Decreased IL-27 Expression in Association with an Increased Th17 Response in Vogt-Koyanagi-Harada Disease

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PURPOSE. IL-27 has emerged as an important regulator of proinflammatory T-cell responses in animal models. We investigated the pathophysiological role of IL-27 in Vogt-Koyanagi-Harada (VKH) disease.

METHODS. IL-27P28 and EBI3 mRNA expression in peripheral blood mononuclear cells (PBMCs) were assayed by RT-PCR. Cytokines in the serum and supernatants of PBMCs, naïve CD4+ T cells and DC-T cocultures were assayed by ELISA. Flow cytometry was used to evaluate the frequencies of IL-17–producing CD4+ T cells.

RESULTS. The active VKH patients showed a decreased IL-27P28 mRNA expression in PBMCs and lower IL-27 expression in the serum and supernatants of PBMCs, but higher Th17 cells in PBMCs. EBI3 mRNA expression was not different among the groups tested. Stimulation of naïve CD4+ T cells under Th17 polarizing conditions showed a higher Th17 cell differentiation in active VKH patients. IL-27 significantly inhibited Th17 cell differentiation. IL-27–treated DCs showed a significant inhibition on Th17 differentiation. There was a significant defect in the Tr1 cell induction as measured by IL-10 in active VKH patients. Treatment with corticosteroids and cyclosporine A (CsA) resolved the intraocular inflammation in association with an upregulation of IL-27 and a downregulation of IL-17. In vitro experiments showed that corticosteroids, but not CsA, significantly upregulated the expression of IL-27.

CONCLUSIONS. The present study suggests that decreased IL-27 expression may result in a higher Th17 in active VKH patients, which may promote the autoimmune response observed in these patients. Manipulation of IL-27 may offer a novel target for treatment of this disease. (*Invest Ophthalmol Vis Sci.* 2012; 53:4668–4675) DOI:10.1167/iovs.12-9863

Uveitis ranks among the leading causes of blindness in the world.[1] Vogt-Koyanagi-Harada (VKH) disease is one of the most common uveitis entities in Asia.[2–7] The pathogenesis of VKH disease has been extensively studied during the past decade and an autoimmune response against melanocytes is considered to be involved in this disease.[8] Early studies suggested that Th1 cells played a role in experimental autoimmune uveitis (EAU), a classical animal model for human uveitis.[9] However, a number of recent studies on EAU showed that IFN-γ, an index cytokine of Th1 cells, provided protection in this model and suggested a critical role for Th17 cells in the pathogenesis of EAU.[10,11] We confirmed these animal data in a study in patients with VKH disease and showed that production of IL-17, a main cytokine of Th17 cells, promoted by IL-23 was an essential mechanism in the development of this disease.[12] In experimental autoimmune encephalomyelitis (EAE), a classical counterpart for human multiple sclerosis, Th17 cells, but not Th1 cells, have also been demonstrated to be critical to the development of this disease.[13-15]

IL-27, one of the members of the IL-12 family, has recently been shown to be an important immunoregulatory cytokine involved in the immune response as well as in the development of inflammation. It is composed of Epstein-Barr virus–induced gene 3 (EBI3), a p40-related protein belonging to the IL-6/IL-12 family, and a unique IL-27p28, a p35-related polypeptide.[16] IL-27R is expressed on monocytes/macrophages, dendritic cells, T and B lymphocytes, NK cells, mast cells, and endothelial cells.[17] Recent studies have shown that IL-27 is an anti-inflammatory cytokine that is important in the regulation of Th17 activation.[18,19] Studies on EAE revealed a significantly severe inflammatory activity in IL-27ra−/− mice as compared with wild mice,[18] and a lower activity when treated by exogenous IL-27.[20] High IL-27 expression was shown at the peak of EAE[21] and EAU,[10] and the expression of this cytokine seems to correlate with disease activity in both animal models. During the development of EAU, IL-27 inhibited uveitogenic T cells and suppressed the expansion of Th17 cells in the retina.[10] These results suggest that the upregulated IL-27 response may be involved in the resolution of the inflammation in these models. However, it is not yet known whether IL-27 also plays a role in the pathogenesis of human uveitis and was therefore the subject of the studies presented here.

MATERIALS AND METHODS

Human Subjects

We analyzed 24 active VKH patients, 7 active VKH patients before and after a 3-month treatment, 24 inactive VKH patients, and 7 patients with idiopathic active anterior uveitis; 28 sex- and age-matched healthy individuals served as controls. Patients and controls were collected

between May 2010 and July 2011. The diagnosis of VKH disease was made according to the diagnostic criteria revised for VKH disease by an international committee on nomenclature.[22] The active VKH patients typically showed mutton fat keratic precipitates, cells and flare in the anterior chamber, iris nodules, and sunset glow fundus, whereas the inactive VKH patients showed only sunset glow fundus and, in some patients, choroidoretinal atrophy.[22] No immunosuppressive agents or prednisone were used in active VKH patients before visiting us and before blood sampling. Blood samples were obtained from inactive patients at least 3 months after termination of all medications. All procedures followed the tenets of the Declaration of Helsinki and were approved by our clinical ethical research committee. Written and informed consents were obtained from all the patients and controls.

