Increased IL-7 Expression in Vogt-Koyanagi-Harada Disease

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PURPOSE. IL-7/IL-7R has been found to be involved in the pathogenesis of several autoimmune diseases. This study was designed to investigate the potential role of IL-7/IL-7R in the pathogenesis of Vogt-Koyanagi-Harada (VKH), an organ-specific autoimmune disease.

METHODS. IL-7 was measured with an enzyme-linked immunosorbent assay (ELISA) in serum obtained from patients with active or inactive VKH and from healthy individuals. The expression of IL-7/IL-7R was measured by flow cytometry (FCM). Cell proliferation was determined after exposure of peripheral blood mononuclear cells (PBMCs) and CD4+ T cells to recombinant IL-7. The levels of IL-17 and IFN-γ levels were detected by ELISA after these cells were cocultured with recombinant IL-7. The influence of recombinant IL-7 on the expansion of Th1 and Th17 cells was evaluated by using FCM.

RESULTS. IL-7 was significantly increased in the serum of patients with active VKH compared with those with inactive VKH (P < 0.001) and normal controls (P < 0.001). However, there was no difference between VKH patients and normal controls in the expression of IL-7Ra on CD4+ T cells. Recombinant IL-7 induced significant cell proliferation and secretion of IL-17 and IFN-γ by PBMCs and CD4+ T cells. It furthermore promoted the expansion of both Th1 and Th17 cells.

CONCLUSIONS. The findings suggest that IL-7 is involved in the pathogenesis of VKH disease. (Invest Ophthalmol Vis Sci. 2012;53:1012–1017) DOI:10.1167/iovs.11-8505

The cytokine IL-7 is a member of the IL-2 family that includes IL-2, IL-4, IL-9, IL-15, and IL-21. It is produced by stromal cells at lymphopoietic sites in the bone marrow, gut, spleen, thymus, and lymph nodes.1 IL-7 has been shown to be a potent immunoregulatory cytokine promoting the expansion of T-cell precursors,2 increasing the diversity of the T-cell receptor repertoire and maintaining T-cell homeostasis.1,3–6 Recent studies have shown that IL-7 plays a role in the development of several chronic autoimmune diseases. An increased level of IL-7 has been observed both systemically and locally in autoimmune diseases, such as juvenile idiopathic arthritis,7 psoriasis,8 rheumatoid arthritis (RA),9–11 spondylarthropathy,12 type 1 diabetes,13 and Sjögren’s syndrome.14 In animal experiments, IL-7 transgenic mice were shown to develop chronic colitis.15 IL-7 or IL-7Ra blockage may ameliorate the severity of inflammation in various autoimmune disease models, such as chronic colitis,16 multiple sclerosis,17 and RA.18

VKH disease is a multisystem disorder mainly affecting pigmented tissues in the eye, and the auditory, integumentary, and central nervous systems. Bilateral granulomatous panuveitis is the hallmark of VKH disease. It frequently results in severely decreased vision or even blindness if not treated properly.19–21 It has been presumed to be induced by an autoimmune response against melanocytes, although its pathogenesis is still not completely understood. Several studies have indicated that CD4+ T helper cells and their cytokines play an important role in the pathogenesis of this disease.22–25

In view of the effect of IL-7 on CD4+ T helper cells and the involvement of this cytokine in autoimmune disease, in the present study, we investigated whether IL-7 was involved in the pathogenesis of VKH disease. Our results showed that an increased IL-7 expression was associated with VKH disease activity and that it can promote the expansion and cytokine secretion of both Th1 and Th17 cells.

MATERIALS AND METHODS

Patients and Controls

Forty-four patients with VKH disease (24 men and 20 women), with an average age of 39.5 years, and 31 healthy individuals (17 men and 14 women), with an average age of 38.7 years, were included in the study. The diagnosis of VKH disease was made according to the diagnostic criteria revised for VKH disease in an international committee on nomenclature.26 Twenty-one patients had active uveitis, as evidenced by diffuse bilateral choroiditis in association with exudative retinal detachment in the first uveitis attack or by mutton fat keratic precipitates, cells in the anterior chamber, and sunset glow fundus in the VKH patients with recurrent episodes. The systemic findings included headache (47.6%), tinnitus (57.1%), dysacusis (38.1%), poliosis (66.7%), alopecia (52.4%), and vitiligo (23.8%). The patients included in the study did not use immunosuppressive agents for at least 1 week or used only a low dosage of corticosteroids (<20 mg/d) before blood sampling. Twenty-three VKH patients did not have any disease activity for at least 3 months after treatment. This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University, Chongqing, China. All procedures complied with the Dec-
laration of Helsinki, and informed consent was obtained from all patients with VKH disease and controls.

