IL-4 abrogates TH17 cell-mediated inflammation by selective silencing of IL-23 in antigen-presenting cells

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Interleukin 4 (IL-4) can suppress delayed-type hypersensitivity reactions (DTHRs), including organ-specific autoimmune diseases in mice and humans. Despite the broadly documented antiinflammatory effect of IL-4, the underlying mode of action remains incompletely understood, as IL-4 also promotes IL-12 production by dendritic cells (DCs) and IFN-γ–producing TH1 cells in vivo. Studying the impact of IL-4 on the polarization of human and mouse DCs, we found that IL-4 exerts opposing effects on the production of either IL-12 or IL-23. While promoting IL-12–producing capacity of DCs, IL-4 completely abrogates IL-23. Bone marrow chimeras proved that IL-4–mediated suppression of DTHRs relies on the signal transducer and activator of transcription 6 (STAT6)-dependent abrogation of IL-23 in antigen-presenting cells. Moreover, IL-4 therapy attenuated DTHRs by STAT6- and activating transcription factor 3 (ATF3)-dependent suppression of the IL-23/TH17 responses despite simultaneous enhancement of IL-12/TH1 responses. As IL-4 therapy also improves psoriasis in humans and suppresses IL-23/TH17 responses without blocking IL-12/TH1, selective IL-4–mediated IL-23/TH17 silencing is promising as treatment against harmful inflammation, while sparing the IL-12–dependent TH1 responses.

IL-4 | TH17 | IL-23 | psoriasis | dendritic cells

IL-4 is a canonical type 2 immune cytokine known for its capacity to induce IgE isotype switching in B cells and to initiate and sustain TH2 cell differentiation (1, 2). IL-4 provides protective immune responses to helminthes (3), and excessive endogenous IL-4 production is linked to TH2-dominated allergic asthma and atopic dermatitis (4) as well as to T-cell immunosuppression in leukemic cutaneous T-cell lymphoma (5). In vivo, IL-4 can suppress organ-specific autoimmune and delayed-type hypersensitivity reactions (DTHRs). Accordingly, IL-4 is absent in naturally occurring DTHRs, such as experimental autoimmune encephalomyelitis (EAE), multiple sclerosis (MS), rheumatoid arthritis (RA), inflammatory bowel disease (IBD), or psoriasis (6–10). Systemic IL-4 immunotherapy improves EAE (11), experimental colitis (12), nonobese diabetes (13), collagen-induced arthritis (14), and hapten-induced contact hypersensitivity (15) in mice and psoriasis in humans (16). The inhibitory effect of IL-4 on the autoimmune DTHRs, however, failed to be completely explained by the redirection of the TH1 immune responses toward IFN-γ–deficient type 2 immune responses. To the contrary, the number of peripheral IFN-γ+CD4+ TH1 cells or serum IFN-γ even increase after IL-4 administration in mice with EAE (11), hemophagocytic lymphohistiocytosis (17), or hapten-

induced contact hypersensitivity (15), and in humans with psoriasis (16); mice with transgenic overexpression of IL-4 exhibit TH2-driven allergic-like inflammatory disease with elevated IFN-γ levels (18), and IL-4 can even instruct protective TH1 immunity in mice with *Leishmania major* infection (19, 20). Thus, although IL-4 might be an important natural inhibitor of many DTHRs, the mode of action by which IL-4 suppresses inflammatory autoimmune disease and DTHRs remains enigmatic. Functional and genetic data now revealed that a significant number of DTHRs that have long been associated with IFN-γ–producing TH1 cells and IL-12p70–producing antigen-presenting cells (APCs), are mediated by IL-17/IL-22–producing TH17 cells and IL-23–producing APCs, rather than by TH1/IL-12 responses (21–23). Consistently, recent reports have correlated the level of disease activity and the absence of IL-4 with the presence of IL-23–producing APCs and IL-23–dependent TH17 cells (24, 25).

Significance

Interleukin 4 (IL-4) has been shown to be highly protective against delayed type hypersensitivity and organ-specific autoimmune and autoinflammatory reactions in mice and humans, but its mode of action has remained controversial and has failed to be explained solely by redirection of TH1/TH17 toward a TH2-type immune response. Here we uncovered that IL-4 selectively suppresses IL-23 transcription and secretion by cells of the innate immune system. We further describe a previously unidentified therapeutic mode of action of IL-4 in TH17-mediated inflammation, and a physiologically highly relevant approach to selectively target IL-23/TH17-dependent inflammation while sparing IL-12 and TH1 immune responses.

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We analyzed the impact of IL-4 on the regulation of IL-23 and T_H17 in DTHRs in mice and in human psoriasis. Unexpectedly, IL-4 abolished the capacity of APCs to produce IL-23, while promoting IL-12p70. This selective inhibition impaired the induction and maintenance of pathogenic T_H17 cells. Bone marrow chimeras with either signal transducer and activator of transcription 6 (STAT6)-deficient APCs or STAT6-deficient T cells proved that IL-4 suppressed T_H17 cells by abrogating IL-23 production in APC. IL-4 therapy of psoriasis in humans also dose-dependently suppressed IL-23 production by APCs and T_H17 cells, while preserving IL-12 and T_H1 immunity. This may open an entirely new approach for a targeted abrogation of harmful IL-23/T_H17 immune reactions without affecting potentially protective IL-12/T_H1 immunity against intracellular parasites (19) and perhaps cancer (26).