Cell Isolation and Culture

PBMCs were prepared from heparinized blood by Ficoll-Hypaque density-gradient centrifugation. To study the production of IL-27, PBMCs were either stimulated with *Staphylococcus aureus* Cowan I (SAC) (Sigma-Aldrich, St. Louis, MO) (0.02%) or cultured in medium alone at a density of 2×10^6 cells/mL for 72 hours and the supernatants were used for IL-27 analysis by ELISA. To detect the level of IL-17 production in the supernatants of PBMCs from VKH patients and healthy controls, PBMCs were cultured with anti-CD3/CD28 (2 μg/mL) (eBioscience, San Diego, CA) for 72 hours, and then the supernatants were harvested for IL-17 detection by ELISA. To determine the effect of both drugs on IL-27 expression in vivo, seven active adult VKH patients were treated by corticosteroids (0.4∼0.6 mg/kg/d) combined with cyclosporine-A (CsA) (3∼5 mg/kg/d). The intraocular inflammation was completely controlled following a 3-month treatment. PBMCs from those patients before and after treatment were suppressed with CsA and/or dexamethasone (Sigma-Aldrich) for 72 hours. To determine the effect of both drugs on IL-27 expression in vitro, PBMCs from six healthy controls were suppressed with CsA and/or dexamethasone for 72 hours. The supernatants were harvested for IL-27 detection by ELISA.

To study the effect of IL-27 on Th17 cell differentiation, naïve CD4+ T cells were separated from PBMCs by magnetic microbeads negative selection according to the manufacturer's instructions (see Supplementary Material and Supplementary Fig. S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-9863/-/DCSupplemental; 93% purity, Miltenyi Biotec, Palo Alto, CA). The cells were subsequently stimulated with anti-CD3/CD28 (2 μg/mL, eBioscience) under Th17 polarizing conditions (IL-6 at 50 ng/mL, IL-23 at 10 ng/mL, IL-1beta at 10 ng/mL, anti IL-4 at 10 μg/mL, and anti–IFN-γ at 10 μg/mL; R&D Systems, Minneapolis, MN) in the presence or absence of recombinant IL-27 (rIL-27) (100 ng/mL, R&D Systems). On day 3, rIL-2 (100 ng/mL, R&D Systems) was added for an additional 4 days. The supernatants were collected for IL-17 detection by ELISA. The cells were harvested for intracellular detection of cytokines by flow cytometry. To investigate the effect of IL-27 in the generation of IL-10 from naïve CD4+ T cells in VKH patients and healthy controls, naïve CD4+ T cells were simulated with anti-CD3/CD28 and rIL-2 in the presence or absence of IL-27 for 7 days. The supernatants were collected for detection of IL-10 by ELISA.

CD14+ monocytes were purified from PBMCs using human microbeads (purity > 93%, Miltenyi Biotec). Human dendritic cells (DCs) were generated from monocytes plated at a density of 1×10^6 cells/mL in RPMI 1640 complete medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 100 U/mL penicillin G/streptomycin (Invitrogen, Carlsbad, CA) in the presence of recombinant human granulocyte macrophage–colony stimulating factor (GM-CSF) (100 ng/mL) and IL-4 (50 ng/mL; R&D Systems). On day 3, half of the culture medium, including GM-CSF and IL-4, was refreshed. On day 6, the cells were stimulated with lipopolysaccharides (LPS) in the presence or absence rIL-27 for 24 hours. Then those DCs were washed three times before coculture with naïve CD4+ T cells at ratio of 1:5 for 7 days. The naïve CD4+ T cells cultured alone served as a control for the coculture. The

culture supernatants were collected for detection of IL-17 by ELISA. The cells were harvested for intracellular analysis of IL-17 by flow cytometry.

Real-Time Quantitative RT-PCR

Total RNA was extracted from freshly isolated PBMCs of controls and the VKH patients using the RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The first-strand cDNA was synthesized for each RNA sample using the Superscript III Reverse Transcriptase system (Invitrogen). Real-time quantitative PCR was performed on the iCycler (Bio-Rad, Veenendaal, The Netherlands) using the Quanti Tect SYBR Green PCR kit (Applied Biosystems). The forward and reverse primers for β-actin were designed using Primer Premier software (Premier Biosoft International) as follows: β-actin forward, 5′-GGATGCAGAAGGAGATCACTG-3′ and β-actin reverse, 5′-CGATCCACACGGAGTACTTG-3′. QuantiTect Primers (Qiagen) were used for EBI3 and P28. The mRNA expression analysis was performed as described previously.[23]

ELISA

Supernatants from PBMCs, stimulated naïve CD4+ T cells, or DC-naïve CD4+ T cocultures were collected and stored at −80°C until the cytokine measurement. The concentrations of IL-17 and IL-10 in the serum or the collected supernatants were measured using a Duoset ELISA development kit (R&D Systems) according to the manufacturer's instructions.

Flow Cytometry Analysis

For intracellular cytokine staining, the cells were pretreated with 50 ng/mL phorbol-12-myristate-13-acetate (PMA) and 1 μg/mL ionomycin for 1 hour at 37°C, brefeldin A (Sigma-Aldrich) for another 4 hours, then washed, fixed, and permeabilized using the Cytofix/Cytoperm kit (eBioscience) according to the manufacturer's instructions. Fluorescent Abs (anti-human CD3-(PerCP)-Cy5.5, anti-human CD8-APC, anti-human IL-17A-PE, and anti-human IgG isotype control Ab) were obtained from BD Biosciences (BD Bioscience, Sunnyvale, CA). Data collection and analysis were performed on a FACScan flow cytometer using CellQuest software (BD Bioscience).

