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ARTICLE

Soluble cytokine receptor levels in aqueous humour of patients with specific autoimmune uveitic entities: sCD30 is a biomarker of granulomatous uveitis

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Abstract

Purpose Soluble cytokine receptors are potential biomarkers for immune activation and have a promising potential as immunotherapeutic agents. We investigated the levels of soluble cytokine receptors in aqueous humour (AH) samples from patients with specific autoimmune uveitic entities.

Methods Patients with active uveitis associated with Behçet's disease (BD) ($n = 13$), sarcoidosis ($n = 8$), HLA-B27-related inflammation ($n = 12$), Vogt–Koyanagi–Harada (VKH) disease ($n = 12$) and control subjects ($n = 9$) were included. AH samples were analyzed with the use of multiplex assays for the proinflammatory cytokine tumour necrosis factor (TNF)-α and the soluble cytokine receptors sCD30, sCD163, sgp130, sIL-6 receptor-α (sIL-6R), sTNFRI and sTNFRII.

Results TNF-α and soluble cytokine receptor AH levels were significantly higher in uveitis patients ($n = 45$) compared with controls ($n = 9$). When nongranulomatous uveitis (BD and HLA-B27-associated uveitis) was compared with granulomatous uveitis (sarcoidosis and VKH disease), the levels of sCD30 and sTNFRI/TNF-α and sTNFRII/TNF-α ratios were significantly enhanced in granulomatous uveitis. Finally, when comparing the profile in the specific uveitis entities, sCD30 levels were highest in patients with VKH disease. sgp130, sCD163, sIL-6R, sTNFRI and sTNFRII did not differ significantly between the four different clinical uveitic subgroups.

Conclusions Soluble cytokine receptors are significantly upregulated in autoimmune uveitis. $CD30^+$ T cells might contribute to the inflammatory process in granulomatous uveitis, particularly in VKH disease. Granulomatous uveitis is also characterized by significantly higher sTNFRs/TNF-α ratios than nongranulomatous uveitis.

Introduction

Patients with autoimmune uveitis present with heterogeneous clinical manifestations of intraocular inflammation and are at risk for severe visual impairment. The patients often also suffer from systemic diseases, such as Behçet's disease (BD), sarcoidosis, human leucocyte antigen (HLA)-B27-associated inflammation and Vogt–Koyanagi–Harada (VKH) disease, which present with different clinical phenotypes. These specific uveitic entities presumably arise as a consequence of different underlying mechanisms, each involving distinct immune molecules [1–4].

Cytokines are key regulators of inflammation and are thought to play important roles in the pathophysiology of various autoimmune uveitis entities [1–5]. Previous studies demonstrated upregulation of proinflammatory cytokines, such as tumour necrosis factor-α (TNF-α), interferon- γ (IFN- γ), interleukin (IL)-6, IL-15 and IL-17 in the aqueous humour (AH) samples from autoimmune uveitis patients [1–5]. Selective blockade of proinflammatory cytokines, therefore, may represent a possible way for clinical intervention. This is exemplified by the introduction and considerable clinical benefits of novel biologicals, such as anti-TNF-α agents and anti-IL-6 receptor (IL-6R) antibody

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for the treatment of autoimmune uveitis resistant to conventional treatment [6–8]. Soluble cytokine receptors have been thoroughly studied in experimental and clinical studies as potential biomarkers of immune activation and/or disease activity in many clinical conditions. Furthermore, soluble cytokine receptors have a promising potential as immunotherapeutic agents for several autoimmune diseases [9].

Cluster of differentiation 30 (CD30), belonging to the TNF–nerve growth factor receptor superfamily, was first characterized as a cell surface antigen on Hodgkin's and Reed–Sternberg cells [10, 11]. Subsequently, CD30 was also found on activated $CD45RO^+$ memory T helper (Th) cells and CD8+ T cells, but is absent on naive and resting T cells [12]. In addition, it was demonstrated that T cells isolated from peripheral blood of healthy individuals expressing CD30 produced more cytokines (IFN-γ or IL-5) than CD30 negative T cells [13]. Activated Th cells expressing CD30 exhibited enhanced helper activity for B cell immunoglobulin production [13]. A soluble form of CD30 is released by $CD30^+$ cells in vitro and in vivo by proteolytic cleavage. The level of sCD30 was demonstrated to correlate well with surface expression of CD30 and can, hence, be applied as a marker for CD30 expression [10, 11].

