of T helper cell differentiation through cytokine receptor inclusion in the immunological synapse. *J. Exp. Med.* 2009; 206:877–892. [PubMed: 19349465]
43. Kaech SM, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. *Nat. Immunol.* 2003; 4:1191–1198. [PubMed: 14625547]
44. Schluns KS, Kieper WC, Jameson SC, Lefrançois L. Interleukin-7 mediates the homeostasis of naïve and memory CD8 T cells in vivo. *Nat. Immunol.* 2000; 1:426–432. [PubMed: 11062503]
45. Geijsen N, Koenderman L, Coffey PJ. Specificity in cytokine signal transduction: lessons learned from the IL-3/IL-5/GM-CSF receptor family. *Cytokine Growth Factor Rev.* 2001; 12:19–25. [PubMed: 11312115]
46. Noben-Trauth N, Shultz LD, Brombacher F, Urban JF Jr, Gu H, Paul WE. An interleukin 4 (IL-4)-independent pathway for CD4⁺ T cell IL-4 production is revealed in IL-4 receptor-deficient mice. *Proc. Natl. Acad. Sci. USA.* 1997; 94:10838–10843. [PubMed: 9380721]
47. Müller U, Stenzel W, Köhler G, Polte T, Blessing M, Mann A, Piehler D, Brombacher F, Alber G. A gene-dosage effect for interleukin-4 receptor α -chain expression has an impact on Th2-mediated allergic inflammation during bronchopulmonary mycosis. *J. Infect. Dis.* 2008; 198:1714–1721. [PubMed: 18954266]
48. Radu DL, Noben-Trauth N, Hu-Li J, Paul WE, Bona CA. A targeted mutation in the IL-4R α gene protects mice against autoimmune diabetes. *Proc. Natl. Acad. Sci. USA.* 2000; 97:12700–12704. [PubMed: 11050183]
49. Mohrs M, Lacy DA, Locksley RM. Stat signals release activated naive Th cells from an anergic checkpoint. *J. Immunol.* 2003; 170:1870–1876. [PubMed: 12574353]
50. Grogan JL, Mohrs M, Harmon B, Lacy DA, Sedat JW, Locksley RM. Early transcription and silencing of cytokine genes underlie polarization of T helper cell subsets. *Immunity.* 2001; 14:205–215. [PubMed: 11290331]
51. Richter A, Löhning M, Radbruch A. Instruction for cytokine expression in T helper lymphocytes in relation to proliferation and cell cycle progression. *J. Exp. Med.* 1999; 190:1439–1450. [PubMed: 10562319]