Statistical Analysis

Data are expressed as the mean ± SD. Results were analyzed using the Student's *t*-test, nonparametric Mann-Whitney, and ANOVA. Values were considered to be significantly different when *P* was less than 0.05.

RESULTS

A Decreased IL-27 Expression Is Associated with an Increased Frequency of Th17 Cells in Active VKH Patients

PBMCs from active VKH patients, inactive VKH patients, and healthy controls were used to assay the mRNA expression of two subunits of IL-27: IL-27p28 and EBI3. The results showed that the mRNA expression of IL-27p28, a unique IL-27 subunit, was significantly decreased in active VKH patients as compared with inactive VKH patients (*P* = 0.004) and healthy controls (*P* = 0.003). However, there was no difference with regard to the IL-27p28 mRNA expression between inactive VKH patients and healthy controls. Concerning the EBI3 mRNA expression, there was no detectable difference among the three tested groups (Fig. 1a).

The IL-27 level in the serum and supernatants of PBMCs from the aforementioned three groups was assayed using ELISA. The results showed that the IL-27 serum level in active
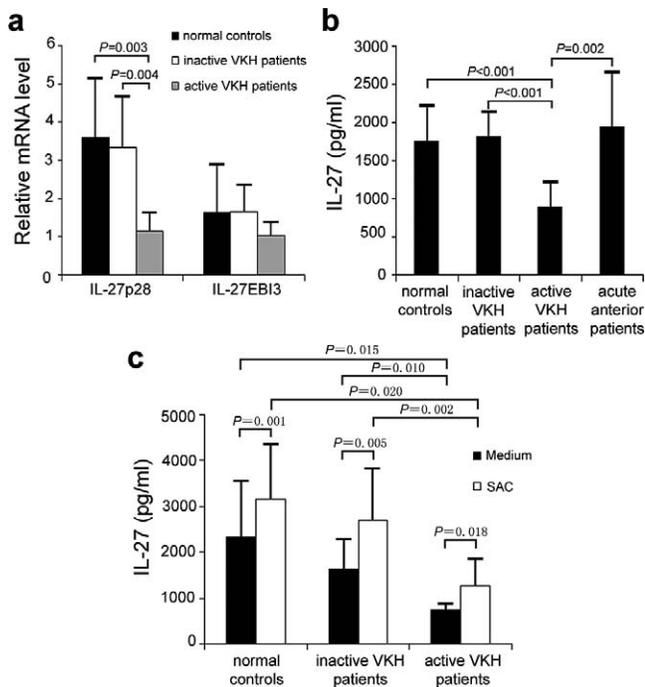
FIGURE 1. IL-27 expression is decreased in active VKH patients. (**a**) Real-time PCR analysis of IL-27p28 mRNA and EBI3 mRNA expression in PBMCs in active VKH patients (*n* = 8), inactive VKH patients (*n* = 8), and healthy controls (*n* = 8). Levels are normalized to β-actin. ANOVA was used for statistical analysis. (**b**) IL-27 serum level in active VKH patients (*n* = 11), inactive VKH patients (*n* = 11), healthy subjects (*n* = 11), and acute anterior uveitis patients (*n* = 7) was measured by ELISA. The Kruskal-Wallis and Mann-Whitney *U* test were used for statistical analysis. (**c**) IL-27 level in the supernatants of PBMCs from active VKH patients (*n* = 9), inactive VKH patients (*n* = 9), and healthy control subjects (*n* = 9) cultured with or without SAC was detected by ELISA. Paired-sample *t*-test for related samples and ANOVA for independent samples were used for statistical analyses (error bars, SD).

VKH patients was significantly lower than that observed in inactive patients (*P* < 0.001) and healthy controls (*P* < 0.001). There was no significant difference between inactive VKH patients and healthy controls concerning the IL-27 serum level. Seven patients with idiopathic active acute anterior uveitis served as a separate control group to evaluate whether the decreased IL-27 expression was also present in another active uveitis entity. The results showed that the IL-27 serum level in these patients was similar to that observed in the inactive VKH patients and healthy controls (Fig. 1b). To determine whether there was a difference concerning the level of IL-27 produced by PBMCs from VKH patients and healthy controls, PBMCs from VKH patients and healthy controls were stimulated with SAC or cultured in medium alone. The results showed that although a basal level of IL-27 was detected in the unstimulated PBMCs from all the patients and controls, a significantly lower expression of IL-27 by PBMCs was noted in active VKH patients as compared with inactive patients (*P* = 0.010) and healthy controls (*P* = 0.015). IL-27 production by PBMCs was markedly increased on SAC stimulation in all the patients and controls. The IL-27 level was, however, still significantly lower in active VKH patients than that in inactive VKH patients (*P* = 0.002) and healthy controls (*P* = 0.020) following SAC stimulation (Fig. 1c). There was no difference concerning the relative percentage increase of IL-27 among the three tested groups.

