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Tissue damage in many human autoimmune diseases is mediated by activated autoantigen-specific Th1 cells. Delineation of the regulatory mechanisms controlling a Th1-biased human immune reaction and its pathologic potential is, therefore, a critical step in the understanding of autoimmune diseases. In this study, we introduce a novel means to investigate human Th1-biased immune reactions in vivo. Intraperitoneal injection of human mononuclear cells into immunodeficient mice generates a xenogeneic Th1-biased human immune response characterized by systemic inflammation and leukocytic infiltrates with a granuloma-like architecture in the liver, and the perigastrointestinal and perirenal fatty tissue. Th1 cell activation was dependent on the presence of APCs and could be blocked by cyclosporine. Importantly, neutralization of endogenously produced IL-4 and IL-10 markedly exaggerated the immune response, whereas exogenous IL-4 and IL-10 inhibited systemic Th1 immunity. Thus, the model described in this paper presents a useful means to analyze the regulation of human immune reactions in an in vivo situation. The results suggest that both IL-4 and IL-10 contribute to controlling the development of a human Th1-biased immune reaction. The Journal of Immunology, 2004, 172: 6427–6434.

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Generation and Regulation of Human Th1-Biased Immune Responses In Vivo: A Critical Role for IL-4 and IL-10¹

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A

utoimmune diseases are caused by failure of self-tolerance and subsequent immune responses against autologous Ags (1). Convincing evidence exists that self-tolerance is an active dynamic state in which potentially pathogenic autoreactive cells are prevented from causing disease by regulatory mechanisms (2). The breakdown of such mechanisms might, therefore, result in the development of pathologic autoimmune reactions. It has become apparent that the destructive effector mechanisms of many systemic autoimmune diseases are mediated by activated autoantigen-specific Th1 cells (2, 3). Therefore, the mechanisms controlling the evolution of Th1-biased immune responses play a critical role in the development of pathogenic autoimmune reactions. Delineation of the mechanisms controlling Th1-mediated immunity has largely been derived from animal models. For example, amelioration of autoimmune diabetes in nonobese diabetic (NOD) mice, a murine model of human insulin-dependent diabetes mellitus, is associated with increased expression of the Th2-derived cytokines IL-4 and IL-5 (4, 5). Moreover, pancreatic expression of IL-4 completely prevents diabetes in NOD mice (6). Injection of IL-4-transduced cells reduces the incidence and severity of collagen-induced arthritis, a model of human inflammatory arthritis (7), and of experimental autoimmune encephalomyelitis (EAE),³ a model of human multiple sclerosis (8). Furthermore, treatment with rIL-4 induces a switch from a Th1-type to a Th2-type response and prevents proteoglycan-induced arthritis, a different model of human inflammatory arthritis (9).

The anti-inflammatory role of another immunomodulatory cytokine, IL-10, has also been shown in animal models. IL-10-deficient mice are more susceptible to EAE when compared with wild-type mice (10). Diabetes induced by adoptively transferred lymphocytes into NOD mice can be prevented by IL-10-transduced islet-specific Th1 lymphocytes (11). Moreover, the effect of regulatory T cells in a transfer model of colitis can be abrogated by neutralizing Abs to TGF-β and IL-10, resulting in the emergence of tissue pathology (12, 13). Although the pathways are complex, there is convincing evidence that anti-inflammatory cytokines play essential roles in regulating the development and perpetuation of chronic Th1-mediated autoimmune responses in animals. Analysis of the role of cytokines in regulating T cell immunity in humans is hampered by the inability to address these questions directly in vivo. In vitro, IL-4 and IL-10 clearly exhibit an anti-inflammatory effect, because they induce expression of the IL-1R antagonist (14, 15) and down-regulate the production of proinflammatory cytokines, such as IL-1 and TNF from human monocytes (16, 17). Moreover, IL-4 has a direct inhibitory effect on the development of human Th1 cells (18), and IL-10 is able to prevent Th1 effector functions by induction of long-lasting T cell unresponsiveness (19). Recent studies aiming to dissect the impact of new treatment approaches in human autoimmune diseases have revealed that clinical benefit may be associated with enhanced Th2 cell differentiation in vivo (20–22). Moreover, administration of IL-4 or IL-10 to patients with psoriasis resulted in improvement of skin disease (23–25). In contrast, treatment of rheumatoid arthritis (RA), a prototype human Th1-biased autoimmune disease, with IL-4 or IL-10 has largely failed to down-modulate inflammation.

³Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; RA, rheumatoid arthritis; GvH, graft vs host.
and to provide clinical benefit (26). Therefore, the precise mechanisms of regulation of Th1-mediated immune reactions in humans remain obscure, and as a result, the impact of targeted interventions remains unpredictable.

To address these issues, we sought to develop a model in which human Th1-biased immune responses could be induced and analyzed in vivo. Inducing T cell activation by transfer of human T cells and APC into SCID mice provided an appropriate model system. In this model, a Th1-biased immune response developed that was associated with lymphocytic infiltrations into several organs. The in vivo evolution of the human Th1 immune response was tightly regulated by endogenously produced IL-4 and IL-10. Further analysis of the reaction showed that both cytokines administered exogenously lead to a down-modulation of human Th1 immunity. Using this novel in vivo model to understand the regulation of a Th1-biased immune response should provide important insights into the regulation of human immune reactions and their pathologic consequences.

Materials and Methods

Reagents and Abs

The following mAbs were used for purification and staining of human cells: anti-CD16, anti-CD19; FITC-conjugated anti-CD3, PE-labeled anti-CD4, FITC-labeled anti-CD4 (Sigma-Aldrich, Taufkirchen, Germany); FITC-labeled anti-CD14, PE-labeled anti-CD25 (Cymbus Biotechnology, Hants, U.K.); FITC-labeled anti-HLA-DR (DAKO, Hamburg, Germany); PE-labeled anti-CD8, PE-labeled anti-IL-4 (MP4-25D2), and FITC-labeled anti-IFN-γ (4S.B3) (BD Pharmingen, Heidelberg, Germany). CFSE was obtained from Molecular Probes (Leiden, The Netherlands). Cyclosporine was purchased from Sigma-Aldrich. Human rIL-4 and the neutralizing mAb to IL-10 were from R&D Systems (Wiesbaden, Germany). Mice congenic for the scid mutation on the NOD genetic background were purchased from M&B (Ry, Denmark). The animals were maintained under pathogen-free conditions in the animal facility of Nikolaus Fiebig Center (Erlangen, Germany). Mice were used at 6–12 wk of age.

Human cell preparation

PBMC were obtained by Ficoll Hypaque (Sigma-Aldrich) gradient centrifugation of heparinized venous blood from young healthy volunteers not taking any medications. For further T cell or monocyte preparation, PBMC were incubated with sheep erythrocytes, and T cells were isolated from the rosette-negative cells by negative selection panning using anti-CD16 and anti-CD19 as previously described (20). Monocytes were purified from the fraction of rosette-negative cells using the monocyte isolation kit from Miltenyi Biotec (Bergisch Gladbach, Germany), according to the manufacturer’s instructions. The frequencies of cell populations within PBMC, and homogeneity and purity of the isolated T cells and monocytes were routinely assessed by flow cytometry. Typically, ≥95% of the T cells were positive for CD3 and CD4, ≥90% of the monocytes stained brightly with a mAb to CD14, and >98% of the cells were viable after the purification procedure. T cells were negative for the activation markers CD25, CD30, CD69, and HLA-DR.

Injection with human cells and treatment of mice

Isolated cell populations were resuspended in PBS and injected i.p. into mice in a total volume of 0.2 ml. For CFSE labeling, 10 × 10^6 PBMC were resuspended in 1 ml of PBS and labeled for 8 min with 10 μM CFSE at room temperature. Cyclosporine treatment was performed daily with 0.02–0.04 mg from days 0 through 14 by i.p. injection. Treatment with IL-4 (0.008–0.2 mg) or IL-10 (0.0016–0.04 mg) was performed daily by i.p. injection of recombinant cytokines from days 14 to 19. Treatment with blocking Abs for human IL-4 or IL-10 (100 μg) was performed by i.p. injections during the 14-day experiments, at days 0, 5, and 10.

