Inhibitory effect of rapamycin and dexamethasone on production of IL-17 and IFN-γ in Vogt–Koyanagi–Harada patients

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Accepted 17 August 2008
Published Online First 19 November 2008

ABSTRACT

Aims: To evaluate the effect of rapamycin (RAPA) and dexamethasone (DEX) on the production of IL-17 and IFN-γ by peripheral blood mononuclear cells (PBMCs) from Vogt–Koyanagi–Harada (VKH) patients and healthy individuals.

Methods: Blood samples were drawn from 10 active VKH patients and 10 healthy individuals. PBMCs were cultured with or without anti-CD3 and anti-CD28 antibodies in the presence or absence of different concentrations of RAPA or DEX for 72 h. IL-17 and IFN-γ concentrations in the supernatants were measured by enzyme-linked immunosorbent assay (ELISA).

Results: The expression of IL-17 and IFN-γ was significantly increased in active VKH patients compared with that in healthy controls. Both RAPA and DEX were able to significantly inhibit the production of IL-17 and IFN-γ by PBMCs from patients and healthy controls. RAPA was able to completely inhibit IL-17 production at a dosage of 10 ng/ml but only partially suppressed IFN-γ production even at a much higher concentration (1000 ng/ml). DEX inhibited the production of both IL-17 and IFN-γ by approximately 70%.

Conclusions: This study indicates that both RAPA and DEX inhibit the production of IL-17 and IFN-γ by PBMCs. RAPA is much stronger in inhibiting the production of IL-17 than DEX.

Vogt–Koyanagi–Harada (VKH) syndrome is characterized by a panuveitis frequently associated with headache, pleocytosis, skin vitiligo, alopecia and inner ear disturbances. It is considered an autoimmune disease.[1] Previous studies have suggested that IFN-γ is involved in the pathogenesis of experimental autoimmune uveitis (EAU) and human uveitis.[2 3] Recent studies have shown that IL-17 is also involved in the pathogenesis of certain autoimmune diseases including VKH syndrome.[4 5]

Rapamycin (RAPA) is a newly developed immunosuppressive agent and has been used for the prevention and treatment of graft rejection, coronary artery disease and certain autoimmune disorders experimentally or clinically.[6 7] It has been shown that RAPA exerts its action primarily via suppression of IL-2 and other growth factors at the late G1 phase.[8 9] Glucocorticoids are commonly used in the treatment of autoimmune diseases including VKH syndrome. It has been demonstrated that glucocorticoids exert their role through multiple mechanisms including inhibition of a variety of cytokines such as IL-2, IL-6, TNF-α and IFN-γ.[10 11] Whether RAPA and glucocorticoids could also inhibit IL-17 production is not yet clear and therefore was the subject of the study presented here. Our results showed that both RAPA and dexamethasone (DEX) inhibited the production of IL-17 as well as IFN-γ. More importantly, we found that RAPA was much stronger in inhibiting IL-17 than DEX.

MATERIALS AND METHODS

Patients

Ten patients with VKH syndrome (five men, five women, with an average age of 36.5) and 10 healthy individuals (six men, four women, with an average age of 41) were included in this study. The diagnosis of VKH syndrome was made according to the revised diagnostic criteria for VKH syndrome.[12] All patients showed active recurrent intraocular inflammation as evidenced by mutton fat keratic precipitates and cells in the anterior chamber. These patients also showed a sunset glow fundus and Dalen–Fuchs nodules. The extraocular findings included tinnitus (50%), poliosis (40%), alopecia (40%), vitiligo (30%) and dysacusis (20%). None of the patients received immunosuppressive drugs for at least 1 week before blood sampling. All procedures followed the tenets of the Declaration of Helsinki, and informed consent was obtained from all patients and control subjects.