Results

Strictly Opposing Effects of IL-4 on Either IL-12 or IL-23 Secretion by Dendritic Cells. To dissect the pro- and antiinflammatory effects of IL-4 on dendritic cells (DCs), we stimulated, with toll-like receptor (TLR) ligands in the presence or absence of IL-4, four distinct DC populations: BDCA-1–expressing DCs (MDC1), BDCA-3–expressing DCs (MDC2), 6-sulfo-LacNAc–expressing DCs (slanDC), and murine bone-marrow derived DCs (mBMDC). IL-4 strongly and significantly induced IL-12p70 production in all four DC subsets, in human DCs 10- to 100-fold and in murine BMDCs about 3-fold (Fig. 1A). Surprisingly, IL-4 simultaneously and almost completely abrogated TLR-triggered IL-23 production in all human and mouse DC populations (Fig. 1B). High concentrations of IL-13, a cytokine that shares a common signaling pathway with IL-4, also suppressed IL-23 secretion in DCs (*SI Appendix*, Fig. S1), which was not the case for IL-5 (*SI Appendix*, Fig. S2A). The opposing effects of IL-4 on the production of either IL-12 or IL-23 were transcriptionally regulated. IL-4 significantly suppressed the TLR-driven induction of *il23a* mRNA ($P = 0.001$), while strongly inducing *il12a* mRNA expression ($P < 0.001$; Fig. 1C). IL-4 also suppressed TLR-induced expression of the common IL-12/23p40 (*il12b*) subunit in most APCs ($P < 0.003$; Fig. 1C). To determine whether IL-4 affects the dynamics of IL-12 or IL-23 induction rather than the total production, we performed time-course studies over 12 h in slanDCs. We observed that, following LPS stimulation, slanDCs started to produce IL-12 and IL-23 after 2–4 h, and IL-23 levels increased more than 100-fold during the first 12 h (Fig. 1D). IL-4 simultaneously suppressed IL-23 but enhanced IL-12p70 production over the entire study period, showing that IL-4 did not alter the dynamics of either IL-12 or IL-23 production. Moreover, the opposing effects of IL-4 were dose dependent and reached saturation at 100 ng IL-4/mL (Fig. 1E). IL-4 also reduced the secretion of other innate cytokines, such as IL-1β and IL-6 (*SI Appendix*, Fig. S2B).

IL-4 Selectively Abrogates the T_H17 Cell-Inducing Capacity of DCs. The hallmark of DC function is the ability to prime naïve T cells and steer T_H cell differentiation into either T_H1, T_H17, or T_H2 cells. To address whether IL-4 affects the capacity of DCs to drive proliferation of naïve T cells, we first used either immature or in vitro matured DCs to stimulate naïve autologous $CD4^+$ $CD45RA^+$ T cells. As expected, immature DCs were less efficient than mature DCs in inducing the proliferation of naïve $CD4^+$ T cells (Fig. 2A). IL-4 did not affect T-cell proliferation induced by either DC population (Fig. 2A). Based on our observation that IL-4 affected the cytokine pattern secreted by DCs, we tested whether IL-4 also affected their capacity to prime naïve T cells for either T_H1, T_H17, or T_H2 differentiation. For this test, we matured DCs in the presence or absence of IL-4, used them to prime naïve $CD4^+$ T cells, expanded the cells, and restimulated such primed $CD4^+$ T cells for cytokine production. Maturation of DCs in the absence of exogenous IL-4 resulted in a DC phenotype that induced both T_H1 and T_H17
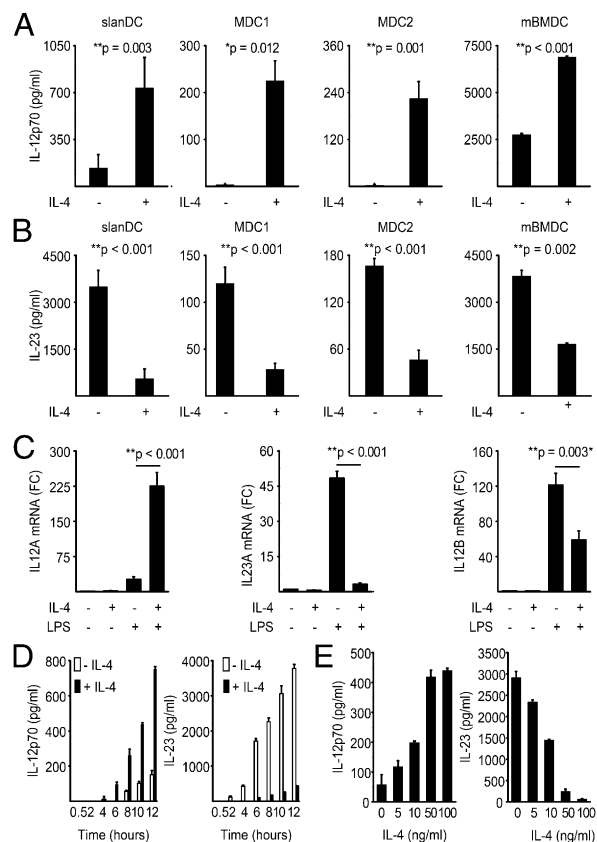