CD163 is a member of the cysteine-rich scavenger receptor family class B and is expressed exclusively by the monocyte–macrophage cell lineage. As an endocytic receptor for haemoglobin–haptoglobin complexes CD163 is proposed to capture free circulating haemoglobin. CD163 may have anti-inflammatory and immunoregulatory properties, since it decreases in vitro activation and proliferation of T lymphocytes. The proteolytic cleavage of the membrane-bound CD163 by matrix metalloproteinases in response to inflammatory stimuli releases sCD163. Thus, sCD163 may be viewed as a biomarker for macrophage activation in several inflammatory disorders [14, 15].

Dysregulated expression of IL-6 is implicated in the development of various chronic inflammatory autoimmune diseases [16–18]. The biological activities of IL-6 are mediated by a receptor complex comprising the IL-6 binding transmembrane glycoprotein IL-6R-α and the transmembrane signal transducer protein gp130 that is shared by additional cytokines of the IL-6 family [19]. The high-affinity complex of IL-6 and IL-6R interacts with two gp130 chains and activates the underlying signal transduction pathways. gp130 is broadly expressed, whereas IL-6R is expressed restrictively in liver cells and certain leucocyte subtypes [19]. Soluble gp130 (sgp130) isoforms are generated by alternative splicing. The soluble IL-6R (sIL-6R) also arises through alternative splicing, in addition to ectodomain shedding. sIL-6R and intact membrane-bound IL-6R bind IL-6 with comparable affinity. Contrary to the soluble receptors of TNF-α, which inhibit the activity of TNF-α, the IL-6/sIL-6R complex can via interaction with gp130 activate target cells, in the absence of the classical membrane-embedded IL-6R. Such cells cannot be activated by IL-6 alone when sIL-6R is not available. This latter process is referred to as IL-6 trans-signalling, as opposed to classical signalling via surface IL-6R. It is increasingly apparent that many of the activities assigned to IL-6 are mediated via sIL-6R [19, 20]. Since sgp130 binds the complex of IL-6/sIL-6R, sgp130 acts as a natural inhibitor of IL-6 trans-signalling in vivo, without interfering with classical signalling [19, 20].

TNF-α recognizes two distinct cell surface receptors, namely TNF-α receptor type I (TNFRI) and type II (TNFRII). In addition to membrane-bound forms, both TNFRI and TNFRII also exist as soluble forms produced by proteolytic cleavage [21, 22]. The soluble receptors sTNFRI and sTNFRII retain their ligand binding capacity and can act as natural inhibitors by sequestering TNF-α and avoiding activation of its membrane-embedded signalling receptors. As TNF-α itself is a principal inducer of sTNFRs shedding and expression, determination of sTNFRI and sTNFRII levels could provide indirect evidence of TNF-α production and reflect the activation state of the TNF-α/TNFR system [21, 22].

We hypothesized that different immunopathogenic mechanisms are involved in each clinical subtype of autoimmune uveitis. Through analysis of the soluble cytokine receptor profile in AH from patients with specific clinical entities of autoimmune uveitis, a better understanding of the underlying immune mechanisms may be obtained. In addition, it may also be of clinical importance for disease diagnosis and identification of potential targets for selective therapy. For these reasons, we analyzed the AH from patients with active uveitis associated with four systemic inflammatory diseases (sarcoidosis, VKH disease, BD and HLA-B27-related inflammation) for the presence of sCD30, sCD163, sgp130, sIL-6R, sTNFRI and sTNFRII. Furthermore, the levels of soluble receptors were correlated with the levels of TNF-α, a major proinflammatory cytokine, and with clinical disease activity.