Cell isolation and culture

Mononuclear cells were isolated from heparinized blood by Ficoll-Hypaque-gradient centrifugation. For determination of IL-17, IFN-γ and IL-13 production, PBMCs were cultured with or without anti-CD3 (OKT3, 5 μg/ml) (eBioscience, San Diego, California) and anti-CD28 antibodies (1 μg/ml) (eBioscience) in the presence or absence of RAPA (1, 10, 50, 100, 500, 1000 ng/ml) (Sigma, St Louis, Missouri), which was diluted as below, or DEX (1, 10, 50, 100, 500, 1000 ng/ml) (Sigma) respectively for 72 h at a concentration of 2×10^6 cells/ml. RAPA was diluted in dimethyl sulfoxide (DMSO) (Sigma) and stored at −20°C in the dark. RAPA was finally diluted with RPMI1640 to the aforementioned concentrations and used for the experiments. PBMCs cultured alone served as negative controls and those cultured with DMSO (5 μg/ml) in the presence of anti-CD3 and anti-CD28 antibodies as DMSO controls. Trypan Blue dyeing was used to evaluate cell viability after 72 hours' culture. An additional experiment was performed to investigate the influence of RAPA and DEX on the production of IL-17 and IFN-γ by PBMCs upon stimulation with four concentrations

of anti-CD3 antibody (10 ng/ml, 100 ng/ml, 1 μg/ml and 5 μg/ml).

Measurement of IL-17, IFN-γ and IL-13 by ELISA

For determination of IL-17, IFN-γ and IL-13 production, the supernatants obtained from the aforementioned cell cultures were collected and used for analysis of these cytokines. Duoset ELISA Development kit (R&D system, Minneapolis, Minnesota) was used for this study. The detection limits were 15.625, 15.625 and 93.75 pg/ml for IL-17, IFN-γ and IL-13, respectively.

Statistical analysis

The Student t test was used for comparison of two independent groups of observations. p Values below 0.05 were considered statistically significant.

RESULTS

IFN-γ in the supernatants of unstimulated PBMCs (negative controls) was barely detectable in both VKH patients and healthy controls. IL-17 in the supernatant of unstimulated PBMCs is detectable in seven out of 10 patients with active 10 VKH syndromes and in five out of 10 healthy controls. The concentration of IL-17 in patients (83.46 (SD 17.96) pg/ml) is significantly higher than that in controls (46.1 (20.15) pg/ml)

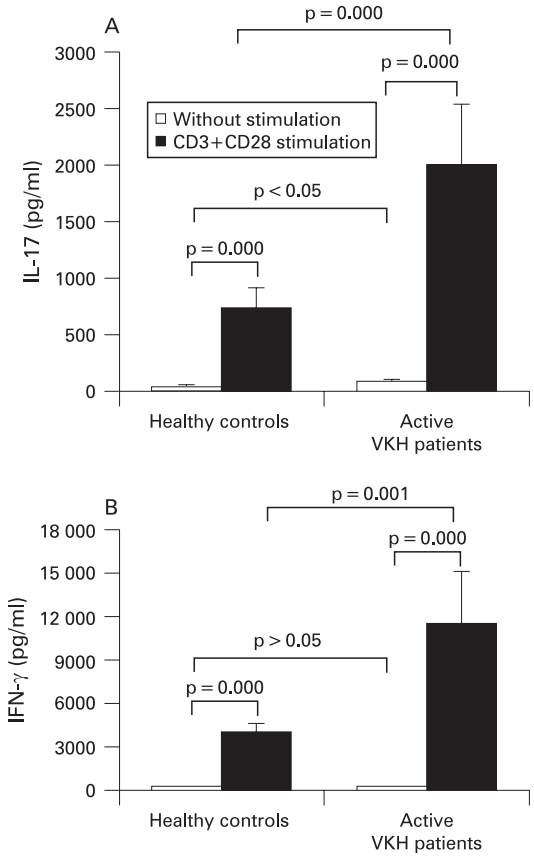

Figure 1 Production of IL-17 and IFN-γ by peripheral blood mononuclear cells (PBMCs) upon stimulation with or without anti-CD3 and anti-CD28 antibodies. Isolated PBMCs were cultured with or without anti-CD3 and anti-CD28 for 72 h. Levels of IL-17 (A) and IFN-γ (B) in supernatants of PBMCs from Vogt–Koyanagi–Harada (VKH) patients (n = 10) and healthy controls (n = 10) were determined by ELISA. Data are expressed as means (SD).