Cytokine determination

Human cells were recovered from the peritoneal cavity of the mice. To assess the acquired capacity of T cells for cytokine production, 2 × 10^6 recovered cells were restimulated with ionomycin (1 μM; Calbiochem, Schwalbach, Germany) and PMA (20 ng/ml; Sigma-Aldrich) for 5 h in the presence of 2 μM monensin (Sigma-Aldrich). Cells were fixed with 4% paraformaldehyde (Sigma-Aldrich), and cytoplasmic human IFN-γ and IL-4 were detected by flow cytometry after intracellular staining with FITC-labeled anti-IFN-γ and PE-labeled anti-IL-4. The numbers of cytokine-producing T cells were determined from the total population of gated lymphocytes. Analysis of extracellular markers revealed that recovered lymphocytes contained <1% CD19-positive B cells.

To analyze the serum levels of the human inflammatory cytokines IFN-γ and TNF-α and of human IL-4, blood was taken from the tail vein of the mice, sera were collected, and the cytokine levels were measured using commercially available high-sensitivity ELISA kits that were highly specific for the human cytokines (sensitivity thresholds, 0.12, 8, and 0.13 pg/ml for TNF-α, IFN-γ, and IL-4, respectively; R&D Systems).

Histopathologic analysis

Mouse tissues were sampled immediately after sacrifice, fixed in 5% neutral buffered formalin, and embedded in paraffin using standard histological procedures. Three-micrometer paraffin sections were stained with H&E for morphological assessment by light microscopy.

For immunohistochemistry, a polyclonal CD3 antigen, monoclonal CD4, CD8, CD20, and CD68 Abs (all specific for human Ags), and a monoclonal eosinophilic peroxidase Ab (all from DAKO) were used. Paraffin sections were dewaxed and subjected to Ag retrieval in 0.1 M citrate buffer (pH 6.0) using a pressure cooker. Following incubation with appropriately diluted primary Abs, sections were incubated with a biotin-labeled goat anti-rabbit serum (for CD3; DAKO) or with biotinylated rabbit anti-mouse IgGs (for all others; DAKO). Bound Abs were detected using a streptavidin-biotinylated alkaline phosphatase complex (DAKO) and Fast Red as a chromogen (Sigma-Aldrich). Stained sections were counterstained with hematoxylin and examined by light microscopy.

Statistical and mathematical analysis

Linear regression was calculated using the InStat computer program (GraphPad, San Diego, CA). Based on CFSE fluorescence intensity, the frequencies (F) of CD4 and CD8 cells that had undergone divisions (from d = 1 for the first cycle to n cell divisions) was calculated as follows:

\[
F = \sum_{d=1}^{n} (2^{-d}) F_d
\]

Results

Development of a human Th1 immune reaction in SCID mice

Previous studies have shown that human PBMC remain functionally active after injection into SCID mice (27). Moreover, transfer of human cells into SCID mice results in the development of a xenogeneic graft-vs-host (GvH) disease (28). For characterization of the developing immune reaction of human cells against mouse tissue, human PBMC were labeled with CFSE and injected into SCID mice (Fig. 1A). Human CD4 and CD8 T cells spontaneously proliferated after introduction into SCID mice. The degree of CD8 T cell expansion exceeded that of CD4 cells. Because CFSE pattern analysis indicated a similar proliferative rate of CD4 and CD8 cells (data not shown), this was likely to be a consequence of increased precursor frequencies of CD8 cells capable of undergoing proliferation in response to murine Ags (Table 1). Of note, the percentage of T cells initially reacting to mouse tissue was different among donors. Additional experiments using nonlabeled PBMC indicated that greater expansion of CD8 compared with CD4 cells led to an inverted CD4/CD8 ratio (Fig. 1B).

Analysis of activation markers on the T cell surface revealed that the frequency of CD4 T cells expressing HLA-DR and CD25 increased with time (Fig. 1C). The percentage of T cells capable of
producing IFN-γ and IL-4 as indicators of a Th1 or a Th2 response, respectively, was determined by intracellular flow cytometry. As demonstrated in Fig. 1D, the frequency of IFN-γ-producing T cells increased with time of the xenogeneic immune reaction. In contrast, IL-4-producing T cells could not be detected by intracytoplasmic staining after ex vivo stimulation in the recovered T cells (data not shown). Importantly, histopathological analysis revealed infiltration of activated human lymphocytes into the portal tracts of the liver, and into the perigastrointestinal and perirenal fatty tissues (Fig. 1, E and F). The lymphocytic infiltrates were frequently organized in granuloma-like structures with palisading human macrophages and central necrosis (Fig. 1F), further indicating the Th1-biased nature of the inflammatory immunity (29–31). By contrast, eosinophils, indicative of Th2 activation, could not be detected in the granulomas (data not shown).