Fig. 1. Strictly opposing effects of IL-4 on either IL-12 or IL-23 secretion by DCs. (A and B) Different subsets of human myeloid DCs and mouse BMDCs were preincubated for 24 h with 100 ng/mL IL-4 and then stimulated with LPS. The IL-12 (A) and IL-23 (B) levels in the culture supernatants were determined by an ELISA. The data shown are from at least three independent experiments, and the results are expressed as the means ± SD. (C) The expression levels of transcripts encoding IL23A, IL12A, and IL12B were determined by quantitative real-time PCR in slanDCs treated as described in A. The values from three independent experiments were calculated relative to the expression levels of the housekeeping gene G6PD and were normalized to the unstimulated control. FC, fold change. (D and E) SlanDCs were treated as described in A, and IL-12p70 and IL-23 secretion was analyzed at the indicated time points (D) or as a function of IL-4 (E). The data are expressed as means ± SD of triplicates and are representative of five independent experiments.

cells, which produced large amounts of either IL-17 and IL-22 or IFN-γ (Fig. 2B), as previously reported (27, 28). Maturation of DCs in the presence of IL-4 (IL-4–DC) resulted in a DC phenotype that failed to induce T_H17 cells ($\leq 0.5\%$) (Fig. 2B); instead the percentage of IFN-γ–producing T_H1 cells increased (Fig. 2C). Moreover, IL-17$^+$ cells within fully differentiated IL-17 secreting cells (*SI Appendix*, Fig. S3) and total $CD4^+$ T cells (*SI Appendix*, Fig. S4) were significantly reduced upon coculture with IL-4–DC. This reduction could be restored by exogenous IL-23 but not by IL-1β and/or IL-6 (*SI Appendix*, Fig. S4).

IL-4–Induced Immune Suppression Strictly Depends on Direct Suppression of IL-23. To analyze the biological relevance of this IL-4–mediated suppression of IL-23 and its effect on the subsequent maintenance of T_H17 cells in vivo, we first studied IL-4–induced immune suppression in 2,4,6-trinitrochlorobenzene (TNCB)-induced DTHRs in C57BL/6 mice. Challenging sensitized mice with TNCB resulted in pronounced ear swelling and skin inflammation characterized by epidermal hyperplasia, subcorneal neutrophilic infiltrates, and angiogenesis (Fig. 3A). Systemic administration of IL-4 during the challenge significantly reduced the ear swelling in
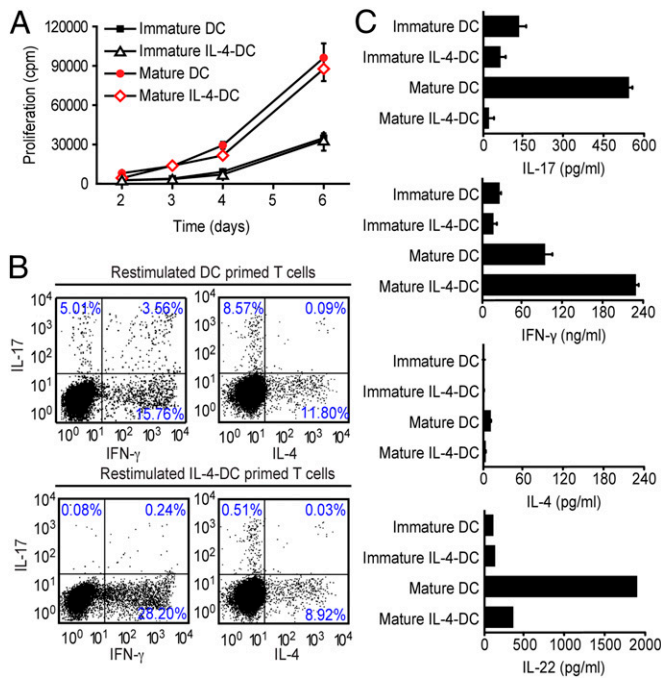
Fig. 2. IL-4 selectively abrogates the T$_H$17 cell-inducing capacity of DCs. (A) DCs stimulated with LPS in the presence or absence of 100 ng/mL IL-4 or control DCs were cocultured with autologous naïve T cells in the presence of staphylococcal enterotoxin B (SEB), and proliferation was determined by 3H-thymidine uptake. (B) DCs stimulated with LPS in the presence or absence of 100 ng/mL IL-4 and control DCs were cocultured with autologous naïve T cells over 12 d. Cytokine production by CD4$^+$ T cells was determined by flow cytometry following restimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin. (C) T cells cocultured with DCs stimulated as indicated in B were reactivated on day 12 with anti-CD3/CD28 for an additional 48 h, and cytokines were analyzed by ELISA. The results are expressed as means ± SD, and the data shown represent independent experiments from three different donors.