52. Ansel KM, Lee DU, Rao A. An epigenetic view of helper T cell differentiation. *Nat. Immunol.* 2003; 4:616–623. [PubMed: 12830136]
53. Liu Z, Liu Q, Hamed H, Anthony RM, Foster A, Finkelman FD, Urban JF Jr, Gause WC. IL-2 and autocrine IL-4 drive the in vivo development of antigen-specific Th2 T cells elicited by nematode parasites. *J. Immunol.* 2005; 174:2242–2249. [PubMed: 15699158]
54. Wojciechowski W, Harris DP, Sprague F, Mousseau B, Makris M, Kusser K, Honjo T, Mohrs K, Mohrs M, Randall T, et al. Cytokine-producing effector B cells regulate type 2 immunity to *H. polygyrus*. *Immunity.* 2009; 30:421–433. [PubMed: 19249230]
55. Cote-Sierra J, Foucras G, Guo L, Chiodetti L, Young HA, Hu-Li J, Zhu J, Paul WE. Interleukin 2 plays a central role in Th2 differentiation. *Proc. Natl. Acad. Sci. USA.* 2004; 101:3880–3885. [PubMed: 15004274]
56. Eisenbarth SC, Zhadkevich A, Ranney P, Herrick CA, Bottomly K. IL-4-dependent Th2 collateral priming to inhaled antigens independent of Toll-like receptor 4 and myeloid differentiation factor 88. *J. Immunol.* 2004; 172:4527–4534. [PubMed: 15034070]
57. Dittrich AM, Chen HC, Xu L, Ranney P, Connolly S, Yarovinsky TO, Bottomly HK. A new mechanism for inhalational priming: IL-4 bypasses innate immune signals. *J. Immunol.* 2008; 181:7307–7315. [PubMed: 18981153]
58. Des Roches A, Paradis L, Menardo JL, Bouges S, Daurés JP, Bousquet J. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. VI. Specific immunotherapy prevents the onset of new sensitizations in children. *J. Allergy Clin. Immunol.* 1997; 99:450–453. [PubMed: 9111487]
59. Pajno GB, Barberio G, De Luca F, Morabito L, Parmiani S. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. *Clin. Exp. Allergy.* 2001; 31:1392–1397. [PubMed: 11591189]
60. Brown M, Hu-Li J, Paul WE. IL-4/Bcell stimulatory factor 1 stimulates T cell growth by an IL-2-independent mechanism. *J. Immunol.* 1988; 141:504–511. [PubMed: 3133410]
61. Fernandez-Botran R, Sanders VM, Oliver KG, Chen YW, Krammer PH, Uhr JW, Vitetta ES. Interleukin 4 mediates autocrine growth of helper T cells after antigenic stimulation. *Proc. Natl. Acad. Sci. USA.* 1986; 83:9689–9693. [PubMed: 2948185]
62. Li J, Huston G, Swain SL. IL-7 promotes the transition of CD4 effectors to persistent memory cells. *J. Exp. Med.* 2003; 198:1807–1815. [PubMed: 14676295]
63. Reinhardt RL, Liang HE, Locksley RM. Cytokine-secreting follicular T cells shape the antibody repertoire. *Nat. Immunol.* 2009; 10:385–393. [PubMed: 19252490]
64. King IL, Mohrs M. IL-4-producing CD4⁺ T cells in reactive lymph nodes during helminth infection are T follicular helper cells. *J Exp Med.* 2009; 206:1001–1007. [PubMed: 19380638]
65. Zaretsky AG, Taylor JJ, King IL, Marshall FA, Mohrs M, Pearce EJ. T follicular helper cells differentiate from Th2 cells in response to helminth antigens. *J Exp Med.* 2009; 206:991–999. [PubMed: 19380637]
66. Lee BO, Haynes L, Eaton SM, Swain SL, Randall TD. The biological outcome of CD40 signaling is dependent on the duration of CD40 ligand expression: reciprocal regulation by interleukin (IL)-4 and IL-12. *J. Exp. Med.* 2002; 196:693–704. [PubMed: 12208883]
67. Mazzucchelli R, Durum SK. Interleukin-7 receptor expression: intelligent design. *Nat. Rev. Immunol.* 2007; 7:144–154. [PubMed: 17259970]