Patients and methods

All patients included in the study were seen at the outpatient clinic of King Abdulaziz University Hospital and all gave informed and written consent. As controls for patients with active uveitis ($n = 45$; $n = 13$ for BD, $n = 12$ for HLA-B27-associated uveitis; $n = 8$ for sarcoidosis; $n = 12$ for VKH disease), patients who had undergone cataract extraction with no prior history of uveitis ($n = 9$) were included. Diagnosis was made as described before following established clinical criteria, with supporting laboratory evidence

Table 1 Demographic and clinical characteristics of all subjects at presentation [4]

Characteristic	Controls ($n = 9$)	Behçet's disease ($n = 13$)	Sarcoidosis ($n = 8$)	HLA-B27 ($n = 12$)	VKH disease ($n = 12$)
• Age in years, mean ± SD [range]	58.2 ± 9.1 [41–70]	28.9 ± 4.2 [23–36]	25.1 ± 6.5 [17–35]	34.3 ± 9.3 [22–50]	28.3 ± 7.2 [18–42]
• Gender					
Male ($n = 34$), n (%)	5 (55.5)	13 (100)	5 (62.5)	5 (41.7)	6 (50.0)
Female ($n = 20$), n (%)	4 (44.4)	0 (0.0)	3 (37.5)	7 (58.3)	6 (50.0)
• Visual acuity					
≥20/40 ($n = 24$), n (%)		2 (15.4)	8 (100)	9 (75.0)	5 (41.7)
<20/40 ($n = 21$), n (%)		11 (84.6)	9 (0.0)	3 (25.0)	7 (58.3)
• Anterior chamber reaction					
≤2 + ($n = 14$), n (%)		5 (38.5)	3 (37.5)	2 (17.0)	4 (33.3)
>2 + ($n = 31$), n (%)		8 (61.5)	5 (62.5)	10 (83.0)	8 (66.7)
• Presence of posterior synechiae ($n = 28$), n (%)		6 (46.2)	6 (75.0)	8 (66.7)	8 (66.7)

VKH Vogt–Koyanagi–Harada

Table 2 Comparisons of TNF-α and soluble cytokine receptor levels and sTNFRs/TNF-α ratios between patients and controls

	All patients ($n = 45$) median (IQR) (pg/ml)	Controls ($n = 9$) median (IQR) (pg/ml)	p value (Mann–Whitney test)
TNF-α	125.7 (54.9–232.0)	6.9 (6.9–12.2)	<0.001[a]
sCD30	172.2 (81.9–378.9)	56.0 (56.0–58.7)	<0.001[a]
sCD163	153,811.7 (44,647.8–283,069.6)	2850.0 (2850.0–2850.0)	<0.001[a]
sgp130	29,953.9 (18,939.3–43,913.8)	11,696.0 (7577.6–15,393.7)	<0.001[a]
sIL-6R	3145.4 (1155.2–5197.9)	297.0 (297.0–317.3)	<0.001[a]
sTNFRI	7712.8 (3689.7–13,426.4)	985.1 (824.1–1734.1)	<0.001[a]
sTNFRII	10,399.2 (1849.5–37,606.3)	221.0 (221.0–221.0)	<0.001[a]
sTNFRI/TNF-α	61.0 (37.0–95.7)	140.8 (96.9–151.8)	0.002[a]
sTNFRII/TNF-α	65.8 (18.1–177.1)	32.0 (24.2–32.0)	0.051

IQR—interquartile range (1st to 3rd quartile)

[a]Statistically significant at 5% level of significance

as needed [4, 23, 24]. In each patient, the uveitis activity was graded according to the criteria of the Standardization of the Uveitis Nomenclature Working group grading scheme [25]. None of the patients was on topical or systemic therapy on presentation. AH sampling was done before the start of therapy. By means of limbic paracentesis 100–200 µl of AH was aspirated and processed as described [1–4]. The demographic and clinical characteristics of the included patients are described in detail in a previous report in Table 1; duplicated with permission from [4].

All procedures were performed according to the tenets of the Declaration of Helsinki and the protocol of this study was approved by the Research Center, College of Medicine, King Saud University.

