Upregulation of Interleukin 21 and Promotion of Interleukin 17 Production in Chronic or Recurrent Vogt-Koyanagi-Harada Disease

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Objectives: To analyze the expression and potential role of interleukin (IL) 21 in the pathogenesis of Vogt-Koyanagi-Harada (VKH) disease.

Methods: Blood samples were obtained from patients with VKH disease and from healthy control subjects. Serum IL-21 level and IL-21 messenger RNA (mRNA) expression by peripheral blood mononuclear cells (PBMCs) were determined by enzyme-linked immunosorbent assay and by reverse transcriptase–polymerase chain reaction, respectively. Interleukin 17 and interferon γ levels in the supernatants of PBMCs and CD4+ T cells cultured with anti-CD3 and anti-CD28 antibodies in the presence or absence of recombinant IL-21 were detected by enzyme-linked immunosorbent assay.

Results: The results showed a significantly increased serum IL-21 level, as well as higher IL-21 mRNA expression by PBMCs, in patients having chronic or recurrent active VKH disease compared with patients having inactive VKH disease and with controls. In vitro experiments showed that recombinant IL-21 significantly increased IL-17 production by PBMCs and by CD4+ T cells from patients and from controls. However, recombinant IL-21 did not affect interferon γ expression by PBMCs or by CD4+ T cells.

Conclusion: Interleukin 21 may be involved in the pathogenesis of chronic or recurrent VKH disease, possibly by promoting IL-17 secretion.

Clinical Relevance: Findings from the present study suggest that IL-21 may be a potential target in the development of therapy for VKH disease.


Vogt-Koyanagi-Harada (VKH) disease is a systemic autoimmune disorder involving the eye, meninges, ear, skin, and hair. Bilateral granulomatous panuveitis is the hallmark of VKH disease and frequently results in severely decreased vision or even blindness if not treated appropriately. The pathogenesis of VKH disease is not completely understood, although extensive investigations have been performed. Results of previous studies suggested that CD4+ helper T cells, subtype 1 (Th1s) are involved in the development of VKH disease. Recent evidence from our group indicated that the CD4+ Th17 and interleukin (IL) 23 pathway may also have an active role in this disease.

Findings from recent studies revealed that IL-21 promotes the differentiation of Th17s in autoimmune diseases. Interleukin 21, a member of the IL-2 family of cytokines, exerts pleiotropic effects on the immune system and has a role in the pathogenesis of several autoimmune diseases, including inflammatory bowel diseases, rheumatoid arthritis, psoriasis, multiple sclerosis, systemic sclerosis, systemic lupus erythematosus, and type 1 diabetes mellitus in nonobese diabetic mice. Distinct aspects of IL-21 are that it is mainly derived from activated CD4+ T cells and that it regulates the development of CD4+ T cells. Earlier studies showed that IL-21 was associated with a predominant Th1 response and with increased interferon (IFN) γ production. The present study was designed to examine whether IL-21 is involved in VKH disease and how it affects IL-17 and IFN-γ expression.

Methods

Patients

Thirty patients with VKH disease (14 men and 16 women [mean age, 33.4 years]) and 21
healthy control subjects (10 men and 11 women [mean age, 32.5 years]) were included in the study. The diagnosis of VKH disease was made according to the revised diagnostic criteria. Among the patients with VKH disease, 15 had complete VKH disease, 13 had incomplete VKH disease, and 2 had probable VKH disease with typical bilateral sunset glow fundus and granulomatous anterior uveitis. Sixteen patients had chronic or recurrent active bilateral intraocular inflammation evidenced by mutton-fat keratic precipitates, cells in the anterior chamber, and aqueous flare in association with sunset glow fundus. Patients with active disease had received no immunosuppressive drugs for at least 1 week before study evaluation and blood drawing. Fourteen patients having inactive VKH disease with typical bilateral sunset glow fundus but without intraocular inflammation for at least 3 months after treatment formed a separate study group. Twelve patients with inactive disease had received no immunosuppressive drugs for at least 3 months, while 2 patients with inactive disease had received a small dosage of oral prednisone (5 mg every other day) for 1 month before blood drawing. This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University, Chongqing, China. All procedures followed the tenets of the Declaration of Helsinki, and informed consent was obtained from all patients with VKH disease and from controls.

SERUM SAMPLE PREPARATION

Blood samples were collected by venipuncture and were processed after clotting for 30 minutes at room temperature. Serum specimens were obtained by centrifugation at 3000g for 10 minutes and stored at −70°C until analysis.