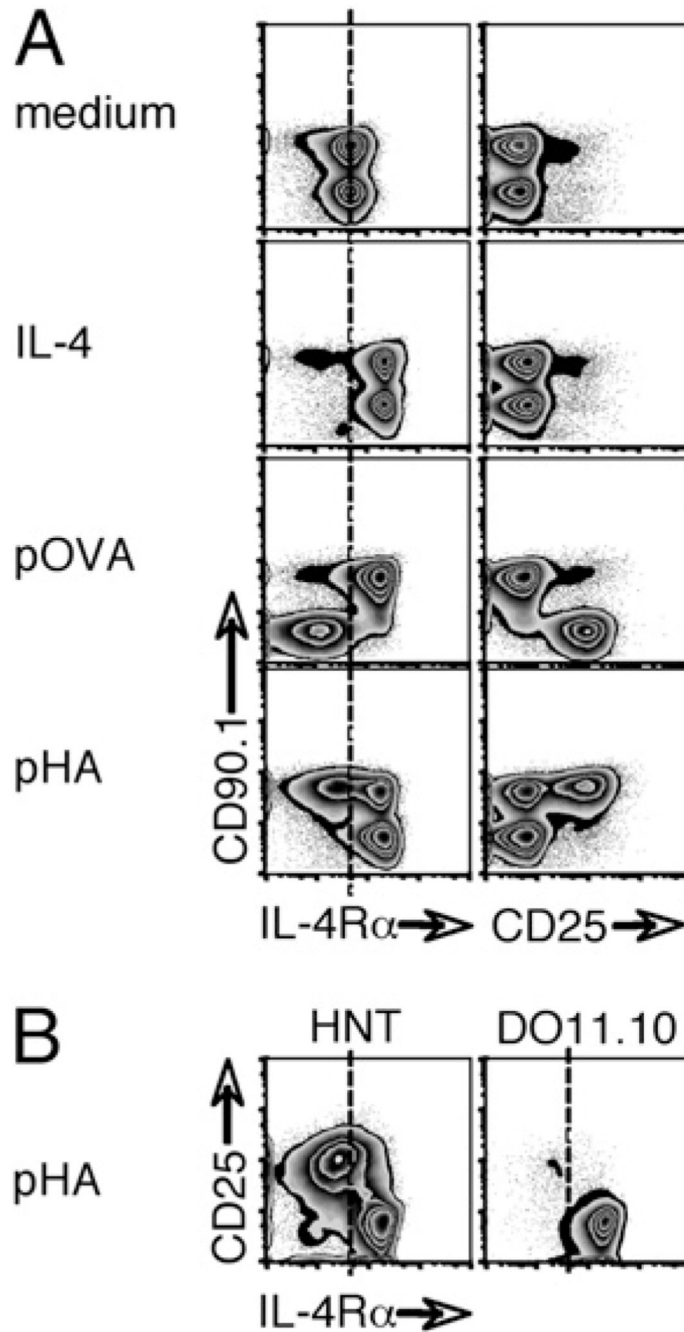
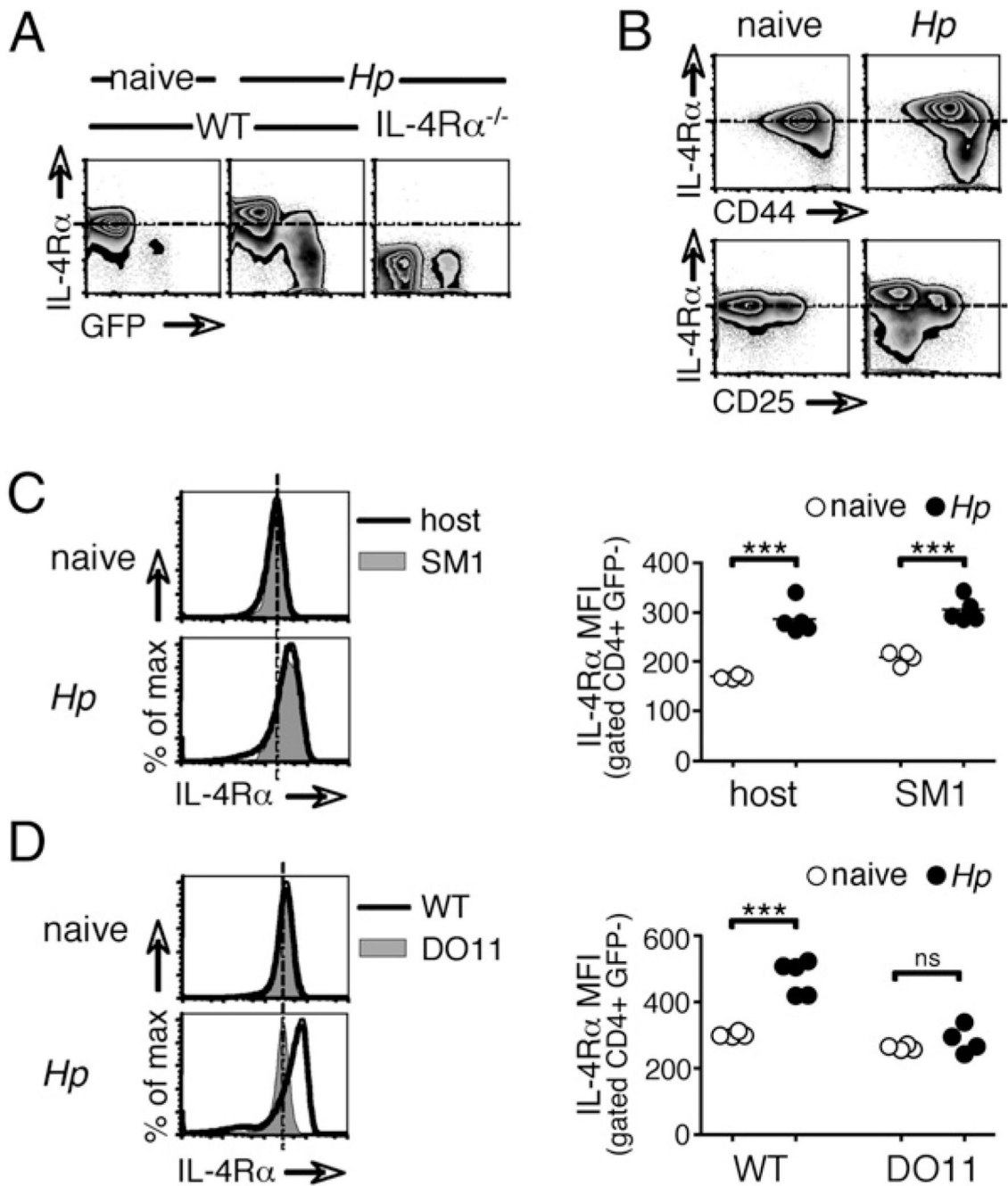