Cytokine and soluble cytokine receptor assays

We determined the profile of TNF-α and the soluble cytokine receptor levels in the AH samples using two multiplex assays. The first multiplex contained detection reagents for the proinflammatory cytokine TNF-α (Bio-plex CatNr 171ak99mr2, BIORAD, Hercules, CA, USA), the second multiplex assessed the soluble cytokine receptors sCD30, sCD163, sgp130, sIL-6R, sTNFRI and sTNFRII (Bio-plex CatNr 171al001m, BIORAD). The two analyses were performed according to the manufacturer's guidelines in separate plates on consecutive days on the same AH samples. In each array, 8 µl of AH was used after dilution with 56 µl of assay buffer (BIORAD). The data were obtained

Table 3 Comparisons of TNF-α and soluble cytokine receptor levels and sTNFRs/TNF-α ratios between controls and the four disease groups

	Controls ($n = 9$) median [IQR] (pg/ml)	Behçet's disease ($n = 13$) median [IQR] (pg/ml) (p value)	Sarcoidosis ($n = 8$) median [IQR] (pg/ml) (p value)	HLA-B27-associated uveitis ($n = 12$) median [IQR] (pg/ml) (p value)	VKH disease ($n = 12$) median [IQR] (pg/ml) (p value)
TNF-α	6.9 [6.9–12.2]	178.3 [59.6–284.3] (<0.001[a])	95.0 [57.7–171.8] (0.011[a])	246.9 [89.2–409.0] (<0.001[a])	56.9 [32.8–131.5] (0.089)
sCD30	56.0 [56.0–58.7]	84.3 [63.9–175.6] (0.234)	355.9 [166.3–701.4] (<0.001[a])	112.4 [61.9–184.8] (0.204)	435.7 [159.3–3206.9] (<0.001[a])
sCD163	2850.0 [2850.0–2850.0]	63,482.3 [29,783.6–236,301.0] (0.009[a])	226,207.7 [55,261.0–477,197.3] (79.4) (<0.001[a])	124,877.8 [35,223.6–339,721.0] (0.002[a])	142,630.2 [80,178.7–278,444.8] (<0.001[a])
sgp130	11,696.0 [7577.6–15,393.7]	32,901.9 [17,703.9–47,432.6] (0.013[a])	39,044.2 [23,884.3–63,347.6] (0.002[a])	24,992.2 [18,915.9–41,513.4] (0.037[a])	25,091.2 [17,680.5–39,159.5] (0.075)
sIL-6R	297.0 [297.0–317.3]	3723.9 [804.0–7139.6] (0.003[a])	3617.4 [1102.8–4589.2] (0.010[a])	2025.3 [1463.8–3716.9] (0.005[a])	4215.6 [1167.8–7106.3] (<0.001[a])
sTNFRI	985.1 [824.1–1734.1]	4947.6 [2897.7–13,623.9] (0.003[a])	9365.0 [3808.7–16,036.4] (0.002[a])	9612.3 [4767.3–16,807.2] (<0.001[a])	6990.4 [3178.5–10,282.7] (0.008[a])
sTNFRII	221.0 [221.0–221.0]	7227.1 [827.4–35,593.1] (0.005[a])	25,890.8 [1784.5–37,941.4] (0.002[a])	13,797.4 [2480.2–36,301.8] (0.001[a])	12,118.8 [2679.5–46,617.0] (<0.001[a])
sTNFRI/TNF-α	140.8 [96.9–151.8]	48.5 [28.7–91.5] (0.031[a])	59.6 [38.5–135.9] (0.479)	46.5 [19.5–67.8] (0.005[a])	90.7 [53.6–131.6] (0.998)
sTNFRII/TNF-α	32.0 [24.2–32.0]	38.5 [13.9–137.4] (0.998)	121.6 [18.7–420.6] (0.651)	41.9 [9.8–137.7] (0.998)	89.8 [53.4–505.3] (0.051)

IQR—interquartile range (1st to 3rd quartile)

VK Vogt–Koyanagi–Harada

[a]Statistically significant at 5% level of significance

and analyzed using the Bio-Plex 200 system and software (BIORAD).