**Cylosporine prevents the human Th1 immune reaction in SCID mice**

To define the role of T cells in the initiation of the xenogeneic GvH reaction in the SCID mouse more precisely, experiments were conducted in which PBMC-injected mice were treated with cyclosporine, an inhibitor of T cell activation (Fig. 2). Cyclosporine treatment had a marked inhibitory effect on the activation of CD4 T cells (Fig. 2A). The effect of cyclosporine was even more pronounced with regard to the expansion of IFN-γ producers, which

**FIGURE 1.** Human PBMC develop a Th1-biased immune reaction after i.p. injection into SCID mice. A total of 50 × 10⁶ human PBMC was injected i.p. into SCID mice. At the indicated time points, human cells were recovered from the peritoneal cavity, and analyzed by flow cytometry. A, Before injection, PBMC were labeled with CFSE. Frequencies of cells with reduced CFSE fluorescence indicative of proliferative cycles were assessed after counterstaining with mAbs to CD4 or CD8. B, The CD4/CD8 ratio in recovered cell populations was calculated after surface staining with mAbs to CD4 and CD8. C, Percentage of CD4 cells expressing the activation marker HLA-DR or CD25. D, The frequency of cells capable of IFN-γ production was assessed by intracellular flow cytometry after a 5-h in vitro stimulation with PMA and ionomycin. One representative of eight independent experiments with different donors is shown. E, Liver sections from day 14 are illustrated after staining with H&E (i–iii) or with a mAb to human CD3 (iv). Polymorphic lymphoid cell infiltrates are observed in a large (i) and in small portal tracts (ii and iii) around small bile ducts. Note that bile duct epithelial cells occasionally show degenerative changes (ii). Immunostaining of a serial section to iii identifies the majority of infiltrating cells as human CD3-positive T cells (iv). Original magnification: i, iii, and iv, ×200; ii, ×400. F. A typical epitheloid cell granuloma from perigastric fatty tissue is illustrated (i, H&E staining). Immunostaining reveals a row of human CD68-positive macrophages surrounding an area of central necrosis (ii). The polymorphic lymphoid cell infiltrate adjacent to the macrophages consists of human CD3-positive T cells (iii) with a large proportion of CD4-positive cells (iv) and a smaller number of CD8-positive cells (v). Adjacent to the granuloma are aggregates of human CD20-positive B cells (vi). Original magnification: i, ii, iii, and vi, ×200; iv and v, ×400.
was completely prevented by cyclosporine (Fig. 2B). As a reflection of the activity of effector T cells in vivo, the inflammatory human cytokines IFN-γ and TNF and human IL-4 were measured in the serum of the animals. The serum level of human IFN-γ was markedly diminished in the cyclosporine-treated mice compared with PBS-treated mice, whereas the serum level of human TNF was only slightly decreased (Fig. 2C). Human IL-4 was not detectable in any of the mice (data not shown). Together, the data strongly indicate that the immune reaction of human PBMC to mouse tissue generates a T cell-mediated, Th1-biased immune response.

The xenogeneic Th1-biased immune response requires APC

The development of a specific immune response requires APC (32). Monocytes represent potential APC in the pool of PBMC. To evaluate the impact of monocytes on the development of the human Th1-biased xenogeneic GVH reaction, T cells and monocytes were purified from the peripheral blood of the same donor and injected into SCID mice separately or at a purposeful ratio of five T cells to one monocyte (Fig. 3, A–C). Activation of CD4 T cells, their differentiation into effectors capable of IFN-γ production, and in vivo secretion of IFN-γ and TNF were all observed only in the mice that had received T cells together with monocytes.