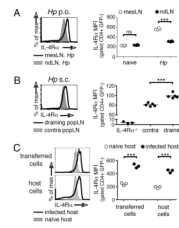
FIGURE 1.

IL-4R α expression is oppositely influenced by cytokine-mediated and antigenic stimulation. *A*, CD4⁺ CD44^{lo} cells sorted from naive DO11.10 animals (CD90.1⁻, specific for the OVA peptide 323–339 [pOVA]) were mixed 1:1 with equivalent cells from HNT mice (CD90.1⁺, specific for the hemagglutinin peptide 126–138 [pHA]) and cultured in the presence of irradiated APCs. Medium was supplemented with recombinant IL-4 or peptide Ag as indicated. Cells were recovered 24 h later and analyzed by flow cytometry for expression of IL-4R α and CD25. *B*, Identical cocultures were established, and pHA-stimulated cells were analyzed for dual expression of IL-4R α and CD25, gating on each transgenic population as shown. In both cases, data are representative of five independent experiments.

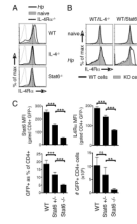
**FIGURE 2.**

Modulation of IL-4R α expression upon infection with a helminth parasite. *A*, WT and IL-4R α ^{-/-} get reporter mice, in which GFP fluorescence denotes IL-4 expression, were infected with *H. polygyrus* for 14 d. mesLN cells were analyzed by flow cytometry. Data shown are gated on CD4⁺ cells. *B*, WT mice were infected and analyzed as in *A*. *C*, CD4⁺ cells from SM1 TCR transgenic mice on a RAG^{-/-} background, which are specific for an irrelevant *Salmonella* Ag, were transferred into congenically distinct, WT recipients 13 d after *H. polygyrus* infection. Twenty-four hours later, mesLN CD4⁺ cells of donor and recipient origins were analyzed for their expression of IL-4R α by flow cytometry. *D*, WT BALB/c or DO11.10 TCR transgenic mice, in which T cells recognize only OVA were