CELL ISOLATION AND CULTURE

Anticoagulated blood samples were obtained using vacuum tubes containing EDTA. Peripheral blood mononuclear cells (PBMCs) were prepared by Ficoll-Hypaque density-gradient centrifugation. CD4+ T cells were purified by magnetic-activated cell sorting using a human CD4+ T-cell isolation kit (Miltenyi Biotec, Palo Alto, California). The PBMCs and CD4+ T cells were stimulated with anti-CD3 (OKT3, 0.5 µg/mL) and anti-CD28 antibodies (15E8, 0.1 µg/mL) (Miltenyi Biotec) in the presence or absence of recombinant (r) IL-21 (30 ng/mL) (PeproTech, Rocky Hill, New Jersey).

Figure 1. Interleukin 21 (IL-21) levels in serum samples from patients with chronic or recurrent active Vogt-Koyanagi-Harada (VKH) disease (n=8), patients with inactive VKH disease (n=8), and healthy control subjects (n=10). Kruskal-Wallis test and Mann-Whitney test were used for statistical analyses.

RNA PREPARATION AND REVERSE TRANSCRIPTASE–POLYMERASE CHAIN REACTION

Total RNA was extracted from freshly isolated PBMCs using a commercially available kit (RNeasy Plus Mini kit; Qiagen, Valencia, California) according to the manufacturer’s instructions. The following primers were used for reverse transcriptase–polymerase chain reaction (RT-PCR): IL-21 forward, 5'-GGCCCACTAAAGTCAGCAATA-3', and IL-21 reverse, 5'-GGGAGTATTCTGTGTTTCTCG-3' (38 cycles at 62°C); β-actin forward, 5'-GGATGCAAGAGATGACCTTG-3'; and β-actin reverse, 5'-CGATCCACAGGAGTACCTTG-3' (30 cycles at 60°C). The ratio of IL-21 RT-PCR product to β-actin product was analyzed to quantify the level of IL-21 messenger RNA (mRNA) expression. The identity of IL-21 and β-actin RT-PCR products was verified by sequencing using a commercially available system (Applied Biosystems model 3730 DNA sequencing system; Invitrogen Biotechnology Co, Shanghai, China).

ENZYME-LINKED IMMUNOSORBENT ASSAY FOR CYTOKINES

The concentration of serum IL-21 in patients and controls was assayed using a human IL-21 enzyme-linked immunosorbent assay kit (DuoSet; eBioscience, San Diego, California) with a detection limit of 31 pg/mL. The concentrations of IL-17 and IFN-γ in cell culture supernatants were measured using enzyme-linked immunosorbent assay development kits (DuoSet; R&D Systems, Minneapolis, Minnesota) with a detection limit of 15 pg/mL.

STATISTICAL ANALYSIS

One-way analysis of variance, paired-sample t test, Kruskal-Wallis test, and Mann-Whitney test were performed using commercially available statistical software (SPSS 12.0; SPSS Inc, Chicago, Illinois). Data are expressed as the mean (SD). P < .05 was considered statistically significant, while P < .05 divided by 3 (P < .02 [modified by Bonferroni correction]) was accepted in multiple comparisons.

RESULTS

Our results suggest that IL-21 is markedly upregulated during active uveitis episodes. Furthermore, rIL-21 may have a role in chronic or recurrent VKH disease by promoting IL-17 production.

IL-21 CONCENTRATION IN SERUM SAMPLES OF PATIENTS WITH VKH DISEASE AND CONTROLS

Interleukin 21 was detected in serum samples from patients with VKH disease and from controls (Figure 1). The mean level of IL-21 in patients with chronic or recurrent active VKH disease was significantly higher (212.2 [67.9] pg/mL) than that in patients with inactive VKH disease (90.1 [23.9] pg/mL) and in controls (90.8 [27.0] pg/mL) (P < .01 for both). There was no significant difference in serum IL-21 level between patients with inactive VKH disease and controls.
EFFECT OF rIL-21 ON IL-17 PRODUCTION

Interleukin 17 production by peripheral blood mononuclear cells (PBMCs) and by CD4\(^+\) T cells. Cells were cultured with anti-CD3 and anti-CD28 antibodies in the presence or absence of recombinant IL-21 for 72 hours. A, Interleukin 17 production by PBMCs from patients with chronic or recurrent active Vogt-Koyanagi-Harada (VKH) disease (n=7), patients with inactive VKH disease (n=7), and healthy control subjects (n=9). B, Interleukin 17 production by CD4\(^+\) T cells from patients with chronic or recurrent active VKH disease (n=7), patients with inactive VKH disease (n=7), and healthy control subjects (n=9). Paired-sample t test for related samples and Mann-Whitney test for independent samples were used for statistical analyses.