TNCB-sensitized mice (Fig. 3 B and C), abrogated the infiltration of polymorphonuclear (PMN) cells, and normalized skin morphology (Fig. 3C). The TNCB challenge caused a strong induction of il23a and il17a mRNA in ear tissues of mice challenged with TNCB (Fig. 3D), and IL-4 treatment during the TNCB challenge suppressed il23a and il17a mRNA (Fig. 3D). Upon TNCB challenge, IL-4 further induced il12a mRNA, whereas ifnγ mRNA was not significantly affected (SI Appendix, Fig. S5). To directly test whether IL-4–mediated suppression of IL-23 also suppressed inflammation, we treated sensitized mice with recombinant mouse (rm) IL-4 during the TNCB challenge. Subsequently, we aimed in half of the IL-4–treated mice to prevent suppression of inflammation via systemic administration of rmIL-23, rmIL-6, or PBS. Neither PBS nor rmIL-6 restored the rmIL-4–mediated suppression of IL-23 and DTHRs to almost background levels (SI Appendix, Fig. S6). In contrast, rmIL-23 fully rescued the cutaneous DTHRs, as determined by the ear swelling responses (Fig. 3 E and F).

IL-4–Responsive APCs Orchestrate IL-4–Induced Suppression of T$_H$17 Responses. Administration of IL-4 induces IL-4–producing T$_H$2 cells when acting directly on T cells (1) and restimulation of these Th2 cells may further provide IL-4 necessary to modulate DTHRs during the effector phase, possibly by affecting APCs (11, 15, 16). We next set up experiments to analyze the role of IL-4 during the effector phase of DTHRs and whether IL-4 suppresses T-cell–mediated inflammation not only by its action on CD4$^+$ T cells but also through the suppression of IL-23 production of APCs in vivo. Therefore, we first generated bone marrow chimeric (BMC)

mice with CD45.1$^+$ hematopoiesis on a CD45.2$^+$ background (CD45.1$^+$ → CD45.2$^+$ mice). Eight weeks after transplantation, engraftment efficiency and lineage reconstitution were >90%, and residual host CD45.2$^+$ BMC was <3% (SI Appendix, Figs. S7 and S8). The chimeric mice developed a typical DTHR showing that the BMC mice could be sensitized normally; in addition, rmIL-4 suppressed cutaneous DTHRs in such CD45.1$^+$ → CD45.2$^+$ mice as in previous experiments (Fig. 4A). To distinguish the effects of IL-4 on either APCs or T cells, we selectively blocked IL-4 signaling in either T cells or APCs of the BMC mice. This was achieved first by generating BMC mice deficient for STAT6 in the T-cell lineage (STAT6$^{-/-}$/Tc$^{-/-}$ → WT mice). In detail, by transplanting BM of STAT6$^{-/-}$ mice into lethally irradiated recipient mice, the T-cell repertoire of STAT6$^{-/-}$ mice was established in BMC mice. By cotransplantation of BM devoid of any T cells from Tc$^{-/-}$ mice into these BMC mice, those chimeric mice had normal IL-4 sensitive STAT6-expressing APCs from the Tc$^{-/-}$ donor organism, but only harbored STAT6^{neg} T cells unresponsive to IL-4 therapy from the STAT6$^{-/-}$ mice. A TNCB challenge in sensitized STAT6$^{-/-}$/Tc$^{-/-}$ → WT chimeric mice resulted in a similar ear swelling response compared with the control chimeric mice (CD45.1$^+$ → WT CD45.2$^+$ mice) (Fig. 4 A and B). Of note, IL-4 significantly reduced ear swelling and abrogated cutaneous inflammation in these sensitized STAT6$^{-/-}$/Tc$^{-/-}$ → WT chimeric mice (Fig. 4B), highlighting a key role for APCs in mediating the beneficial effect of IL-4–induced immune deviation in the effector phase of DTHRs. Next, to further study the role of APCs in this process, we generated BMC mice with a mixed STAT6$^{-/-}$/MHCII$^{-/-}$ hematopoiesis on a wild-type background (STAT6$^{-/-}$/MHC II$^{-/-}$ → WT mice), in which IL-4
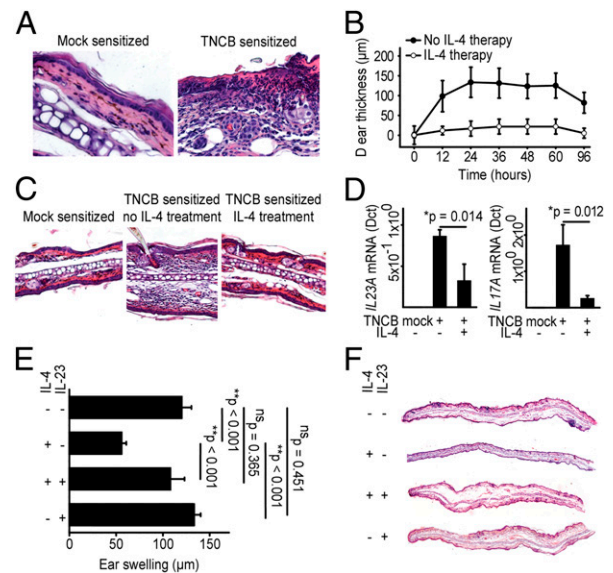