To characterize the role of monocytes in the development of the human xenogeneic Th1-biased reaction in greater detail, we conducted experiments with different ratios of purified T cells to monocytes, keeping the absolute number of injected T cells constant (Fig. 3D). Levels of human cytokines indicative of an in vivo Th1-biased effector cell activation (IFN-γ and TNF) were measured in the serum of the animals. A marked increase in the concentration of IFN-γ and TNF was detected in the serum of the animals that had been injected with mixtures that contained higher ratios of monocytes to T cells. Notably, when the in vivo production of IFN-γ from PBMC of various donors was analyzed as a function of the monocyte-to-T cell ratio within the injected PBMC, the IFN-γ concentration in the serum of the animals correlated directly with this ratio (Fig. 3E). Thus, the ratio of human monocytes to T cells regulates the strength of the Th1-mediated xenogeneic reaction.

IL-4 and IL-10 are regulators of human Th1 immunity

A large body of in vitro data suggests that the development of a human Th1-mediated immune reaction is controlled by cytokines, such as IL-4 and IL-10 (26). However, conclusive evidence from in vivo human immune responses has not been provided to date. To address this question, human endogenously produced IL-4 and IL-10 were neutralized during the development of the xenogeneic Th1-biased reaction resulting from the injection of PBMC into SCID mice by mAb that were specific for the human cytokines with no cross-reactivity to murine IL-4 and IL-10 (Fig. 4). The blockade of endogenous human IL-4 led to a significant enhancement of the Th1-biased immune response (Fig. 4, A–C). Whereas the frequency of activated CD4 T cells within the cells recovered from anti-IL-4-treated mice was comparable with that of control mice (Fig. 4A), neutralization of endogenous IL-4 resulted in a significantly increased frequency of T cells capable of IFN-γ production (1.2 ± 0.2-fold increase, p < 0.004; B). When the in vivo activity of effector T cells was assessed, the effect of IL-4 neutralization became even more pronounced, because neutralization of IL-4 resulted in a >2-fold increase in the serum concentrations of human IFN-γ (3.0 ± 2.4-fold increase, p < 0.05) and human TNF (2.2 ± 1.5-fold increase, p = 0.06) in the serum of the mice (Fig. 4C). Because IFN-γ production is not affected directly by IL-4 (33), this is likely to be a consequence of markedly increased numbers of activated cytokine-producing T cells rather than of enhanced secretion of human IFN-γ and TNF by the activated T cells.

Neutralization of endogenously produced IL-10 during the xenogeneic human Th1-biased immune response did not interfere with the activation of CD4 T cells, because the frequencies of HLA-DR-positive CD4 T cells recovered from control mice and from anti-IL-10-treated mice were comparable (Fig. 4D). Inhibition of IL-10, however, resulted in a significant increase of the frequencies of effector T cells capable of IFN-γ production (1.2 ± 0.3-fold increase, p < 0.02; Fig. 4E). Consequently, blockade of IL-10 resulted in a tendency for increased Th1-mediated effector functions in vivo as documented by increased serum levels of IFN-γ (1.9 ± 1.0-fold increase, p = 0.08) and TNF (1.8 ± 0.8-fold increase, p < 0.05; Fig. 4F). Thus, the development of a Th1-biased human immune reaction is tightly controlled by the endogenously produced anti-inflammatory cytokines IL-4 and IL-10. It should be noted that no IL-10 mRNA could be detected in recovered cells even when using highly sensitive real-time PCR, and

Table 1. Frequencies of T cells initiating spontaneous proliferation after introduction into SCID mice

<table>
<thead>
<tr>
<th>CD4 Cells (%)</th>
<th>CD8 Cells (%)</th>
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<tbody>
<tr>
<td>Donor 1</td>
<td>0.46</td>
</tr>
<tr>
<td>Donor 2</td>
<td>1.09</td>
</tr>
</tbody>
</table>

FIGURE 2. Cyclosporine prevents the Th1-biased human immune response in SCID mice. A total of 50 × 10⁶ freshly isolated human PBMC was injected into SCID mice. Mice were treated daily by i.p. injection with cyclosporine or PBS as control. Analysis was performed on day 14. Human cells were recovered from the peritoneal cavity and analyzed by flow cytometry for extracellular expression of CD4 and HLA-DR (A), and for cytoplasmic IFN-γ after in vitro stimulation with PMA and ionomycin (B). For comparison, values from freshly isolated PBMC are shown (Before). C, Serum levels of the human inflammatory cytokines IFN-γ and TNF were determined by ELISA. One representative of six independent experiments with different donors is shown.
IL-4 mRNA was found only at a very low level (data not shown), suggesting that the mechanisms of action of IL-4 and IL-10 on the development of a human Th1-biased immune response, although different, are very precise and efficient processes.