patients having chronic or recurrent active VKH disease compared with patients having inactive VKH disease and with controls (P<.01 for both). Significant augmentation of IL-17 production by PBMCs on exposure to rIL-21 was found in all 3 study groups (P<.05 for all) (Figure 3A). Increased percentages of IL-17 production by PBMCs on stimulation by rIL-21 were 19.1% in patients with chronic or recurrent active VKH disease, 17.0% in patients with inactive VKH disease, and 25.5% in controls. Using analysis of variance, there was no difference among the 3 study groups in the magnitude of rIL-21 effect on IL-17 production by PBMCs. A further study was performed to examine the effect of rIL-21 on IL-17 production by activated CD4\(^+\) T cells. Consistent with the BMC results, the IL-17 level in the supernatants of cultured CD4\(^+\) T cells was significantly higher in patients with chronic or recurrent active VKH disease than in patients with inactive VKH disease or in controls (P<.01 for both) (Figure 3B). Recombinant IL-21 induced activated CD4\(^+\) T cells to secrete a much higher

EXPRESSION OF IL-21 mRNA BY PBMCs FROM PATIENTS WITH VKH DISEASE AND FROM CONTROLS

DNA sequencing and BLAST analysis (http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/information3.html) showed that RT-PCR products were identified as 100% homologous with the known IL-21 mRNA sequence. The IL-21 mRNA band intensity was normalized compared with the β-actin mRNA band. The mean intensity ratio of IL-21 RT-PCR product to β-actin product in patients with chronic or recurrent active VKH disease (0.68 [0.11]) was markedly higher than that in patients with inactive VKH disease (0.52 [0.08]) and in controls (0.52 [0.03]) (P<.01 for both) (Figure 2). No ratio difference was found between patients with inactive VKH disease and controls.

A, Reverse transcriptase–polymerase chain reaction gel images from patients with chronic or recurrent active VKH disease (n=8), patients with inactive VKH disease (n=8), and healthy control subjects (n=8). B, Quantitative analysis of reverse transcriptase–polymerase chain reaction data using commercially available software (Quantity One; Bio-Rad, Hercules, California). Values for β-actin were set to 1. bp indicates base pair; mRNA, messenger RNA. Analysis of variance was used for statistical analyses.
among the 3 study groups using the Kruskal-Wallis test. The present study investigated the expression of IL-21 in patients with VKH disease and its effect on the expression of IL-17 and IFN-γ. The results revealed significantly higher expression of IL-21 at the protein and mRNA levels in patients having chronic or recurrent active VKH disease compared with patients having inactive VKH disease and with controls. Recombinant IL-21 significantly stimulated IL-17 secretion by PBMCs and by CD4+ T cells from patients and from controls. However, it did not affect IFN-γ production by PBMCs or by CD4+ T cells.

As a multifunctional cytokine produced mostly by activated natural killer T cells and by CD4+ T cells, IL-21 is involved in various immune inflammatory diseases. To test whether IL-21 has a role in the development of VKH disease, we evaluated the expression of IL-21 at the protein and mRNA levels in patients having VKH disease or without active uveitis and in controls. Increased expression of IL-21 was observed in serum samples of patients with chronic or recurrent active VKH disease. Consistent with serum specimen results, significantly higher IL-21 mRNA expression was observed in patients with chronic or recurrent active VKH disease. These results indicated that increased expression of peripheral circulating IL-21 correlated with VKH disease activity. Our findings are in line with evidence showing increased IL-21 expression in serum or PBMCs in animal models and in patients with systemic lupus erythematosus, primary Sjögren syndrome, and psoriasis. Interleukin 21 has also been reported to be overexpressed in affected tissues of patients with Crohn disease and with psoriasis. These data collectively reveal that IL-21 is associated with the activity of autoimmune diseases. It is notable that serum IL-21 reference levels from difference laboratories range from 16.5 pg/mL to 1051 pg/mL. This wide range may be attributed to different techniques used in the experiments or to different populations tested.

In light of the association between increased serum IL-21 and VKH disease activity, we extended our research to the role of IL-21 in this disease. Because Th17s have a role in the pathogenesis of VKH disease, we first analyzed the effect of IL-21 on the production of IL-17. Consistent with a previous study, increased IL-17 was observed in the active stage of VKH disease, suggesting a correlation between upregulated IL-21 and IL-17 production. The results herein showed that rIL-21 markedly stimulated IL-17 production by PBMCs from patients with VKH disease and from controls. Because CD4+ T cells have a critical role in the development of VKH disease, we further tested the effect of IL-21 on IL-17 production by these cells. Similar results were observed with PBMCs and with isolated CD4+ T cells. These findings validate the promotional effect of rIL-21 on IL-17 production, as reported in various autoimmune diseases. Unexpectedly, we failed to find any difference in the magnitude of the effect of rIL-21 on IL-17 production among the 3 study groups. These results seem