Fig. 3. IL-4–induced immune suppression strictly depends on direct suppression of IL-23. (A) Representative H&E stains (40×) from skin inflammation after challenge with TNCB in TNCB-sensitized C57BL/6 mice (B and C). Time course (B) of the ear swelling and representative H&E stains (20×) (C) after TNCB challenge in sensitized C57BL/6 mice, treated intraperitoneally with either PBS or IL-4 during challenge. The results in B are means ± SD. (D) Expression of transcripts encoding IL-23A and IL-17A in DTHR ear samples in mice treated with IL-4 as described in B. Quantitative real-time PCR was performed, and the data are expressed in relative units (Δct) compared with the housekeeping gene. The results are the means ± SD. (E and F) Ear swelling (E) and representative H&E stains (F) 24 h after challenge with TNCB in sensitized C57BL/6 mice. Mice were treated intraperitoneally with either PBS or IL-4 as in B. Additionally, IL-23 was applied to the groups where indicated. The data are expressed as means ± SD.
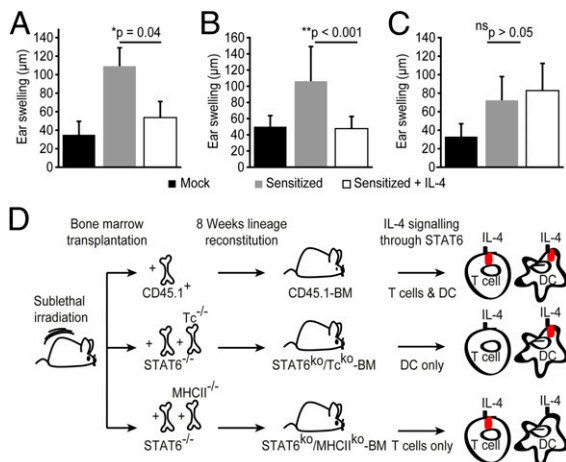
Fig. 4. IL-4 responsive APCs orchestrate IL-4–induced suppression of T_H17 responses. (A–C) Ear swelling after TNCB challenge in sensitized C57BL/6 mice bone marrow (BM) chimeric mice. IL-4 treatment during challenge was administered in some of the groups as indicated. Data from control CD45.1[+] BM chimeric mice on wild-type nonhematopoietic background are presented in A. Data from STAT6^{−/−}/Tc^{−/−} BM chimeric mice are presented in B, and data from STAT6^{−/−}/MHCII^{−/−} BM chimeric mice are presented in C. Data are expressed as mean ± SD and represent two independent experiments. At least six mice per group have been analyzed. (D) Schematic presentation of the experimental approach for the generation of bone marrow chimeric mice.

signaling was completely abrogated in functional MHCII[pos] APCs. TNCB challenge after sensitization of those chimeric mice resulted in increased ear swelling comparable to what we observed in the other two chimeric mouse models (Fig. 4 A–C). However, when we treated the STAT6^{−/−}/MHC II^{−/−} → WT mice with IL-4, cutaneous inflammation failed to improve, and the ear swelling was not reduced but remained comparable to that of STAT6^{−/−}/MHC II^{−/−} → WT mice not treated with IL-4 (Fig. 4C). This demonstrates the indispensable role for APCs in regulating the antiinflammatory therapeutic effect of IL-4 during DTHR elicitation. Fig. 4D presents schematically the experimental approach for the generation of the BMC mice. In vitro coculture assays of DCs and T cells from either WT or STAT6^{−/−} mice, provided additional evidence for the mode of action of IL-4 on DCs. Both WT and STAT6^{−/−} T cells secreted less IL-17 upon coculture with IL-4–exposed WT DCs, but not when cultured with STAT6^{−/−} DCs (*SI Appendix*, Fig. S9).

IL-4–Mediated Suppression of IL-23 Is Partly Mediated Through ATF3. ATF3 is a repressor of *il6*, but also *tnf* and *il23b* transcription in TLR4-stimulated macrophages (29, 30). ATF3 blocks *il23b* transcription by binding to repressive promotor elements near the genes coding for the *il23b* subunit in macrophages and possibly other APCs (29, 31). Because IL-4 significantly suppresses *il23b* transcription (Fig. 1C), and because both IL-23 and ATF3 could be modulated by cyclic adenosine monophosphate (32–34), we assessed whether the effects of IL-4 could be mediated through ATF3. Indeed, IL-4 markedly up-regulated ATF3 mRNA expression and protein production in murine and human DCs, and in murine RAW264.7 cells (Fig. 5 A–D). To determine the functional relevance of ATF3 on IL-23 production, we stimulated DCs from either WT or ATF3^{−/−} mice with LPS and assessed mRNA expression and production of IL-23. Even in the absence of IL-4, ATF3-deficient DCs produced higher amounts of IL-23 than WT DCs, and IL-4 suppressed transcription and production of IL-23 only in the ATF3-competent, but not in the ATF3-deficient DCs (Fig. 5E). Accordingly, IL-4 reduced the TNCB-mediated DTHR (Fig. 5 F and G) and *il17* mRNA (*SI Appendix*, Fig. S10) in WT mice but not in ATF3^{−/−} mice.