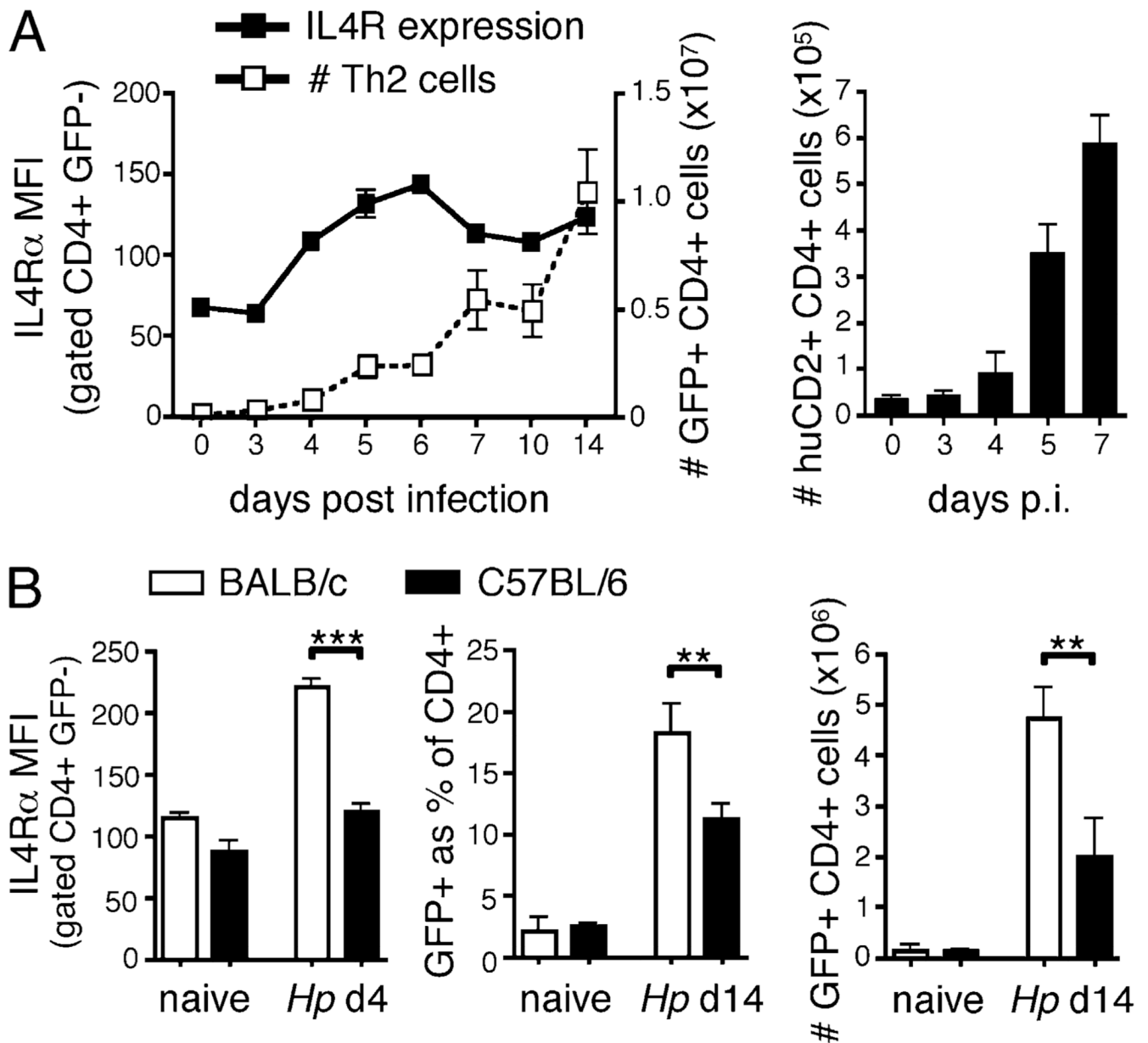
infected with *H. polygyrus*, and 14 d later, CD4⁺ cells from mesLNs were analyzed by flow cytometry. Data in all of the panels are representative of two or more independent experiments.