**Serum Preparation**

Serum was obtained by centrifugation at 3000g for 10 minutes after 30 minutes of clotting and was stored at −80°C. At the end of the study, all samples were analyzed simultaneously.

**Cells Isolation and Culture**

PBMCs were isolated from anticoagulant whole blood using density gradient centrifugation (Ficoll-Hypaque; TDBSciences, Tianjin, China). CD4⁺ T cells were purified with a human CD4⁺ T cell isolation kit by magnetic-assisted cell sorting (MACS) according to the manufacturer's instructions (Miltenyi Biotec, Palo Alto, CA). The purity of isolated CD4⁺ T cells, identified by flow cytometry (FCM), was more than 95%. PBMCs and CD4⁺ T cells were resuspended at a concentration of 1 × 10⁵ cells/mL in RPMI 1640 medium (Gibco; Invitrogen, Carlsbad, CA) containing 1-glutamine (2 mM), penicillin/streptomycin (100 U/mL), and 10% fetal calf serum. PBMCs and CD4⁺ T cells were cultured with or without recombinant human IL-7 (rIL-7; R&D Systems, Minneapolis, MN) in the presence of anti-CD3 (OKT3, 0.5 μg/mL) and anti-CD28 antibodies (15E8, 0.1 μg/mL; Miltenyi Biotec).

**Proliferation Assay**

PBMCs and CD4⁺ T cells were treated with or without rIL-7 in the presence of anti-CD3 and anti-CD28 antibodies for 3 days, and proliferation was measured by cell counting (Cell Counting Kit 8; Sigma-Aldrich, St. Louis, MO). Twenty microliters WST-8 (2-(2-methoxy-4-nitrophenyl)-5-(4-nitrophenyl)-2H-tetrazolium, monosodium salt) was added to 200 μL complete culture medium. The absorbance was determined at 450 nm with an ELISA plate reader (SpectraMax M2, Molecular Devices, Sunnyvale, CA).

**Cytokine Analysis**

IL-7 levels were measured in serum from patients with active (n = 19) or inactive (n = 19) VKH and normal controls (n = 20) by using a commercially available high-sensitivity ELISA kit (R&D Systems) according to manufacturer's instructions. Patients were randomly selected and matched with controls according to age and sex. The detection limit of the assay used was 0.25 pg/mL. The expression of IL-17 and IFN-γ in cell culture supernatants was detected using ELISA development kits (DuoSet; R&D Systems, Minneapolis, MN) with a detection limit of 15.6 pg/mL.

**Flow Cytometry**

IL-7Rα expression was analyzed by flow cytometry. Isolated PBMCs were stained with anti-CD3, anti-CD4, and anti-IL-7Rα antibodies or the appropriate isotypes (eBioscience, San Diego, CA) for 30 minutes for the analysis of IL-7Rα expression.

**Statistical Analysis**

Data are expressed as the mean ± SD. The analysis was performed using SPSS 13.0. One-way ANOVA, Student’s t-test, Kruskal-Wallis, and Mann-Whitney U-tests were applied. Differences reaching P < 0.05 were statistically significant.

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**RESULTS**

**Serum IL-7 Is Increased in Patients with Active VKH**

The IL-7 expression in the serum was determined by ELISA. The level of IL-7 was significantly higher in active (20.3 ± 3.1 pg/mL) than in inactive (13.4 ± 1.9 pg/mL) VKH patients and normal controls (10.9 ± 5.1 pg/mL, both P < 0.001). There was no significant difference in serum level of IL-7 between the patients with inactive VKH and the normal controls (P = 0.063; Fig. 1).

IL-7Rα expression on CD4⁺ T cells from PBMCs was evaluated by FCM. The expression, both the percentage and mean fluorescence intensity (MFI), was not different between the patients with active VKH and the normal controls (Fig. 2).