We subsequently examined the frequency of IL-17–producing CD4+ T cells in PBMCs after stimulation with PMA and ionomycin. The results showed a significantly increased

frequency of IL-17–producing CD4+ T cells in PBMCs from active VKH patients as compared with inactive VKH patients (*P* = 0.006) and healthy controls (*P* = 0.006). No significant difference was observed between inactive VKH patients and healthy controls concerning the frequency of Th17 cells in PBMCs (Figs. 2A, 2B). ELISA analysis of the supernatants of stimulated PBMCs from VKH patients and healthy controls revealed a similar higher production of IL-17 by PBMCs from active VKH patients as compared with inactive VKH patients (*P* < 0.001) and healthy controls (*P* < 0.001; Fig. 2C).

Recombinant IL-27 Inhibits Th17 Cell Differentiation and IL-17 Production in Both VKH Patients and Healthy Controls

Previous studies have revealed that IL-27 is a negative regulator of Th17 cell differentiation in animals.[10,18,19] The aforementioned results showed a decreased expression of IL-27 and an increased frequency of IL-17–producing CD4+ T cells and increased IL-17 expression by PBMCs from active VKH patients. Further experiments were designed to examine the influence of IL-27 on Th17 cell differentiation and IL-17 expression by naïve CD4+ T cells from VKH patients and healthy controls. Highly purified naïve CD4+ T cells from VKH patients with or without active uveitis and healthy controls were stimulated with anti-CD3/CD28 under Th17 polarizing conditions in the presence or absence of rIL-27. Intracellular staining analysis revealed that a significant increased frequency of IL-17–producing CD4+ T cells from naïve CD4+ T cells under Th17 polarizing conditions was observed in active VKH patients as compared with that from inactive VKH patients (*P* = 0.024) and healthy controls (*P* = 0.024). There was no significant difference between inactive VKH patients and healthy controls. The addition of rIL-27 significantly inhibited the frequency of IL-17–producing CD4+ T cells from naïve CD4+ T cells under Th17 polarizing conditions from VKH patients with (*P* = 0.043) or without (*P* = 0.002) active uveitis and healthy controls (*P* = 0.043). However, no detectable difference was observed concerning the inhibitory percentages among the three groups (Figs. 3A, 3B). We further examined the IL-17 expression in the cell supernatants of stimulated naïve CD4+ T cells by ELISA and showed results similar to those obtained by flow cytometry (Fig. 3C).

As DCs can lead to the differentiation of naïve CD4+ T cells into Th17 cells, we performed a set of experiments to elucidate the role of IL-27 in this pathway. Immature DCs generated from monocytes stimulated with GM-CSF and IL-4 were treated with LPS alone or LPS and IL-27 for 24 hours and then cocultured with naïve CD4+ T cells for IL-17 measurement by flow cytometry and ELISA. The results showed that IL-27–treated DCs significantly inhibited the frequency of IL-17–producing CD4+ T cells as compared with control DCs (*P* = 0.009; Figs. 4A, 4B). ELISA analysis revealed that the expression of IL-17 in the culture supernatants was also decreased in naïve CD4+ T cells cocultured with IL-27–treated DCs as compared with those cocultured with control DCs (*P* = 0.003, Fig. 4C). The levels of IL-17 were under the detection limit in the supernatants of naïve CD4+ T cells alone.