(p<0.05). A significantly increased production of IL-17 and IFN-γ was observed upon anti-CD3 and anti-CD28 antibody stimulation in the tested samples (both p<0.001). Moreover, the expression of IL-17 and IFN-γ was significantly increased in active VKH patients compared with that in healthy controls (p<0.001; p = 0.001, respectively) (fig 1A,B).

RAPA caused a significant reduction in IL-17 in both VKH patients and healthy controls. The production of IL-17 was almost totally blocked (>97%) by RAPA at a dosage of 10 ng/ml (p<0.001) (fig 2A). RAPA was also able to inhibit the production of IFN-γ. However, it inhibited the IFN-γ production up to approximately 80% in VKH patients even at a high RAPA concentration (1000 ng/ml) (fig 2B).

The study on influence of RAPA and DEX on the production of IL-17 and IFN-γ by PBMCs stimulated with different concentrations of anti-CD3 antibody showed a similar result. RAPA (10 ng/ml) was able to completely inhibit the production of IL-17 at any one of the four concentrations of anti-CD3 antibodies, whereas it only partially inhibited the IFN-γ production. DEX only partly inhibited the production of both IL-17 and IFN-γ no matter which concentration of anti-CD3 antibody was used.

No influence was found on the production of IL-17 and IFN-γ by PBMCs when cultured with DMSO alone (fig 2A,B). After a 72 h culture with different concentrations of RAPA or DMSO in the presence of anti-CD3 and anti-CD28 antibodies, the results showed that about 85% of PBMCs were still viable in both VKH patients and healthy controls (fig 3). IL-13 was not detectable in cell cultures from both VKH patients and controls (data not shown).

DEX was also able to significantly inhibit the production of IL-17 and IFN-γ by PBMCs stimulated with anti-CD3 and anti-CD28 antibodies (both p = 0.001). A gradually increased inhibition was observed from 1 to 100 ng/ml of DEX (figs 4A,B, 5A,B). The maximal inhibition of DEX was about 70% in VKH patients. Much higher concentrations (more than 100 ng/ml) of DEX did not increase its inhibitory effect (fig 4A,B). The results for the DEX experiment also showed that the concentrations used did not influence the viability of the PBMCs (fig 3).

Comparing the effect of RAPA and DEX on the production of IL-17 and IFN-γ, we found that the former was much stronger than the latter in terms of both volume concentrations and mole concentrations (data not shown).

DISCUSSION

The present study showed that the expression of IL-17 and IFN-γ was significantly increased in VKH patients with active uveitis. RAPA and DEX could effectively inhibit the production of both IL-17 and IFN-γ. Importantly, RAPA was shown to be much stronger in inhibiting the production of these two cytokines.

Previous studies suggest that IFN-γ, a classical Th1 cytokine, plays an important role in the development of certain autoimmune and inflammatory diseases including VKH syndrome.[13 14] Recent studies from our laboratory revealed that IL-17 was also actively involved in this disease.[5] The present study is an extension of our earlier findings in a new group of VKH patients and confirms our earlier observations showing that the expression of both IL-17 and IFN-γ is increased in VKH.

RAPA is a novel immunosuppressive agent. It has been demonstrated that RAPA can effectively suppress the production of cytokines including IL-2 and IL-12.[15] In this study we examined whether RAPA could inhibit the production of IL-17