**FIGURE 3.**

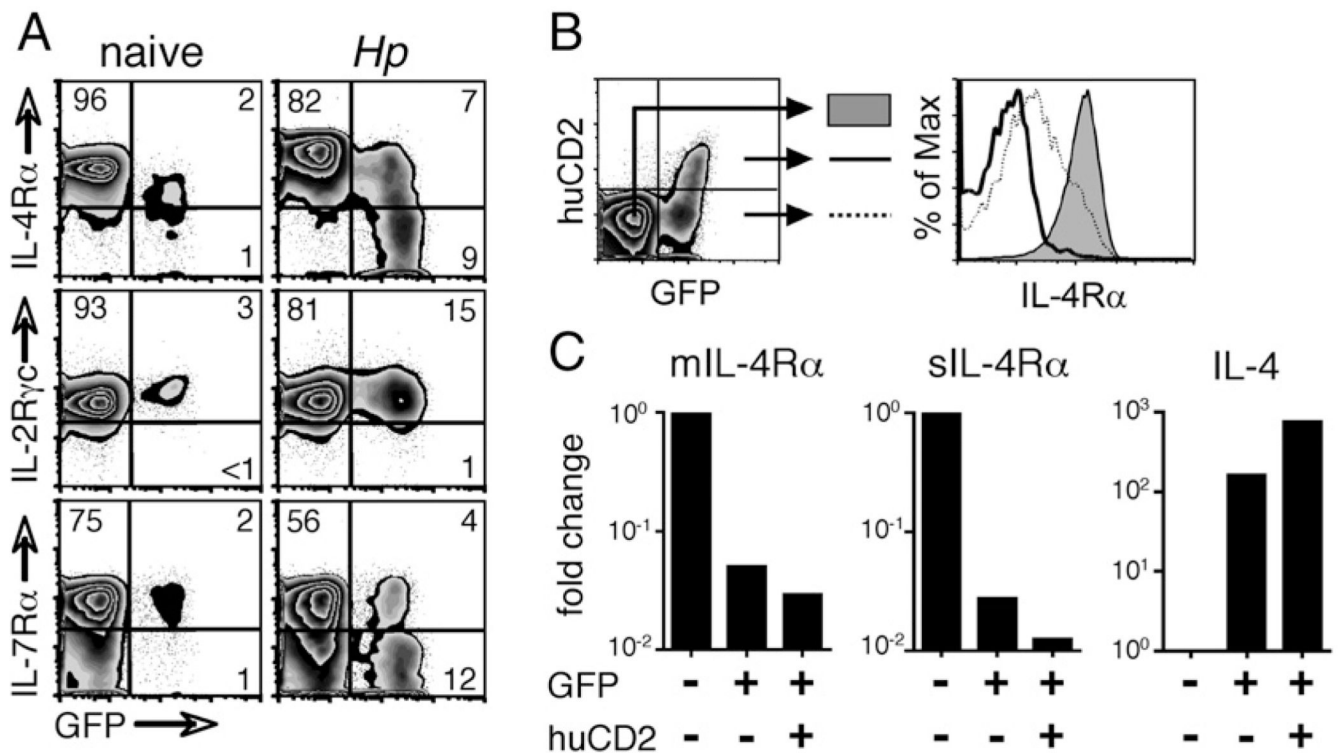
Increased expression of IL-4R α is determined by the local environment. *A*, IL-4R α expression on CD4⁺ cells from mesLNs or pooled nondraining lymph nodes (cervical, axial, brachial, inguinal, and popliteal) was compared 14 d after oral *H. polygyrus* infection and (*B*) from draining and contralateral popliteal lymph nodes 7 d after s.c. immunization in the footpad with *H. polygyrus* larvae. *C*, CD4⁺ IL-4R α ^{hi} cells were isolated from mesLNs 14 d after *H. polygyrus* infection and transferred into either 14 d infected or naive recipients. CD4⁺ cells in the recipient mesLNs were analyzed 24 h later by flow cytometry. Data are representative of three or more independent experiments.