IL-27 Induces IL-10 Production by Naïve CD4+ T Cells

To determine the effect of IL-27 on the immunosuppressive cytokine IL-10, naïve CD4+ T cells were stimulated with anti-CD3/CD28 and IL-2 in the presence or absence of IL-27. The results showed that rIL-27 significantly induced the expression of IL-10 (*P* = 0.014, Fig. 5A). We further analyzed IL-10
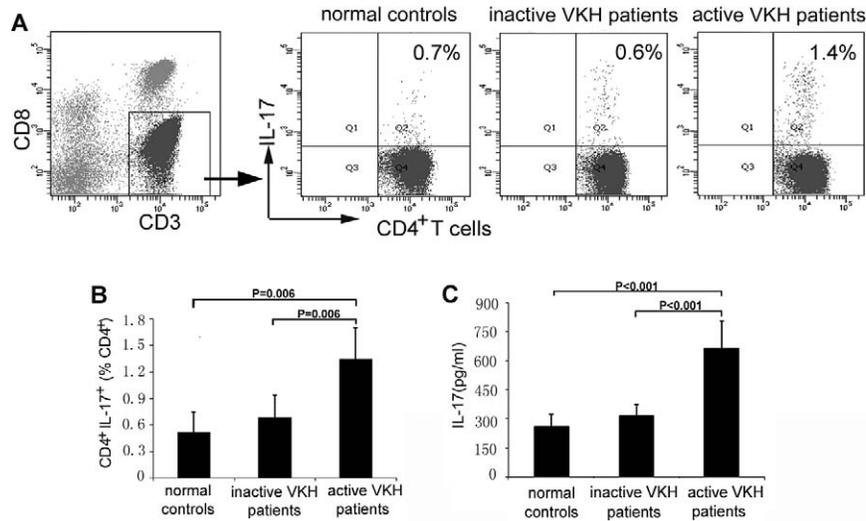
FIGURE 2. The frequency of IL-17–producing CD4^{+} T cells and IL-17 production was increased in active VKH patients. (**A**) Detection of intracellular expression of IL-17 in PBMC from active VKH patients ($n = 6$), inactive VKH patients ($n = 6$), and healthy control subjects ($n = 6$) by flow cytometry, dot plots of a representative subject for each group. The Kruskal-Wallis and Mann-Whitney tests were used for statistical analyses. (**B**) Quantitative analysis of the percentage of IL-17–expressing CD4^{+} T cells. (**C**) IL-17 production in the supernatants of PBMCs from active VKH patients ($n = 6$), inactive VKH patients ($n = 6$), and healthy control subjects ($n = 6$) cultured with anti-CD3/CD28 for 72 hours was detected by ELISA. ANOVA was used for statistical analysis (error bars, SD).
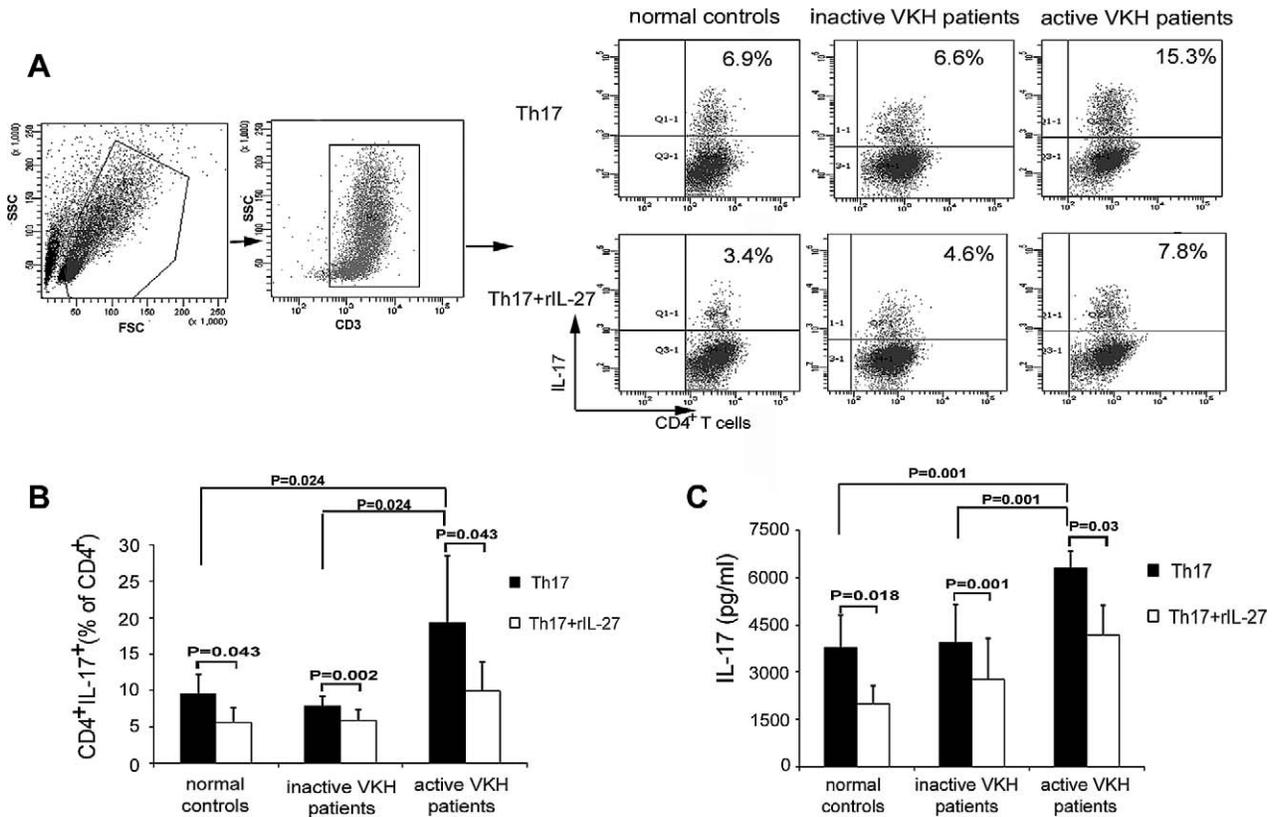