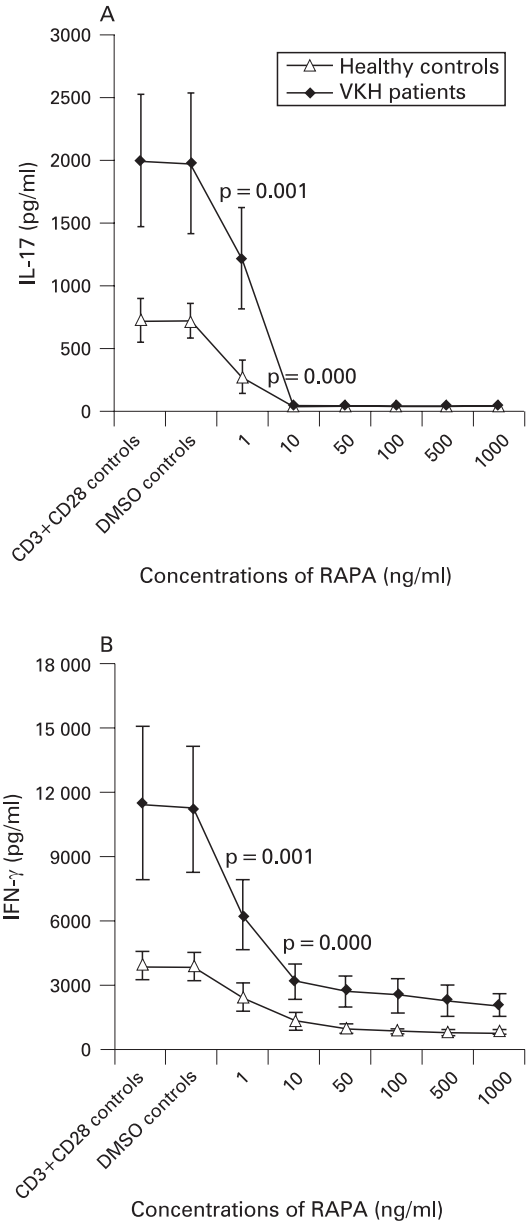

Figure 2 Effect of rapamycin (RAPA) on the production of IL-17 and IFN-γ in vitro. Isolated peripheral blood mononuclear cells from Vogt–Koyanagi–Harada (VKH) patients and healthy controls were cultured with anti-CD3 and anti-CD28 antibodies at a density of 2×10^6 cells/ml in the presence of different concentrations of RAPA (1, 10, 50, 100, 500, 1000 ng/ml) for 72 h. The levels of these two cytokines in the supernatant were detected using ELISA analysis. (A) The inhibiting rate of RAPA on the production of IL-17 is over 97% at 10 ng/ml in VKH patients. (B) The inhibiting rate of RAPA on the production of IFN-γ is about 80% at 10 ng/ml in VKH patients. Data are expressed as means (SD).

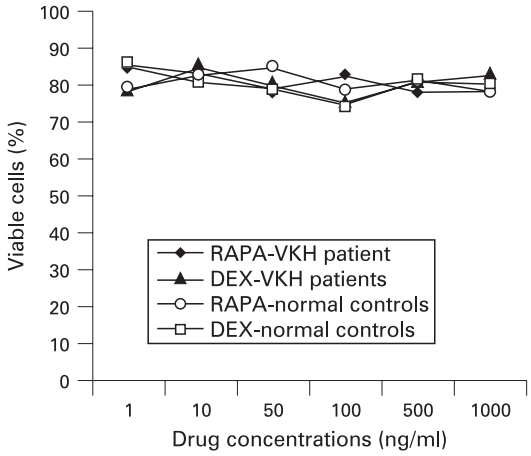

Figure 3 Cell viability of peripheral blood mononuclear cells assessed using Trypan Blue, with baseline cell viabilities in the absence of added drugs. The data shown in this figure are representative of three independent experiments. DEX, dexamethasone; RAPA, rapamycin; VKH, Vogt–Koyanagi–Harada.

(Th17 cells) and IFN-γ (Th1 cells) in vitro. The results showed that it could effectively inhibit the production of both IL-17 and IFN-γ by PBMCs cultured for a period of 72 h. However, suitable doses with excellent inhibitory effects at different culture time points are as yet unknown. More studies are needed to clarify this issue. The potent suppressive role of RAPA may explain the inhibition on the induction of EAU previously reported by Roberge *et al*.[16] Previous findings also revealed that IFN-γ played a role in the development of EAU. A recent study reported by Amadi-Obi *et al*[17] showed that IL-17 was also involved in the development of EAU. Taken together, the above-mentioned results suggest that the inhibiting effect of RAPA on EAU may be due to its suppression of both IL-17 and IFN-γ. Interestingly, we found that RAPA almost completely blocked IL-17 production at 10 ng/ml. This concentration of RAPA has also been reported by others to effectively inhibit the production of certain cytokines such as IL-2 and IL-10.[18 19]