IL-4 down-modulates established Th1-biased immunity

Neutralization of endogenous IL-4 led to an unbalanced and exaggerated development of the xenogeneic human Th1-biased immune response (Fig. 4, A–C), suggesting the potential of this cytokine to function as an immune modulator in humans. Therefore, we sought to delineate the effect of IL-4 as a means to down-modulate an established Th1-mediated immune response. SCID mice were injected with PBMC, and starting at day 14, they were treated daily for 5 days with recombinant human IL-4 (Fig. 5). A 5-day treatment with IL-4 did not alter the frequency of HLA-DR-expressing T cells (Fig. 5A). However, the Th1-biased differentiation of T cells into IFN-γ-producing effectors was significantly inhibited in response to IL-4 treatment (reduction to 92% of control, p < 0.03; Fig. 5B). Moreover, IL-4 caused a significant suppression of the Th1-biased effector functions in vivo, as

**FIGURE 3.** Monocytes are required for the development of the Th1-biased immune reaction of human cells in SCID mice. Human T cells and monocytes were purified by negative selection from PBMC. A–C, T cells (50 × 10⁶) and monocytes (10 × 10⁶) from the same donor were injected either separately or together into mice. As a control, mice were injected with PBMC that contained 50 × 10⁶ T cells. Analysis was performed on day 14. Human cells were recovered from the peritoneal cavity and analyzed by flow cytometry for CD4 and HLA-DR (A), and cytoplasmic IFN-γ (B). C, Serum levels of the human inflammatory cytokines IFN-γ and TNF were determined by ELISA. One representative of three independent experiments with different donors is shown. n.d., Nondetectable. D, A total of 50 × 10⁶ T cells was injected together with different numbers of monocytes (6.25 × 10⁶, 12.5 × 10⁶, or 25 × 10⁶, respectively) from the same donor into mice. Serum levels of the human inflammatory cytokines IFN-γ and TNF were determined at day 14 by ELISA. E, Serum concentrations of human IFN-γ (day 14) were plotted as a function of the monocyte-to-T cell ratio within the injected PBMC from 19 independent experiments, and the linear regression was calculated. The serum IFN-γ concentration significantly correlated with the monocyte-to-T cell ratio of the inoculum.

**FIGURE 4.** Endogenously produced IL-4 and IL-10 control the development of the human Th1-biased immune reaction. A total of 50 × 10⁶ freshly isolated human PBMC was injected into SCID mice. At days 0, 5, and 10, mice were treated with mAbs to human IL-4 (anti-IL-4) (A–C) or human IL-10 (anti-IL-10) (D–F). As a control, animals were treated with isotype-matched control mAbs (IgG). Analysis was performed on day 14. Human cells were recovered from the peritoneal cavity and analyzed by flow cytometry for CD4 and HLA-DR (A and D), and cytoplasmic IFN-γ (B and E). C and F, Serum levels of the human inflammatory cytokines IFN-γ and TNF were determined by ELISA. One representative of 13 independent experiments for anti-IL-4 and one of 13 independent experiments for anti-IL-10 with different donors are shown.
IL-10 has been shown to be a very powerful inhibitor of IFN-γ response. Together, the data indicate that administration of exogenous IL-4 after in vitro stimulation with PMA and ionomycin significantly inhibited the in vivo production of IFN-γ. (reduction to 51%, assessed by measurement of serum levels of these cytokines (34). The data from the anti-IL-10 experiments (Fig. 4, D–F) suggest a similar mechanism of IL-10 activity in humans. To test this hypothesis, we investigated the effect of exogenous human IL-10 on an established Th1-mediated immune reaction of human PBMC (Fig. 6). IL-10 treatment reduced the frequency of HLA-DR-positive CD4 T cells but, in contrast to IL-4, had no effect on the differentiation of cells capable of producing IFN-γ (Fig. 6, A and B). However, IL-10 treatment significantly inhibited the in vivo production of IFN-γ and TNF as assessed by measurement of serum levels of these cytokines (reduction to 51 ± 19 and 60 ± 32%, respectively; p < 0.002 and p < 0.03; Fig. 6C). Thus, IL-10 might inhibit effector functions of the human Th1-mediated immune response by preventing the production of these inflammatory cytokines.