FIGURE 3. Recombinant IL-27 inhibited Th17 cell differentiation in both VKH patients and healthy controls. Naive CD4+ T cells from active VKH patients ($n = 7$), inactive VKH patients ($n = 7$), and healthy controls ($n = 7$) were stimulated with anti-CD3, anti-CD28, rIL-2, and Th17 polarizing conditions (rIL-1β, rIL-6, rIL-23, anti–IL-4, and anti–IFN-γ) in the presence or absence of rIL-27 for 6 days. (**A**) Detection of intracellular expression of IL-17 in these stimulated naïve CD4^{+}T cells by flow cytometry, dot plots of a representative subject for each group. (**B**) Quantitative analysis of the percentage of IL-17–expressing CD4^{+} T cells. (**C**) IL-17 production in the supernatants was detected by ELISA. Paired-sample *t*-test for related samples and Mann-Whitney test for independent samples were used for statistical analyses (error bars, SD).
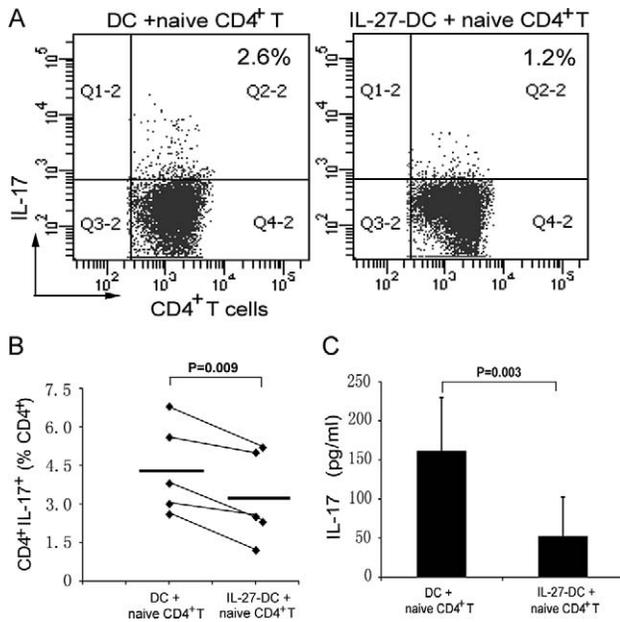
FIGURE 4. Allogeneic naïve CD4$^+$ T cells ($n = 5$) were isolated and cocultured with DCs or IL-27–treated DCs for 7 days. (A) Detection of intracellular expression of IL-17 in those naïve CD4$^+$ T cells by flow cytometry, dot plots of a representative subject. (B) Quantitative analysis of the percentage of IL-17–producing CD4$^+$ T cells. (C) Detection of IL-17 production in the culture supernatants by ELISA. Paired-sample *t*-test for related samples was used for statistical analyses (error bars, SD).

Upregulated IL-27 Production by Corticosteroids and CsA Is Associated with Regression of Intraocular Inflammation in VKH Patients

As corticosteroids and CsA are both effective in the treatment of VKH disease, experiments were designed to examine whether this was associated with changes in the expression of IL-27. The IL-27 level in the serum and supernatants of PBMCs from seven active VKH patients before and after treatment was assayed using ELISA. The results showed that the serum level of IL-27 from patients after treatment was significantly increased as compared with that before treatment ($P = 0.012$, Fig. 6A). An increased expression of IL-27 by PBMCs with or without SAC stimulation was also observed following this treatment as compared with that before treatment ($P = 0.026$, $P = 0.007$ respectively, Fig. 6B). An experiment with PBMCs from six healthy controls was used to examine the influence of dexamethasone and CsA on the IL-27 expression in vitro. The results showed that corticosteroids significantly enhanced the expression of IL-27 by PBMCs and the stimulated effect seemed to reach its plateau at a concentration of 50 ng/mL (Fig. 6C); however, we failed to find any influence of CsA on the expression of IL-27 by PBMCs at the tested concentrations. An experiment with corticosteroids at a concentration of 5 ng/mL, which was able to stimulate the expression of IL-27, was used to evaluate whether CsA at different concentrations could lead to a further stimulation of "already activated PBMCs" to produce IL-27. The results showed that CsA did not have any influence on the expression of IL-27 by the PBMCs already "activated" by corticosteroids at the three tested concentrations (Fig. 6D).

DISCUSSION

In this study, we showed a significantly decreased expression of IL-27 in association with an increased Th17 cell response in active VKH patients. We further revealed that IL-27 could significantly inhibit Th17 cell differentiation in a direct manner as well as by modulating DCs and that it was able to induce IL-10 production by naïve CD4$^+$ T cells. Additionally, treatment with corticosteroids and CsA resulted in a resolution of