Statistical analysis

All statistical analyses were performed using SPSS version 21.0 software (IBM, Armonk, NY, USA). Shapiro–Wilk test and normal Q–Q plots were used to test for the normality distribution of the data. Since the data were not normally distributed, they are presented as median and interquartile range (1st–3rd quartiles) and fold increase, where fold increase was calculated by dividing cytokine levels of the uveitis patients by the cataract patient control levels. The Kruskal–Wallis test was used to compare different disease categories. Subsequently, the Mann–Whitney test was used to compare two independent groups and Bonferroni test was used to adjust the p values for multiple comparison corrections. Correlations between variables were assessed by computing Spearman correlation coefficients. A p value less than 0.05 indicated statistical significance.

Results

Levels of TNF-α and soluble cytokine receptors in AH samples from uveitis patients versus controls operated for cataract

When considering the whole group of patients, TNF-α and soluble cytokine receptor levels were significantly enhanced in AH of patients compared with controls (Table 2). Compared with controls, TNF-α, sCD163, sgp130, sIL-6R, sTNFRI and sTNFRII levels were significantly higher in BD and HLA-B-27-associated uveitis. The levels of TNF-α, sCD30, sCD163, sgp130, sIL-6R, sTNFRI and sTNFRII were significantly elevated in sarcoidosis. The levels of sCD30, sCD163, sIL-6R, sTNFRI and sTNFRII were significantly higher in VKH disease (Table 3).

Levels of TNF-α and soluble cytokine receptors in AH samples from patients with specific autoimmune uveitic entities

Next, the Kruskal–Wallis test was applied to compare the distribution of TNF-α and soluble cytokine receptor levels among the four disease groups. Among the cytokine and soluble cytokine receptors analyzed, TNF-α and sCD30 differed significantly between patients with BD, sarcoidosis, HLA-B27-associated uveitis and VKH disease ($p = 0.029$; $p = 0.001$, respectively) (Fig. 1a). TNF-α levels were significantly increased in HLA-B27-associated uveitis compared with VKH disease (Mann–Whitney test;