IL-4 Therapy of Psoriasis Abrogates Intralesional IL-23 and IL-17 in Human Skin.

IL-4 suppresses IL-23 production in mouse and human DCs and abrogates their capacity to induce/maintain T_H17 responses. Moreover, rmIL-4 suppresses DTHRs by suppressing IL-23 and downstream IL-17 during contact hypersensitivity in mice. We therefore asked whether this mode of immune suppression also translates to human autoimmune diseases, namely psoriasis, which is a disease strongly improved by IL-4 therapy or the mAb-mediated blockade of either IL-17 or IL-23 (35). To this end, we studied a unique population of patients with psoriasis who had successfully been treated with increasing doses of systemically administered IL-4. Consistent with recent data (36), *il23a* and *il17a* mRNA were both increased in psoriasis skin lesions (*SI Appendix*, Fig. S11A). Confocal laser scanning microscopy colocalized the abundant IL-23 protein with HLA-DR–expressing APCs, and the IL-17 protein with CD3^{+} T cells (*SI Appendix*, Figs. S11B and S12) in psoriasis plaques, but not in healthy skin (*SI Appendix*, Figs. S13 and S14). In addition to T_H1 and T_H17 cells, the psoriasis plaques contained numerous polymorphonuclear cells and shared many similarities with the TNCB-induced DTHR. Because IL-4 therapy improves psoriasis without suppressing IFN-γ–expressing T cells in the peripheral blood (16), we asked whether effective IL-4 therapy improved psoriasis by suppressing IL-23 and IL-17 in lesional skin. We examined the effect of IL-4 therapy on the expression and production of IL-17/IL-23 in a cohort of 22 patients with psoriasis (i.e., 19 patients, 3 drop-outs). The study was designed as a dose-escalation study, where patients were treated for psoriasis systemically with increasing doses of IL-4 over 6 wk. The therapy was initiated with either 0.05, 0.1, 0.2, 0.3, or 0.5 µg/kg of IL-4
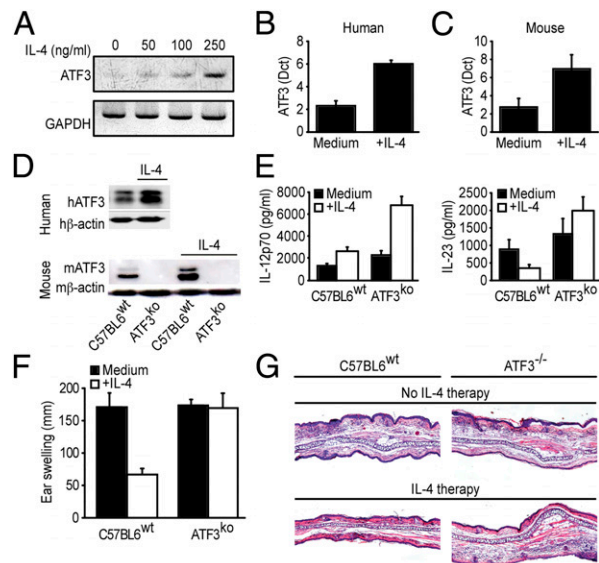

Fig. 5. IL-4–mediated suppression of IL-23 is partly mediated through activating transcription factor 3 (ATF3). (A) Representative data from a semiquantitative PCR for ATF3 mRNA expression in murine RAW264.7 cells, analyzed as a function of IL-4. (B and C) Quantification of ATF3 mRNA expression in LPS-stimulated human (B) and murine (C) DCs. Data are expressed in relative units (Δct) compared with the housekeeping gene. The results are the means ± SD. (D) Human DCs and BMDCs from either C57BL6 WT mice or ATF3^{−/−} mice were preincubated with IL-4 and then stimulated with LPS. ATF3 protein was analyzed semiquantitatively by Western blotting. Representative data from three independent experiments are shown. (E) BMDCs from either C57BL6 WT mice or ATF3^{−/−} mice were treated as in D. IL-12 and IL-23 levels in the culture supernatants were determined by ELISA. The data shown are from three independent experiments, and the results are expressed as the means ± SD. (F and G) Ear swelling (F) and representative H&E stains (G) 48 h after challenge with TNCB in sensitized C57BL/6 and ATF3^{−/−} mice. Mice were treated intraperitoneally with either PBS or IL-4 as in Fig. 3B. The data are expressed as means ± SD.