production by IL-27–treated naïve CD4$^+$ T cells from VKH patients with or without active uveitis and healthy controls. A significantly decreased expression of IL-10 by IL-27–treated naïve CD4$^+$ T cells was observed in active VKH patients as compared with inactive VKH patients ($P = 0.001$) and healthy controls ($P = 0.030$). No detectable difference was observed between inactive VKH patients and healthy controls (Fig. 5B).
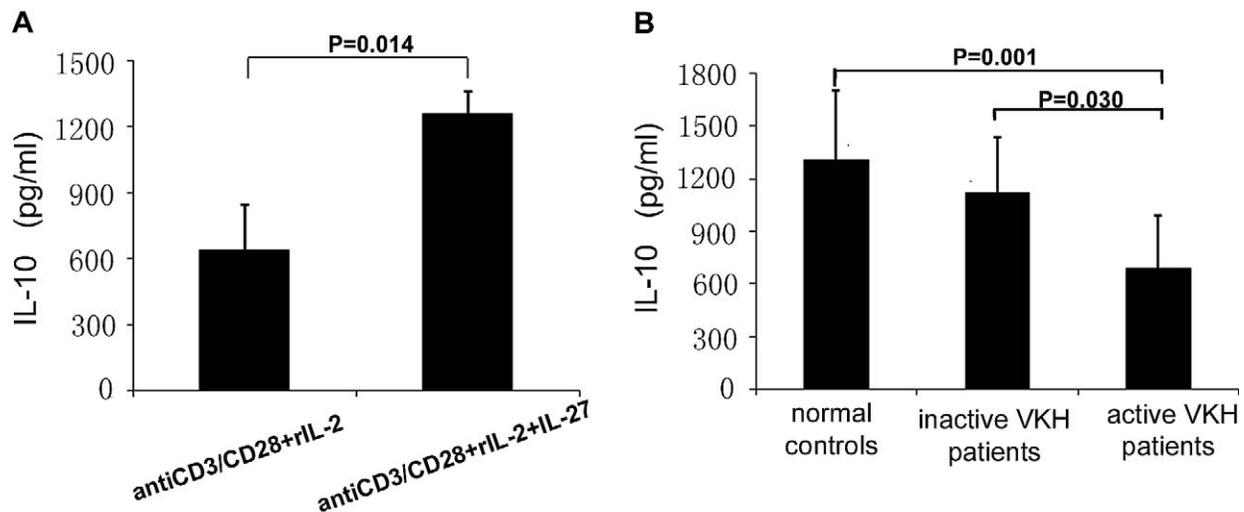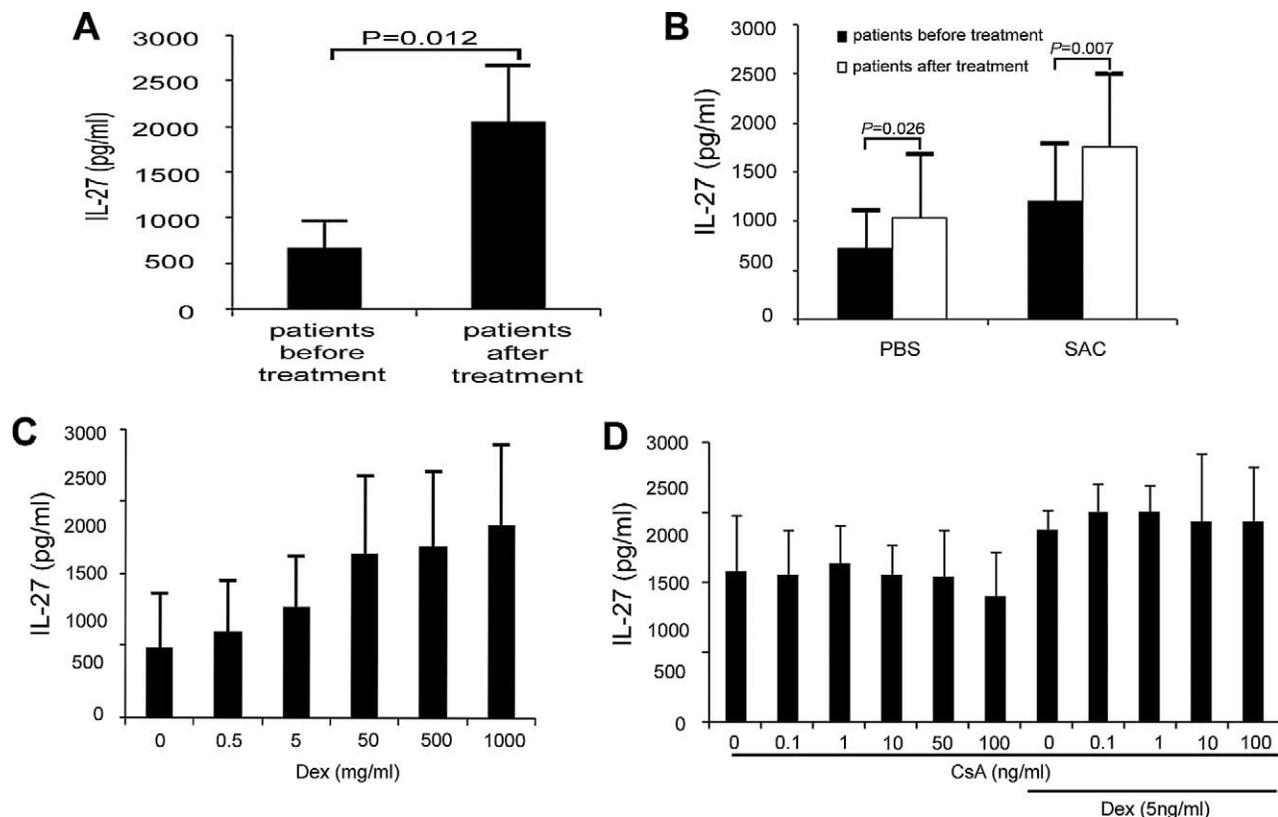

FIGURE 5. IL-27 induces IL-10 production by naïve CD4$^+$ T cells. (A) Detection of IL-10 in the supernatants of naïve CD4$^+$ T cells from healthy controls ($n = 5$) stimulated with anti-CD3/CD28 and rIL-2 in the presence or absence of IL-27 for 7 days by ELISA. Paired-sample *t*-test for related samples was used for statistical analyses. (B) Detection of IL-10 in the supernatants of naïve CD4$^+$ T cells from active VKH patients ($n = 8$), inactive VKH patients ($n = 8$), and healthy controls ($n = 9$) stimulated with anti-CD3/CD28, rIL-2, and IL-27 for 7 days by ELISA. ANOVA was used for statistical analysis (error bars, SD).