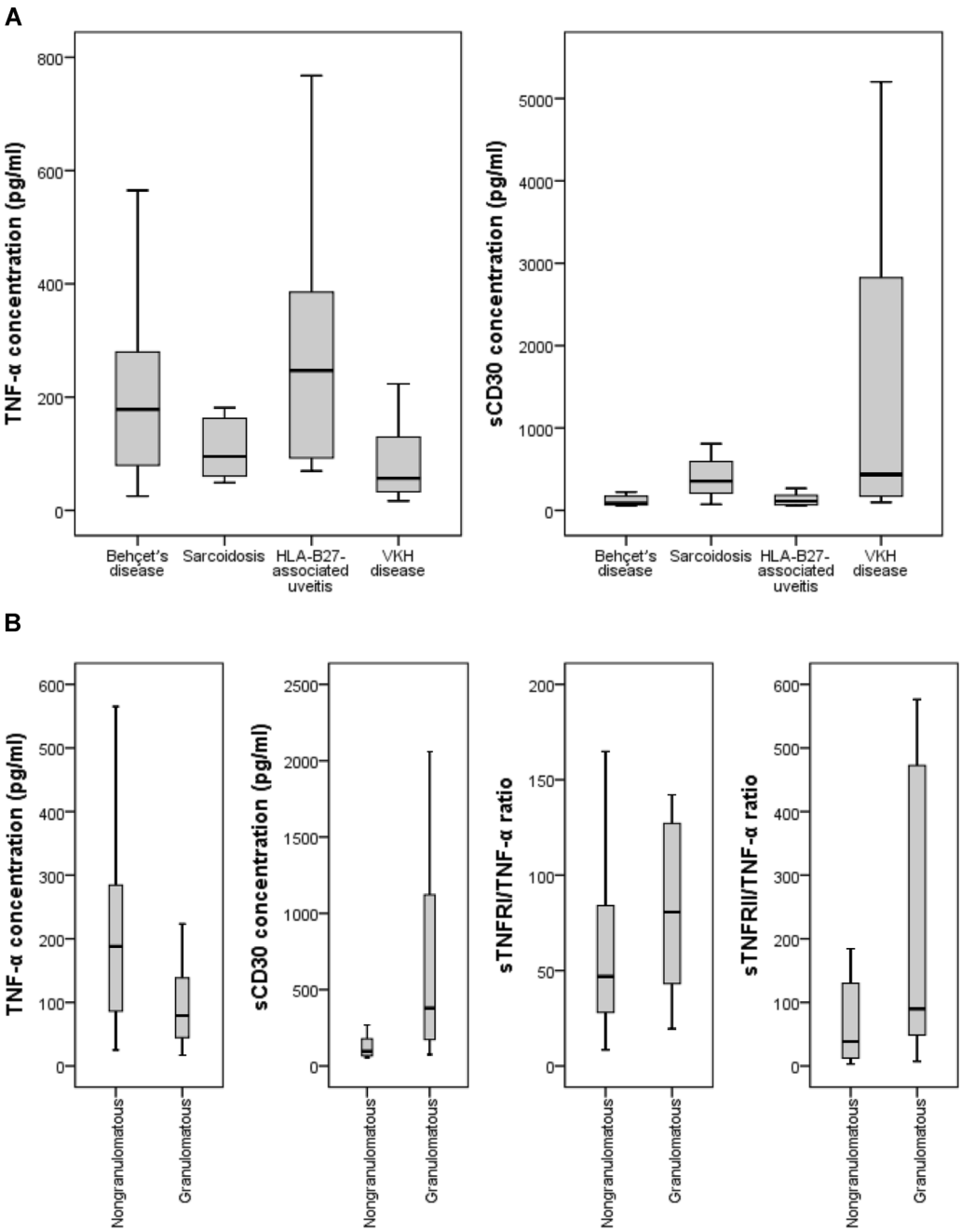

Fig. 1 sCD30 and TNF-α levels and the ratios of TNF-α to its soluble receptors in aqueous humour of patients with active autoimmune uveitic entities. Distribution of sCD30 and TNF-α levels in the four different clinical uveitic entities (Kruskal–Wallis test; **a**). Comparison of nongranulomatous uveitis versus granulomatous uveitis (Mann–Whitney test; **b**)

$p = 0.023$). sCD30 levels in VKH disease were significantly higher than in BD and HLA-B27-associated uveitis (Mann–Whitney test; $p = 0.003$; $p = 0.009$, respectively).

Evaluation of TNF-α versus soluble TNF-α receptors ratios

Because the biological activity of TNF-α is regulated by sTNFRI and sTNFRII in vivo, the ratios of TNF-α to its soluble receptors were calculated for the AH samples. When considering the whole patient group, the sTNFRI/TNF-α ratio was significantly higher in the AH of controls than in uveitis patients. On the other hand, the sTNFRII/TNF-α ratio was increased in uveitis patients compared with controls, but significance was not reached (Table 2). In the four disease groups, the sTNFRI/TNF-α ratio was significantly higher in controls than in BD and HLA-B27-associated uveitis. However, the sTNFRI/TNF-α ratio did

Table 4 Comparisons of TNF-α and soluble cytokine receptor levels and sTNFRs/TNF-α ratios between nongranulomatous uveitis (Behçet's disease and HLA-B27-associated uveitis) and granulomatous uveitis (sarcoidosis and Vogt–Koyanagi–Harada disease)

	Nongranulomatous uveitis ($n = 25$) median [IQR] (pg/ml)	Granulomatous uveitis ($n = 20$) median [IQR] (pg/ml)	p value (Mann-Whitney test)
TNF-α	187.9 [79.5–289.0]	79.2 [42.7–141.1]	0.009[a]
sCD30	96.1 [63.9–178.5]	378.9 [159.3–1277.5]	<0.001[a]
sCD163	95,943.9 [31,899.7–271,050.5]	163,513.2 [76,302.0–331,708.7]	0.132
sgp130	31,003.7 [18,324.2–43,034.2]	29097.6 [19,816.3–48,644.3]	0.732
sIL-6R	2134.1 [1159.7–4962.3]	3824.3 [1153.1–6538.4]	0.465
sTNFRI	7364.2 [3881.0–14,993.2]	7755.1 [3383.5–11,757.6]	0.599
sTNFRII	7227.1 [1418.7–36,222.3]	14,999.0 [2649.4–39,744.9]	0.398
sTNFRI/TNF-α	46.9 [21.5–85.4]	80.7 [42.9–131.6]	0.028[a]
sTNFRII/TNF-α	38.5 [11.4–132.6]	89.8 [46.3–488.1]	0.019[a]