### Discussion

Modern immunological research is dependent on the use of animal models to dissect the complex, but tightly regulated cellular and molecular interactions that occur during an immune response. However, animal models cannot provide all of the answers that are required to understand human immunity completely, or to translate basic research to useful clinical applications efficiently. Therefore, it is essential to develop reliable models of human immunity to foster the translation of fundamental principles into appropriate clinical applications. In this study, we introduce a convenient and reliable model to analyze human immune responses in vivo. The system provides the opportunity to investigate in detail the mechanisms involved in regulating human immunity and, in particular, the analysis of the development of human Th1 responses at the molecular level. Thus, the model represents a novel and useful tool for the study of human immunity.

Human tissue engrafted into SCID mice has served as a model for in vivo human research in HIV, transplantation, and tumor biology (27, 28, 35, 36). The goal of most such investigations was to establish functional human tissue in nonhuman living organisms. However, little attention was paid to the xenogeneic reaction that occurs after engraftment of immunocompetent human cells into mice, although it has been shown that immunocompromised recipients of xenogeneic cells develop severe T cell-dependent GvH disease (28, 37, 38). To induce human immune reactions in vivo, therefore, we transferred human PBMC into SCID mice and could, for the first time, conclusively show that a human APC-dependent Th1-biased cellular immune response develops. The magnitude of this Th1-biased immune reaction is comparable to that of physiological immune reactions in the human body (39, 40). For example, the serum IFN-γ level in RA and osteoarthritis patients varies in the range of 7–16 pg/ml (40). Even the strongest inducer of IFN-γ production, IL-12, induces IFN-γ serum levels in a comparable range (40–140 pg/ml) (39). Moreover, the development of the human immune response was characterized by lymphocytic infiltrations into different murine organs. The frequent granuloma-like architecture of the infiltrates with a central necrotic area surrounded by dense accumulation of T cells and macrophages is reminiscent of granulomatous lesions in tuberculosis, sarcoidosis, and Wegener’s granulomatosis, all of which are characterized by Th1-mediated granuloma formation (29–31, 41, 42).

Systemic Th1 inflammation evolved reliably and consistently after i.p. injection of PBMC from healthy individuals into SCID

### FIGURE 5. Exogenous IL-4 diminishes the human Th1-biased immune reaction. A total of 50 × 10⁶ freshly isolated human PBMC was injected into SCID mice. Starting at day 14, mice were treated daily with human IL-4 for 5 days. As a control, animals were treated with PBS. Analysis was performed on day 19. Human cells were recovered from the peritoneal cavity and analyzed by flow cytometry for CD4 and HLA-DR (A), and cytoplasmic IFN-γ (B). C, Serum levels of the human inflammatory cytokines IFN-γ and TNF were determined by ELISA. One representative of six independent experiments with PBMC from different donors is shown.

**FIGURE 6.** Exogenous IL-10 diminishes the human Th1-biased immune reaction. A total of 50 × 10⁶ freshly isolated human PBMC was injected into SCID mice. Starting at day 14, mice were treated daily with human IL-10 for 5 days. As a control, animals were treated with PBS. Analysis was performed on day 19. Human cells were recovered from the peritoneal cavity and analyzed by flow cytometry for CD4 and HLA-DR (A), and cytoplasmic IFN-γ (B). C, Serum levels of the human inflammatory cytokines IFN-γ and TNF were determined by ELISA. One representative of six independent experiments with PBMC from different donors is shown.
mice. However, the degree of Th1 inflammation varied, largely depending on the ratio of injected APC to T cells (Fig. 3), which emphasizes the specific nature of the immune response. Of note, PBMC from a small minority of donors repeatedly failed to develop systemic inflammation in the SCID mice, despite normal frequencies and numbers of monocytes and T cells. Whether the lack of response of the PBMC from this subset of donors reflects specific anergy to murine Ags or the presence of regulatory cells of some kind remains to be delineated. With the exception noted, the model is robust in that injection of PBMC from the vast majority of donors results in spontaneous systemic Th1 inflammation.