**rIL-7 Promotes Cell Proliferation of PBMCs and CD4⁺ T Cells**

PBMCs separated from the patients with active VKH and the healthy controls were cultured with different concentrations of rIL-7 (0–100ng/mL) in the presence of anti-CD3 and anti-CD28 antibodies for 3 days to detect cell proliferation. rIL-7 induced a dose-dependent proliferation of PBMCs both in the patients with active VKH and the normal controls (Fig. 3A). rIL-7 at 10 ng/mL and 100 ng/mL induced a significant proliferation of PBMCs. A concentration of 10 ng/mL was used in the following experiments. Both PBMCs and CD4⁺ T cells from the patients with active VKH showed a significantly higher cell proliferation compared with those cells in inactive patients and normal controls. rIL-7 (10 ng/mL) promoted significant cell proliferation of PBMCs from all the VKH patients and the normal controls. There was no difference in the ability of rIL-7 to promote cell proliferation among the three groups (Fig. 3B). A similar result was also observed in experiments using CD4⁺ T cells (Fig. 3C).

**rIL-7 Promotes IL-17 Production**

As the serum IL-7 level was increased in the patients with active VKH, we further investigated whether IL-7 induces inflammatory cytokine secretion. PBMCs from the VKH patients were cultured with or without rIL-7, and IL-17 production was measured using ELISA (Fig. 3D).

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**FIGURE 1.** IL-7 levels in serum from patients with active (n = 19), or inactive (n = 19) VKH and normal controls (n = 20) as measured by ELISA.
and the normal controls were cultured with anti-CD3 and -CD28 antibodies in the presence or absence of rIL-7 for 3 days, and supernatants were collected to detect the expression of IL-17. The expression of IL-17 was significantly higher in the patients with active VKH (904.5 ± 281.7 pg/mL) compared with those with inactive VKH (293.3 ± 150.8 pg/mL) and the normal controls (282.7 ± 104.1 pg/mL, both P = 0.002). rIL-7 significantly increased the production of IL-17 by PBMCs in all three groups (active VKH: from 904.5 pg/mL to 1242.4 pg/mL, P = 0.005; inactive VKH: from 293.3 pg/mL to 399.9 pg/mL).

**FIGURE 2.** FCM analysis of IL-7Rα expression on CD4+ T cells from PBMCs. (A) The number of IL-7Rα+ cells among the CD4+ T cells of patients with active VKH and normal controls. Data are representative of six independent experiments. Percentage (B) and MFI (C) of IL-7Rα+ cells among the CD4+ T cells of patients with active VKH (n = 6) and normal controls (n = 6).

**FIGURE 3.** Effect of rIL-7 on the cell proliferation of PBMCs and CD4+ T cells from VKH patients and normal controls. Cells were cultured with anti-CD3 and -CD28 antibodies in the presence or absence of rIL-7 for 3 days, and proliferation was determined. (A) Proliferation of PBMCs from patients with active VKH (n = 6) and normal controls (n = 7) cultured with various concentrations of rIL-7. (B) Proliferation of PBMCs from patients with active (n = 10) or inactive (n = 11) VKH and normal controls (n = 11) cultured with or without rIL-7 (10 ng/mL). (C) Proliferation of CD4+ T cells from patients with active (n = 8) or inactive (n = 8) VKH and normal controls (n = 8), cultured with or without rIL-7 (10 ng/mL).
**FIGURE 4.** Effect of rIL-7 on the IL-17 production by PBMCs and CD4⁺ T cells from VKH patients and normal controls. Cells were cultured with or without rIL-7 in the presence of anti-CD3 and anti-CD28 antibodies for 3 days, and IL-17 was measured by ELISA. (A) IL-17 production by PBMCs from patients with active (n = 10) or inactive (n = 11) VKH and normal controls (n = 11). (B) IL-17 production by CD4⁺ T cells from patients with active (n = 8) or inactive (n = 8) VKH and normal controls (n = 8).