IQR—interquartile range (1st to 3rd quartile)

[a]Statistically significant at 5% level of significance

not differ significantly between controls and sarcoidosis or between controls and VKH disease. The sTNFRII/TNF-α ratio did not differ significantly between controls and individual disease groups (Table 3).

Next, we evaluated the distribution of sTNFRI/TNF-α and sTNFRII/TNF-α ratios among the four disease groups (Kruskal–Wallis test). No statistically significant differences were observed.

Comparisons of TNF-α and soluble cytokine receptor levels and sTNFRs/TNF-α ratios in nongranulomatous uveitis versus granulomatous uveitis

When analysis was done according to subdivision of the patients into those with granulomatous uveitis (sarcoidosis and VKH disease) ($n = 20$) and those with non-granulomatous uveitis (BD and HLA-B27-associated uveitis) ($n = 25$), sCD30 levels and sTNFRI/TNF-α and sTNFRII/TNF-α ratios in granulomatous uveitis were significantly higher than those in nongranulomatous uveitis. On the other hand, TNF-α levels in nongranulomatous uveitis were significantly higher than those in granulomatous uveitis. Levels of sCD163, sgp130, sIL-6R, sTNFRI and sTNFRII were similar in granulomatous and non-granulomatous uveitic diseases (Table 4 and Fig. 1b).

Correlations

In the whole group of patients, the levels of TNF-α correlated significantly with the levels of sTNFRI ($r = 0.72$; $p < 0.001$) and sTNFRII ($r = 0.64$; $p < 0.001$). Significant positive correlations were found between AH levels of TNF-α ($r = 0.624$; $p < 0.001$), sIL-6R ($r = 0.384$; $p = 0.009$), sTNFRI ($r = 0.378$; $p = 0.01$) and sTNFRII ($r = 0.364$; $p = 0.014$) and clinical disease activity. On the other hand, there was a significant negative correlation between sTNFRI/TNF-α ratio and clinical disease activity ($r = -0.337$; $p = 0.027$). There were no significant correlations between clinical disease activity and sCD30, sCD163, sgp130 and the sTNFRII/TNF-α ratio.

Discussion

In the present study of 45 patients, elevated levels of sCD30 in the AH samples from patients with autoimmune uveitis might reflect a recruitment of $CD30^+$ T cells into the ocular inflammatory microenvironment of patients with uveitis. Our subgroup analysis showed that sCD30 levels were significantly higher in patients with VKH disease than in patients with BD and patients with HLA-B27-associated uveitis. Among the four disease groups, the levels of sCD30 were elevated 7.8-fold, 6.4-fold, 1.5-fold and 2.0-fold in patients with VKH disease, sarcoidosis, BD and HLA-B27-associated uveitis, respectively, compared with controls. In addition, sCD30 levels were significantly higher in patients with granulomatous uveitis associated with sarcoidosis and VKH disease than in patients with nongranulomatous uveitis associated with BD and HLA-B27-associated uveitis. Our findings suggest that $CD30^+$ T cells contribute to the inflammatory process in patients with granulomatous uveitis, particularly in VKH disease. Similarly, previous studies demonstrated that $CD30^+$ T cells are involved in the pathogenesis of several granulomatous inflammatory diseases [26–28]. Recently, Shinoda et al. [29] demonstrated that CD30 knockout mice showed milder symptoms in a model of experimental autoimmune encephalomyelitis and less antigen-specific Th1 and Th17 cells were induced. These findings suggest that CD30 expression on $CD4^+$ T cells is implicated in the pathogenesis of autoimmune diseases of the central nervous system.