**FIGURE 4.**

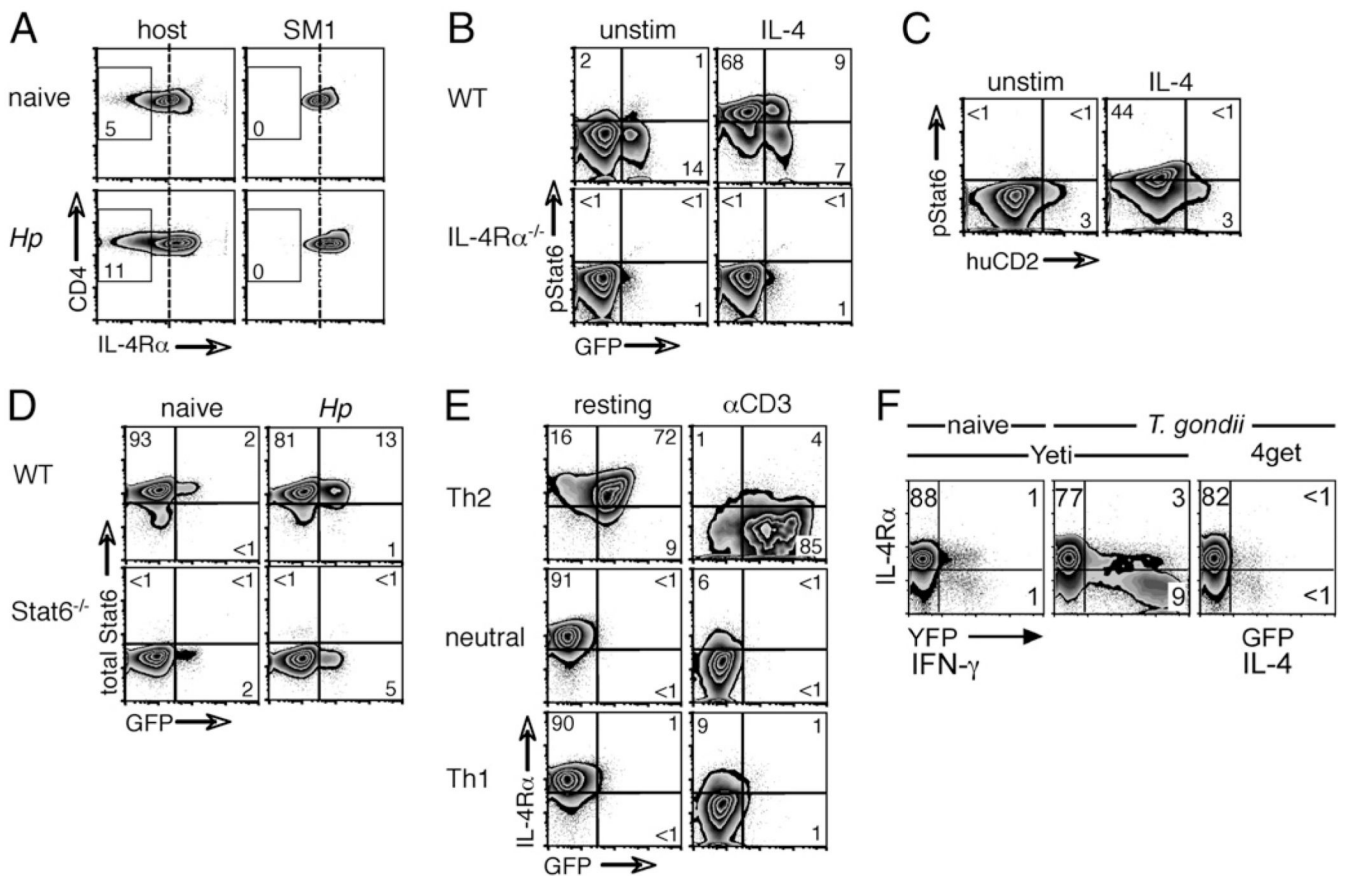
IL-4R α upregulation in vivo is driven by IL-4 and STAT6 signaling. *A*, WT, STAT6^{-/-}, and IL-4^{-/-} mice were infected with *H. polygyrus*, and CD4⁺ cells from mesLNs were analyzed 14 d later by flow cytometry. Filled histograms indicate the staining of cells from naive mice of each genotype; solid line, *H. polygyrus* day 14; dashed line, cells from infected IL-4R α ^{-/-} mice as a negative control. *B*, Mixed BM chimeras were generated by reconstituting CD90.1⁺ recipients with equal parts of CD90.1⁺ WT and either CD90.2⁺ IL-4^{-/-} or CD90.2⁺ STAT6^{-/-} BM. Reconstituted mice were infected with *H. polygyrus* and CD4⁺ cells from mesLNs analyzed 14 d later by flow cytometry. *C*, WT, STAT6^{+/-}, and STAT6^{-/-} 4get mice were infected with *H. polygyrus* and analyzed by flow cytometry 14 d later. The mean fluorescence of intracellular total Stat6 and surface IL-4R α staining on bystander CD4⁺ GFP⁻ cells from the mesLNs is shown, together with the total number of CD4⁺ GFP⁺ cells in the mesLNs. Error bars indicate the SD of five mice per group. Data in all of the panels are representative of two independent experiments.