and increased to the next level after 3 wk, except in the last group (16). Systemic IL-4 therapy significantly improved psoriasis in a dose-dependent manner and normalized the skin morphology (16) (see also Fig. 6A). Cryopreserved tissue sections of these study patients revealed that untreated psoriasis plaques contained abundant IL-23 that colocalized with HLA-DR–expressing APCs and abundant IL-17 protein that colocalized with CD3+ T cells (Fig. 6B and *SI Appendix*, Fig. S15A). After 6 wk of IL-4 therapy, both IL-23 and IL-17 protein were almost undetectable (Fig. 6C and *SI Appendix*, Fig. S15B), suggesting that IL-4 therapy suppressed IL-23 and IL-17 production also in human skin. We further analyzed the tissue samples from the study patients for the expression of *il17a*, *atf3*, *il23a*, or *il12a* mRNA. The dose-escalation design of the study allowed us to correlate local mRNA changes for each of the three cytokines (i.e., *IL17a*, *IL23a*, and *IL12a*) and of the transcription factor *atf3* with the IL-4 treatment dose. IL-4 therapy suppressed *il23a* mRNA expression in a dose-dependent manner, with 20% suppression at 0.05 µg/kg IL-4 and almost 90% suppression at 0.5 µg/kg of IL-4 (Fig. 6D). Similarly, we detected a dose-dependent up-regulation of *atf3* expression in the analyzed tissue (Fig. 6E). Consistent with the *il23a* mRNA suppression, IL-4 therapy dose-dependently suppressed *il17a* mRNA expression (Fig. 6F). As predicted by the in vitro and animal data shown above, IL-4 therapy increased *il12a* mRNA expression in human skin during the 6 wk of IL-4 therapy (Fig. 6G). Finally, at low concentrations, IL-4 induced an IL23A/IL12A ratio of >1 (1.7 at 0.05 µg/kg IL-4), but at high IL-4 concentrations, IL-4 therapy induced a very low IL23A/IL12A ratio (0.05 at 0.5 µg/kg IL-4), a finding that could be important for the design of future IL-4 treatment regimens in humans (Fig. 6H).

Discussion

IL-4 reverts both T_H1 and T_H17 cell-mediated pathology and this effect is associated with the induction of IL-4–producing T_H2 cells in mice and humans (13–16, 37, 38). The underlying mechanism was attributed to inhibition and replacement of pathologic T_H1 and T_H17 cells and their respective cytokines by T_H2 cells and IL-4. However, this concept fails to completely explain the therapeutic effects observed, because IL-4 exerts opposing regulatory effects on T cells and DCs; IL-4 abrogates IFN-γ induction upon direct interaction with T cells (1). In contrast, IL-4 promotes IL-12 production by DCs, thus indirectly promoting IFN-γ in mice and humans (11, 19, 39–41). These effects are not exclusive; IL-4– and IL-4–producing T_H2 cells efficiently improve established T_H1/T_H17 mediated inflammation in mice and humans, while enhancing both IL-12 and IFN-γ (15, 16). These phenomena are highly suggestive of a regulatory mechanism whereby IL-4 selectively prevents T_H17 immunity, while sparing IL-12/T_H1 immunity.

We addressed this question by analyzing in detail the effect of IL-4 on the regulation of IL-23 and T_H17 cells. Starting with human DC subsets, we found that IL-4 had exactly opposing effects on IL-12 and IL-23. Whereas IL-4 induced IL-12, it abolished the induction of IL-23 and abrogated the capacity of DCs to maintain T_H17 but not T_H1 cells. Our observations are in agreement with reports suggesting different roles of IL-4 on DC-derived IL-12 and IL-23 (42–45), but go far beyond the former studies. We confirmed the biological relevance of this regulation in an in vivo experimental setting and demonstrated that IL-4 therapy could abrogate cutaneous inflammation in the elicitation phase of DTHRs. In extensive bone marrow reconstitution experiments, we elucidated the effects of IL-4 on the different immune cells and could demonstrate a previously unidentified in vivo mode of action of IL-4 on APCs. This is important because our data show for the first time to our knowledge that antigen-presenting innate immune cells are indispensable for the immunosuppressive effect of IL-4 therapy. Activation of APCs in an IL-4–deprived or IL-4–dominated inflammatory milieu dictated their capacity to orchestrate T_H17 induction, which supports previous data suggesting that the amount of IL-4 ultimately determines whether immune responses promote or attenuate inflammatory autoimmune diseases (19, 39, 46, 47).