FIGURE 6. The effect of corticosteroids and CsA on the production of IL-27 in vivo and in vitro. (**A**) IL-27 serum level from VKH patients ($n = 7$) before and after treatment. (**B**) IL-27 production by PBMCs from VKH patients ($n = 7$) before and after treatment cultured with or without SAC for 72 hours. (**A**, **B**) Paired *t*-test was used for statistical analysis. (**C**) IL-27 production by cultured PBMCs from healthy controls ($n = 6$) in the presence of different concentrations of dexamethasone (DEX) for 72 hours. (**D**) IL-27 production by cultured PBMCs from healthy controls ($n = 5$) in the presence of different concentrations of CsA alone or combined with 5 ng/mL DEX for 72 hours. All IL-27 expression was measured by ELISA (error bars, SD).

intraocular inflammation in association with an increased IL-27 expression in VKH patients. Finally, in vitro experiments showed that PBMC expression of IL-27 was induced by corticosteroids, but not by CsA. These results seem to show that a decreased IL-27 expression may be involved in the pathogenesis of VKH disease by modulating the Th17 cell response.

VKH disease is an autoimmune disease that provides a good model to study the role of cytokines during inflammation. The transparency of the ocular media allows a noninvasive measurement of the inflammatory activity. Earlier studies concerning the role of cytokines in uveitis have included different disease entities, which might be mediated by different immunopathologic pathways. The abundance of VKH patients in our hospital allows us to study the role of cytokines in a single disease entity. Here we investigated the role of IL-27 in the development of VKH disease. The first experiment showed a decreased IL-27P28 mRNA in PBMCs in active VKH patients. However, the expression of EBI3, another subunit of IL-27, was not different among the three tested groups. Our findings are in line with studies showing that IL-27 secretion is dependent on regulated transcription of the unique subunit IL-27p28, whereas the subunit EBI3 is constitutively expressed. Consistent with the mRNA results, a significant decreased level of IL-27 protein in serum and supernatants of stimulated PBMCs was observed in active VKH patients. Furthermore, the decreased IL-27 expression was detected only in active VKH disease but not in acute anterior uveitis and these results seem to suggest that a decreased IL-27 expression may be an important

mechanism involved in VKH development. Our results are in line with evidence showing a decreased IL-27 expression in patients with active systemic lupus erythematosus.[24] However these findings are in disagreement with animal studies showing an increased IL-27 expression in PBMCs and retinas from EAU mice as compared with control mice. The discrepant findings may be due to differences in the immunological mechanisms between clinical uveitis and animal models. Moreover, we found that an increased expression of IL-17 and frequency of Th17 cells in PBMCs was observed in active VKH patients. The higher circulating IL-17 levels in blood may originate from TH17 cells, although part of the IL-17 production may also be attributed to NK cells.[25] These data collectively suggest that the decreased IL-27 expression in association with increased Th17 cells in active patients, as observed in the present study, correlates with the active intraocular inflammation.

As Th17 cells are necessary for the development of VKH disease, we further evaluated the correlation of IL-27 with the Th17 cell response and the influence of IL-27 on these cells. The results showed that a decreased IL-27 expression correlated with an upregulated Th17 cell response in active VKH patients. We found that rIL-27 was able to significantly inhibit Th17 cell differentiation in a direct manner as well as by modulating DCs. Our results are consistent with previous studies showing that IL-27 could inhibit IL-17 production.[18,26] Taken together, it is likely that the upregulated Th17 cell response, which is critical to VKH disease development, is, at least partially, attributed to the decreased IL-27 expression.

It has been reported that IL-27 could induce Tr1 cells in humans, evidenced by promoting IL-10 production. Our experiments showed that IL-27 significantly induced the release of IL-10 from naïve CD4^{+} T cells on stimulation with antiCD3/CD28 and IL-2, a Tr1 cell polarizing condition. Similar findings have been observed earlier by others.[26] Interestingly, we found a decreased IL-10 production by naïve CD4^{+} T cells under Tr1 cell polarizing conditions in active VKH patients as compared with inactive VKH patients and healthy controls. This result suggests that VKH patients have a decreased sensitivity in their ability to induce Tr1 cells from naïve CD4^{+} T cells on IL-27 stimulation. This observation is similar to findings observed in multiple sclerosis, whereby it was shown that there was a defect in the induction of Tr1 cells on CD46 activation, as evidenced a defect in the IL-10 production.[27] Earlier we reported a decreased frequency and function of CD4^{+}CD25^{high} Treg in active VKH patients.[28] These results seem to suggest that a deficiency in the immunoregulatory mechanisms as represented by CD4^{+}CD25^{high} Treg cells and IL-27–induced Tr1 cells combined with an increase in the expression of proinflammatory factors, such as an enhanced Th17 cell response, may all contribute to the development of VKH disease.

Since the treatment of VKH patients with corticosteroids combined with CsA generally leads to a resolution of the intraocular inflammation, we further examined whether these drugs could regulate the expression of IL-27. Here we showed that the resolution of intraocular inflammation after treatment with corticosteroids and CsA was associated with an increased serum IL-27 level. Previously we showed that corticosteroids and CsA were able to inhibit Th17 cell responses.[29] The in vitro experiments showed that only corticosteroids, but not CsA, promoted the "in vitro" production of IL-27. These results suggest that immunosuppressive agents can resolve intraocular inflammation in autoimmune disease by upregulating IL-27 and downregulating the Th17 cell response. On the other hand, it is likely that different immunosuppressive agents may have distinctive mechanisms in the treatment of VKH disease.

In conclusion, our study shows that a deficiency in the IL-27 expression was associated with disease activity in VKH disease and that this may lead to an increased Th17 cell response. Our study provides preliminary data supporting the rationale for IL-27 treatment of diseases mediated by a Th17 cell response.

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