Glucocorticoids are classical immunosuppressive agents and have been widely used for the treatment of autoimmune diseases. It has been demonstrated that they can inhibit the production of a number of cytokines including IL-2, IL-4 and IFN-γ.[20] In this study, we investigated its inhibitory effect on the production of IL-17 as well as IFN-γ in vitro. The result showed that DEX equally inhibited these two cytokines. Our results on IFN-γ are generally consistent with those observed in healthy individuals, patients with asthma and experimental studies in mice.[20–22] The effect of DEX on IL-17 found in our study is similar to that reported by Suzuki *et al*.[23] They found that the expression of IL-17 was significantly attenuated by the treatment of mice with DEX.

Comparing the effect of DEX and RAPA on IL-17 and IFN-γ, we found the following results. First, both RAPA and DEX inhibited Th1 cells and Th17 cells. Second, RAPA had a stronger role in inhibiting both cytokines than DEX in terms of both volume concentrations and mole concentrations. Third, RAPA was found to have a stronger role in suppressing IL-17 than inhibiting IFN-γ. All these results suggest that RAPA may be more effective in the treatment of diseases mediated by IL-17 and IFN-γ than DEX. More importantly, RAPA may be more suitable for diseases mediated predominantly by IL-17. Our results may partially explain the better results of RAPA in treating glucocorticoid-non-sensitive patients with autoimmune diseases including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).[24 25]

Our results also showed that IL-13, a typical cytokine for Th2 cells, was not detectable in patients with VKH syndrome. In vitro experiments showed that both RAPA and DEX did not influence the expression of this cytokine (data not shown). Therefore, it is unlikely that RAPA and glucocorticoids exert their roles via upregulating Th2 cytokines.