A striking observation in this study was the finding that the in vivo development of human Th1 immunity is tightly controlled by endogenously produced IL-4 and IL-10 to a similar extent. Our data suggest that the role of IL-4 in the regulation of Th1 immunity in humans is much more pronounced than in the mouse. In the mouse, IL-10 neutralization results in an accelerated onset of collagen-induced arthritis and increased severity, whereas IL-4 blockade is without effect (43). Moreover, IL-10-deficient mice are more susceptible and develop more severe EAE when compared with IL-4-deficient mice (10). In contrast, our findings using neutralizing Abs to IL-4 and IL-10 suggest that, in humans, the regulatory role of these two cytokines for Th1-mediated immunity is weighted differently.

Supportive indications for this conclusion derive from the analysis of patients with systemic autoimmune diseases, which are characterized by unresolved inflammation mediated by Th1 autoimmune activity. A typical feature of human autoimmune diseases appears to be the absence of the Th2 cytokine IL-4 together with simultaneous up-regulation of IL-10 (21, 44–48), which, in contrast to the situation in the mouse, is not a typical Th2 cytokine in humans (49). When IL-4 is measured directly in the synovial fluid or in PBMC of RA patients, it is virtually absent (21, 44). Moreover, we have demonstrated that memory CD4 T cells from patients with RA show an impaired differentiation into IL-4-producing cells (45). In marked contrast, high levels of functionally active IL-10 are detected in RA synovial fluid and in the peripheral circulation of RA patients (46, 47). In vitro analysis of mononuclear cells from multiple sclerosis patients shows elevated numbers of cells producing IL-10 (48). Thus, although IL-10 is produced in substantial amounts in human autoimmune diseases, it appears to be insufficient to control an autoimmune reaction in the absence of IL-4.

Analysis of IL-4- and IL-10-induced down-modulation of the established human Th1-biased immune response described in this study suggests that the mechanism of action of these cytokines is different. Whereas the IL-4 effect was evident at the steps of T cell differentiation into IFN-γ-producing effectors, the immunomodulatory effect of IL-10 was detectable only at the level of Th1-mediated effector functions, e.g., in vivo production of inflammatory cytokines. Interestingly, the central necrotic areas of the granulomas were reduced by IL-10, but not by IL-4 treatment (data not shown), further suggesting a direct anti-inflammatory role of IL-10. IL-4 and IL-10 exert a broad spectrum of different rather than overlapping immunomodulatory activities (33, 34). Whereas IL-4 predominantly targets T cells as effectors of an immune reaction, the action of IL-10, in contrast, rather focuses on APC, such as macrophages. The most pronounced regulatory capacity of IL-4 is the direct negative regulation of Th1 differentiation from CD4 T cells (50). In contrast, IL-10 modulates expression of cytokines, such as IL-12, and surface molecules by myeloid cells, with important consequences for their ability to activate and sustain immune responses. Most effects of IL-10 on T cells are, therefore, indirect and are mediated via its down-regulatory activity on APC function (34). In accordance with these findings, the IL-10-mediated immune suppression observed in the current study is likely the result of the inhibition of unique functions of APC that are required for IFN-γ secretion in vivo. However, T cell activation and differentiation into Th1 cells appear to be intact. In contrast, the immunosuppressive effect of IL-4 imparts suppression of T cell activation and differentiation.

Together, the system described in this study presents a useful tool to investigate in detail the mechanisms involved in regulating human immunity. The data demonstrate that both endogenous IL-4 and IL-10 control human Th1-mediated immune reactions. As a logical consequence, rIL-4 and rIL-10 administered during an established immune response repress Th1-mediated effector functions by inhibiting proinflammatory cytokine production, although by different ways. However, whereas IL-10-based treatment, although effective, might carry the risks associated with general immunosuppression, such as enhanced susceptibility to infectious agents (34), the immunosuppressive action of IL-4 appears to be more selective, because IL-4 targets primarily T cells undergoing activation through their Ag receptor (33, 51).

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