![Image](image-url)

**FIGURE 5.** The effect of rIL-7 on the IFN-γ production by PBMCs and CD4⁺ T cells from VKH patients and normal controls. Cells were cultured with or without rIL-7 in the presence of anti-CD3 and anti-CD28 antibodies for 3 days, and IFN-γ was measured by ELISA. (A) IFN-γ production by PBMCs from patients with active (n = 10) or inactive (n = 11) VKH and normal controls (n = 11). (B) IFN-γ production by CD4⁺ T cells from patients with active (n = 8) or inactive (n = 8) VKH and normal controls (n = 8).

\[ P < 0.001; \text{control: from 282.7 pg/mL to 389.4 pg/mL, } P < 0.001 \]

No difference in the relative increase in IL-17 production was observed between the groups tested. A further study was performed to examine the effect of rIL-7 on the production of IL-17 by isolated CD4⁺ T cells activated by anti-CD3 and -CD28 antibodies. Consistent with the aforementioned results in PBMCs, the production of IL-17 by CD4⁺ T cells was significantly higher in the patients with active VKH (619.6 ± 178.1 pg/mL) than in those with inactive VKH (220.7 ± 85.9 pg/mL) and the normal controls (213.2 ± 57.9 pg/mL, both \( P < 0.001 \)). rIL-7 further increased the secretion of IL-17 by activated CD4⁺ T cells from the patients with active (from 619.6 pg/mL to 1035.7 pg/mL) or inactive (from 220.7 pg/mL to 358.3 pg/mL) VKH and the normal controls (from 213.2 pg/mL to 549.3 pg/mL; all \( P < 0.001 \); Fig. 4). Similar to our results in the experiments using PBMCs, we did not find any difference concerning the ability of rIL-7 to promote the secretion of IL-17 by isolated CD4⁺ T cells among the three groups.

**rIL-7 Promotes IFN-γ Production**

We also measured the expression of IFN-γ in the supernatants of PBMCs and CD4⁺ T cells after stimulation with anti-CD3 and anti-CD28 antibodies. The production of IFN-γ by PBMCs and CD4⁺ T cells was significantly higher in the patients with active VKH (PBMCs: 4.6 ± 1.7 ng/mL; CD4⁺ T cells: 2.0 ± 0.8 ng/mL) than in those with inactive VKH (PBMCs: 2.1 ± 1.1 ng/mL, \( P = 0.003 \); CD4⁺ T cells: 0.6 ± 0.1 ng/mL, \( P = 0.005 \)) and the normal controls (PBMCs: 1.9 ± 0.6 ng/mL, \( P = 0.001 \); CD4⁺ T cells: 0.5 ± 0.3 ng/mL, \( P = 0.004 \)). IL-7 significantly upregulated the production of IFN-γ by PBMCs (active VKH: from 4.6 ng/mL to 7.4 ng/mL, \( P < 0.001 \); inactive VKH: from 2.1 ng/mL to 3.4 ng/mL, \( P < 0.001 \); control: from 1.9 ng/mL to 3.1 ng/mL, \( P = 0.003 \)) and CD4⁺ T cells (active VKH: from 2.0 ng/mL to 5.3 ng/mL, \( P = 0.012 \); inactive VKH: from 0.6 ng/mL to 1.7 ng/mL, \( P < 0.001 \); control: from 0.5 ng/mL to 1.6 ng/mL, \( P < 0.001 \)). However, no difference was found in relative increase among the groups (Fig. 5).

**Influence of rIL-7 on Expansion of Th1 and Th17 Cells**

Purified CD4⁺ T cells were treated with or without rIL-7 to evaluate its influence on Th1 and Th17 cell expansion. The results showed that the percentage of Th1 and Th17 cells was already significantly higher in the patients with active VKH than in the normal controls (Th1 cells: 15.9% ± 2.6% versus 9.3% ± 2.5%, \( P = 0.001 \); Th17 cells: 1.9% ± 0.3% versus 1.1% ± 0.5%, \( P = 0.003 \)) in the absence of rIL-7. Furthermore, the intensity of IL-17 and IFN-γ expression of CD4⁺ T cells indicated by MFI was higher in the patients with active VKH. The percentage of IL-17⁺ IFN-γ⁺ cells was also higher in the patients with active VKH than in the normal controls, but the difference was not statistically significant (0.2% ± 0.1% versus 0.1% ± 0.1%, \( P = 0.073 \)). The addition of rIL-7 significantly promoted the expansion of Th1, Th17 cells, and IL-17⁺ IFN-γ⁺ cells both in the VKH patients (Th1 cells: from 15.9% to 28.6%, \( P = 0.008 \); Th17 cells: from 1.9% to 3.5%, \( P = 0.001 \); IL-17⁺ IFN-γ⁺ cells: from 0.2% to 0.8%, \( P = 0.014 \)) and the normal controls (Th1 cells: from 9.3% to 14.9%, \( P = 0.002 \); Th17 cells: from 1.1% to 1.7%, \( P = 0.018 \); IL-17⁺ IFN-γ⁺ cells: from 0.1% to 0.3%, \( P = 0.009 \); Fig. 6). However, there was no difference between the two groups in the relative increase.