**FIGURE 5.**

Early IL-4R α upregulation predicts subsequent Th2 differentiation. *A*, WT 4get or 4get/Kn2 dual-reporter mice, in which GFP and huCD2 indicate IL-4 transcription and translation, respectively, were infected with *H. polygyrus*, and their mesLN CD4⁺ cells analyzed by flow cytometry at the indicated time points. *B*, 4get mice were bred on a BALB/c or C57BL/6 background, infected with *H. polygyrus*, and their mesLN cells analyzed 4 and 14 d later. In both panels, IL-4R α expression is shown as the mean fluorescence of staining on CD4⁺ GFP⁻ cells. Error bars indicate the SD of 3–5 mice per group, and data are representative of two independent experiments.

**FIGURE 6.**

Highly activated Th2 cells lose IL-4Rα expression. **A**, WT 4get mice were infected with *H. polygyrus*, and 14 d later, CD4⁺ cells from mesLNs were analyzed for their expression of IL-4Rα, IL-2Rγc, and IL-7Rα. **B**, 4get/Kn2 dual-reporter mice were also infected, and on day 14, mesLN CD4⁺ cells were sorted into three populations as shown (GFP⁻ huCD2⁻, filled histogram; GFP⁺ huCD2⁻, dashed line; GFP⁺ huCD2⁺, solid line). IL-4Rα expression on each population was measured by flow cytometry. **C**, Populations sorted as in **B** were lysed, their RNA was purified and reverse-transcribed, and their expression of the membrane-associated and soluble alternative splice variants of the IL-4Rα transcript and of IL-4 was assessed by quantitative PCR. Data are shown as a fold change in expression relative to that of GFP⁻ huCD2⁻ cells. In all of the panels, data are representative of at least two independent experiments.

**FIGURE 7.**

IL-4R α downregulation is triggered by Ag encounter and terminates IL-4R signaling. **A**, Mixed BM chimeras were generated by reconstituting WT CD90.2⁺ C57BL/6 recipients with a 90:10 mix of WT CD90.2⁺ and SM1 TCR transgenic CD90.1⁺ BM. Chimeras were infected with *H. polygyrus*, and 14 d later, CD4⁺ cells from mesLNs were analyzed for IL-4R α expression by flow cytometry. Data shown are gated on CD4⁺ cells, and the numbers indicate the percentage of IL-4R α ^{lo} cells within each population. **B**, WT and IL-4R α ^{-/-} 4get mice were infected with *H. polygyrus*, and mesLN cells were isolated on day 14. Cells were first incubated with anti-IL-4 to remove endogenous STAT6 phosphorylation and then either incubated in medium alone or stimulated with IL-4 for 15 min. Resulting STAT6 phosphorylation was assessed by intracellular staining and flow cytometry. **C**, WT 4get/Kn2 dual-reporter mice were infected with *H. polygyrus*, and mesLN cells were harvested, stimulated, and stained as in **B**. **D**, WT and STAT6^{-/-} 4get mice were infected with *H. polygyrus*, and mesLN cells were isolated on day 14. Total STAT6 expression was assessed by intracellular staining and flow cytometry. **E**, Naive CD4⁺ cells from 4get mice were activated with α CD3 + α CD28 in Th2, neutral, or Th1 conditions. Cells were washed, rested, and on day 7 either cultured in medium alone or restimulated with α CD3. IL-4R α expression was measured 24 h later by flow cytometry. **F**, Yeti IFN- γ and 4get IL-4 reporter mice were infected with the Th1-inducing protozoan *T. gondii*, and 7 d later, CD4⁺ cells from mesLNs were analyzed for their expression of YFP or GFP and IL-4R α . Data in all of the panels are representative of at least two independent experiments.