**Figure 4.** Interferon γ (IFN-γ) production by peripheral blood mononuclear cells (PBMCs) and by CD4+ T cells. Cells were cultured with anti-CD3 and anti-CD28 antibodies in the presence or absence of recombinant interleukin 21 for 72 hours. A, Interferon γ production by PBMCs from patients with chronic or recurrent active Vogt-Koyanagi-Harada (VKH) disease (n=7), patients with inactive VKH disease (n=7), and healthy control subjects (n=9). B, Interferon γ production by CD4+ T cells from patients with chronic or recurrent active VKH disease (n=7), patients with inactive VKH disease (n=7), and healthy controls (n=9). Paired-sample t test for related samples and Mann-Whitney test for independent samples were used for statistical analyses.

amount of IL-17 (P < .05 for all). However, similar to the PBMC results, no difference was found in the magnitude of rIL-21 effect on IL-17 production by CD4+ T cells among the 3 study groups using the Kruskal-Wallis test.

**EFFECT OF rIL-21 ON IFN-γ PRODUCTION**

We also detected IFN-γ in the supernatants of PBMCs and CD4+ T cells following stimulation with anti-CD3 and anti-CD28 antibodies (Figure 4). Interferon γ production by PBMCs and by CD4+ T cells was significantly higher in patients with chronic or recurrent active VKH disease than in patients with inactive VKH disease and in controls (P < .02 for all). However, rIL-21 did not affect IFN-γ production by PBMCs and by CD4+ T cells in patients with VKH disease or in controls.
to suggest that increased IL-17 production by PBMCs or by CD4+ T cells may be due to upregulated IL-21 rather than by higher sensitivity of these cells to this cytokine in this disease. In contrast, other studies24,25 failed to observe an effect of IL-21 on T17s. This discrepancy may be explained by different experimental approaches and varied disease backgrounds in the studies.

Because T17s are actively involved in VKH disease,2,3 our study further examined whether rIL-21 affected the expression of IFN-γ, a typical cytokine of T17s, in patients with VKH disease. The results showed significantly increased IFN-γ production by PBMCs and CD4+ T cells activated by anti-CD3 and anti-CD28 antibodies in patients having chronic or recurrent active VKH disease compared with patients having inactive VKH disease and with controls. This finding was consistent with previously reported results.6 However, we found no effect of rIL-21 on IFN-γ production by PBMCs or by CD4+ T cells from patients with VKH disease and from controls. This result was generally consistent with findings in healthy individuals.6,13 However, IL-21 has been shown to potently promote IFN-γ expression by activated T cells from patients with rheumatoid arthritis13 and with inflammatory bowel diseases.6 Contrary to these results, other investigators have suggested a suppressive effect of IL-21 on IFN-γ production by T17s in their developmental stage26 or by T cells in 2 animal models of rheumatoid arthritis treated with IL-21 receptor Fc fusion protein to block IL-21.12 These discrepant findings are not yet completely understood. The involvement of different mechanisms in different diseases may be an explanation.

In conclusion, the present study revealed an association of upregulated IL-21 with increased IL-17 in patients having chronic or recurrent VKH disease with active intraocular inflammation. Recombinant IL-21 effectively and equally stimulated IL-17 production by PBMCs and by CD4+ T cells from patients with VKH disease and from controls. However, rIL-21 did not affect IFN-γ expression by PBMCs or by CD4+ T cells. These results suggest that IL-21 may be involved in the pathogenesis of chronic or recurrent VKH disease, possibly by promoting IL-17 secretion. Recent evidence indicates that IL-21 is critical for differentiation and function of follicular T17s and that these cells are also involved in the development of autoimmune diseases.27 Future studies should investigate whether IL-21 may have a role in VKH disease through modulation of follicular T17s. In addition, IL-21 has been shown to affect CD8+ T cells, natural killer T cells, and B cells, which are all involved in autoimmune diseases.10 More studies are needed to clarify the effect of IL-21 on these cells and their possible role in VKH disease.

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From the Archives of the Archives

Iridencleisis in chronic glaucoma. HOLTH (Christiana) reports two cases of progressive loss of vision and contraction of visual field in spite of iridectomy and sclerectomy. The disease process was cut short by iridencleisis which was followed by prompt improvement of sight and fields. The incarcration should be combined with flap-shaped or meridional iridotomies unless iridectomy has preceded, wholly subconjunctival and about 10 mm from the limbus. The lance knife is advised. Holth has never lost an eye nor seen sympathetic ophthalmia develop after this procedure.