Psoriasis is characterized by the absence of IL-4, and both T_H1 and T_H17 cells prevail in the skin (6). However, the exact roles of either T_H1 or T_H17 cells remain to be defined. Our data have identified a sequence of immunological events, triggered by IL-4 that selectively impaired the IL-23/IL-17 axis and relieved T_H17-mediated pathology, while promoting IL-12 and T_H1 cytokines. This is relevant because IL-23, but not IL-12, also mediates inflammation in the absence of T cells (10), at least under experimental conditions, and via intracutaneous injection induces a psoriasis-like skin disease in mice (48). It is well accepted that IL-4 can suppress T_H1 and T_H17 and promote T_H2 when directly acting on T cells (1, 49). Our data further showed that IL-4 also indirectly prevented the maintenance of IL-17–producing T_H17 cells by abrogating the expression and production of the T_H17 cell-associated cytokine IL-23 in APCs. Together with our data, the high degree of efficacy of the anti–IL-12/IL-23p40 monoclonal antibody in the treatment of psoriasis further emphasizes the crucial role for IL-23 in disease progression (50). These findings do not exclude a role for IL-12, T_H1 cells, or T_H1 cytokines in psoriasis but rather confirm that the therapeutic silencing of IL-23 (for example by IL-4 or newly engineered IL-4 superkines, currently under investigation) (51) is promising for psoriasis and other T_H17/IL-23–associated autoimmune diseases.
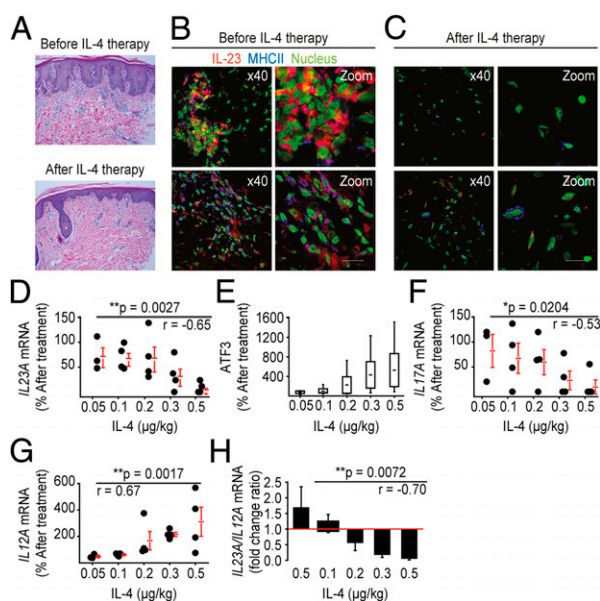

Fig. 6. IL-4 therapy of psoriasis abrogates intralesional IL-23 and IL-17 in human skin. (A) Representative H&E stains from colocalized biopsies of psoriatic skin before (A) and after systemic IL-4 treatment. (B and C) Visualization of colocalized IL-23 (red) and MHC II (blue) and IL-17 (red) and CD3 (blue) in human psoriatic skin lesions before (B) and after (C) IL-4 therapy. The nuclei are stained with green-fluorescent YO-PRO-1 stain. For colorblind-accessible images, please refer to *SI Appendix*, Fig. S9. (D–G) RT-PCR expression of transcripts encoding IL-23A, ATF3, IL-17A, and IL-12A in psoriatic skin samples before and after different doses of IL-4 therapy. The expression of the target gene within psoriasis tissue before treatment (relative to the housekeeping gene G6PD) was set to 100%, and the expression after treatment is presented as a percentage of this value. Each dot represents one pair of specimens from a single study patient; the horizontal bars indicate the means ± SEM *P < 0.05, **P < 0.01. (H) Ratio of IL23A (percent after treatment) to IL12A (percent after treatment) as detected by quantitative real-time PCR in the skin samples from the study patients treated with different doses of IL-4. The data are expressed as the means ± SEM *P < 0.05, **P < 0.01. r = Pearson correlation coefficient.

Materials and Methods

Institutional Review Board Approval. All human studies were approved by the ethics committees of the Medical School of Ludwig Maximilian University of Munich and of the Eberhard Karls University of Tübingen (110/2009B02; 116/ 2005; 383/2007B02; 418/2009B02), and written informed consent was obtained. The animal experiments were in compliance with both European Union and German law and were approved by local authorities (Regierungspräsidium Tübingen, HT5/10; HT1/13).

Reconstitution Experiments. $Tcrb^{-/-}Tcrd^{-/-}$ ($Tc^{-/-}$) mice, $STAT6^{-/-}$ mice, and $CD45.2^+ C57BL/6$ mice were purchased from The Jackson Laboratory. $MHCII^{-/-}$ mice were a gift from Ludger Klein (Institute of Immunology, Ludwig Maximillian University, Munich). Recipient mice were lethally irradiated at 7.0 Gy and bone marrow cells (10^6 cells per recipient) of donor mice were i.v. injected into recipient mice. Donor hematopoietic cells were either bone marrow cells from $CD45.1^+$ mice, a 1:1 mixture of bone marrow cells from $STAT6^{-/-}$ and $Tc^{-/-}$ mice, or a 1:1 mixture of $STAT6^{-/-}$ and $MHCII^{-/-}$ mice. To confirm the chimerism of mice, flow cytometry was made for analysis of $CD45.2^+$ (recipient mice) and $CD45.1^+$ (donor mice). TNCB sensitization experiments were performed 8 wk after irradiation.

A detailed description of all other experimental procedures and the statistical analysis is given in *SI Appendix*.

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