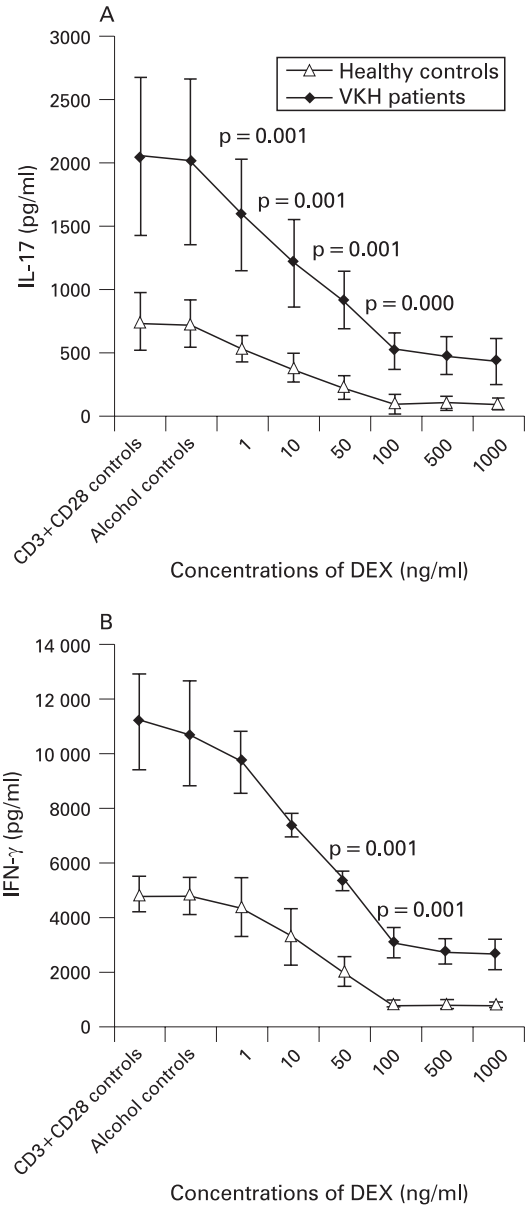

Figure 4 Effect of dexamethasone (DEX) on the production of IL-17 and IFN-γ in vitro. Isolated peripheral blood mononuclear cells from Vogt–Koyanagi–Harada (VKH) patients and healthy controls were cultured with anti-CD3 and anti-CD28 antibodies at a density of 2×10^6 cells/ml in the presence of different concentrations of DEX (1, 10, 50, 100, 500, 1000 ng/ml) for 72 h. The levels of these two cytokines in the supernatant were detected using ELISA analysis. (A) The inhibiting rate of DEX on the production of IL-17 is about 70% at 100 ng/ml in VKH patients. (B) The inhibiting rate of DEX on the production of IFN-γ is about 70% at 100 ng/ml in VKH patients. Data are expressed as means (SD).

In conclusion, our study showed that both RAPA and DEX significantly inhibited the Th17 cells and Th1 cells, whereas they did not influence the viability of these cells. More importantly RAPA was much stronger in suppressing both Th17 cells and Th1 cells in vitro. This study suggests that RAPA may be an effective drug in the treatment of diseases mediated by Th17 cells and Th1 cells. However, our study did not answer the question as to whether both drugs could inhibit cellular proliferation or CD25 expression. More studies are needed to clarify these issues.

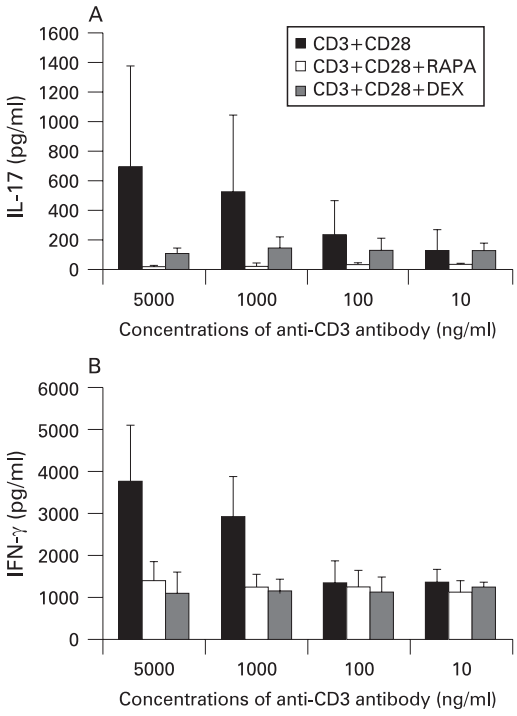

Figure 5 Isolated peripheral blood mononuclear cells from four healthy donors cultured with four different concentrations of anti-CD3 (10, 100, 1000, 5000 ng/ml) and anti-CD28 (1 μg/ml) antibodies at a density of 2×10^6 cells/ml in the absence or presence of rapamycin (RAPA) (10 ng/ml) and dexamethasone (DEX) (100 ng/ml) for 72 h. The levels of these two cytokines in the supernatant were detected using ELISA analysis. The production of IL-17 and IFN-γ showed a dose-dependent manner stimulated by four concentrations of anti-CD3 antibody. (A) The production of IL-17 was almost totally blocked by RAPA not DEX at any concentration of anti-CD3 antibody. (B) The production of IFN-γ was partially inhibited by both drugs at any concentration of anti-CD3 antibody. Data are expressed as means (SD).

Acknowledgements: This study was supported in part of the Fund of Project of Science and Technology of Guangdong Province (2005B60302009), Key project of Natural Science Foundation (30630064), Project of International Cooperation in Science and Technology, Guangdong Province (2006A50107001) and National supporting project of PR China (2007BAI18B10).

Competing interests: None.

Ethics approval: Ethics approval was provided by the Clinical Ethical Research Committee of Zhongshan Ophthalmic Center.

Patient consent: Obtained.

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Br J Ophthalmol 2009 93: 249-253 originally published online November 19, 2008
doi: 10.1136/bjo.2008.142489