**DISCUSSION**

In this study, we investigated the expression of IL-7 and the possible role of this cytokine in the pathogenesis of VKH disease. The result showed that the serum IL-7 level was almost
twice as high in the patients with active VKH as in the patients with inactive disease or the controls. No difference was found in the expression of its receptor (IL-7Rα) on CD4+ T cells between the patients with active VKH and the controls. In vitro experiments showed that rIL-7 significantly promoted cell proliferation as well as the production of IL-17 and IFN-γ by PBMCs and CD4+ T cells and augmented the expansion of Th1, Th17, and IL-17 IFN-γ+ cells. Both Th1 and Th17 lymphocyte populations have been shown to be involved in the pathogenesis of autoimmune diseases such as VKH.27 A possible role for IL-17 IFN-γ+ cells in VKH disease is not clear and is not supported by our preliminary data.

The role of IL-7 and its receptor IL-7R has already been reported in several autoimmune diseases but not yet in clinical uveitis.16–18,26 The only study of the role of IL-7 in uveitis comes from an experimental animal model for human uveitis, whereby IL-7 was shown to support the growth and expansion of Ag-specific CD8 T cells.29 To our knowledge our study is the first to address the role of IL-7 in clinical uveitis. We chose to study the role of IL-7 in VKH disease, which has been presumed to be induced by an autoimmune response against melanocytes. VKH disease is quite common in China20 and it is assumed to be induced by an autoimmune response against melanocytes. VKH disease is quite common in China20 and it is

Figure 6. The effect of rIL-7 on the expansion of Th1 and Th17 cells. Purified CD4+ T cells from patients with active VKH (n = 6) and normal controls (n = 6) were cultured with or without rIL-7 for 3 days. The frequency of Th1 and Th17 cells was analyzed by FCM. (A) A representative patient with data near the mean of each group in (B, C). (B) The results represent the percentages of IL-17+, IFN-γ+, and IL-17 IFN-γ+ cells among the CD4+ T cells and (C) the intensity of IL-17 and IFN-γ expression in the CD4+ T cells.

In vitro experiments showed that rIL-7 was able to show an effect on the relative increase in proliferation or cytokine production. These findings are in agreement with our observation that the expression of the IL-7 receptor on CD4+ T cells was different between the controls and the VKH patients. The expression of IL-7Rα on T cells is increased in synovial tissue of RA patients compared with patients with noninflammatory arthritis patients.28 Although this discrepancy between systemic and local expression of IL-7Rα is not clear, this result seems to show that the sensitivity of the VKH patients and the normal controls was not different in the interaction between IL-7 and its receptor. Local accumulation of CD4+ T cells expressing high levels of IL-7Rα in the target tissue may provide further evidence regarding the role of IL-7 and its receptor IL-7Rα in VKH disease.
Why IL-7 is increased in the serum of patients with active VKH is unclear. It is produced by nonhemopoietic cells, but not by leukocytes and whether the high IL-7 levels reflect the amount of stromal cells or their state of activation remains to be investigated. IL-7 not only influences the proliferation and cytokine release of T cells but also plays an important role in the expansion of the T-cell receptor repertoire. Further studies should investigate whether IL-7 acts in VKH disease through modulation of the cytokines secreted by DCs, such as IL-23 and IL-6, which are both critical for Th1 differentiation from naïve T cells. In addition, IL-7 has been shown to affect various cells that are involved in the pathogenesis of autoimmune disease such as CD8+ T cells, regulatory T cells, natural killer T cells and B cells. More studies are needed to clarify the influence of IL-7 on these various cell types and how it affects their possible role in the pathogenesis of VKH disease.

Acknowledgments

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References