Previous reports demonstrated high serum levels of sCD30 in both Th1- and Th2-dominated disorders [10, 11]. Pellegrini et al. [30] reported that CD30 may be an essential costimulatory molecule and marker for an important immunoregulatory subpopulation of T cells which controls the Th1/Th2 balance in immune responses. Increased levels of sCD30 have been reported in several autoimmune diseases [31–37]. In addition, elevated sCD30 serum concentrations correlated with clinical and laboratory parameters of disease activity [31, 33–35].

Macrophages activated by classical pathways (M1) have proinflammatory properties. Conversely, alternatively activated M2 macrophages which express CD163 have anti-inflammatory, antioxidant, tissue repair, proangiogenic and fibrosing roles. Therefore, sCD163 may represent a marker for alternatively activated macrophages [14, 15]. The value of sCD163 as a marker of macrophage activation has been confirmed in several inflammatory conditions [38–47]. The present study is the first to demonstrate high levels of sCD163 in the ocular microenvironment of patients with autoimmune uveitis, sCD163 levels being elevated about 50-fold, compared with controls. These data suggest that active autoimmune uveitis may be associated with increased macrophage activation and are consistent with previous reports that macrophages are involved in the pathogenesis of autoimmune uveitis [48]. The detected rise in sCD163 may be due to upregulation and/or augmented shedding of CD163. Increased shedding might be correlated to elevated levels of CD163-cleaving proteinases in the ocular inflammatory microenvironment of these patients.

Several studies demonstrated that IL-6 trans-signalling via the sIL-6R is critically involved in the initiation and maintenance of several inflammatory and autoimmune diseases [19, 20]. Elevated sIL-6R levels have been documented in synovial fluids of patients with rheumatoid arthritis [49, 50]. In addition, elevated levels of IL-6 and sIL-6R correlated with the degree of joint destruction in rheumatoid arthritis [49]. In a previous study, we demonstrated upregulation of the proinflammatory cytokine IL-6 in AH samples from patients with autoimmune uveitis [1]. In the present study, sIL-6R levels were elevated 10.6-fold compared with controls, whereas levels of the inhibitory sgp130 were elevated only 2.6-fold. These findings suggest that the regulation of IL-6 trans-signalling may be distorted in autoimmune uveitis. Interestingly, selective targeting of sIL-6R-mediated events may represent a novel avenue for therapeutic intervention [19, 20]. In animal models of arthritis, specific inhibition of sIL-6R-mediated signalling by intra-articular injection of sgp130 effectively suppressed all parameters of disease severity [51]. Similarly, in animal models of colitis, application of a gp130-Fc fusion protein specifically neutralizating sIL-6R suppressed colitis activity and induced apoptosis of lamina propria T cells. These results suggest that a T cell activation pathway driven by the IL-6/sIL-6R complex supports chronic intestinal inflammation [52]. Collectively, these findings suggest that specific therapeutic targeting of sIL-6R may be explored as a novel strategy for the treatment of autoimmune uveitis. Indeed, neutralizing antibodies against IL-6R have proven to be effective in uveitis [8].

In the present study, the levels of sTNFRI and sTNFRII were elevated 7.8-fold and 47.1-fold, respectively, compared with controls. Our results might reflect the cellular attempt to antagonize the effect of TNF-α by cleavage of the surface receptors. Similarly, several studies reported increased levels of sTNFRI and sTNFRII in inflammatory and autoimmune diseases [53–61]. Moreover, sTNFRI and sTNFRII levels correlated with disease activity [53, 55–59]. Our findings also suggest the preferential shedding of sTNFRII in patients with autoimmune uveitis. These results are in agreement with previous studies that demonstrated higher levels of sTNFRII than sTNFRI levels in the synovial fluid of patients with rheumatoid arthritis [55, 56]. Cellular responses to TNF-α are influenced by, amongst other factors, the extracellular concentrations of sTNFRI and sTNFRII [21, 22]. Indeed, elevated concentrations of sTNFRs have been demonstrated to inhibit the activity of TNF-α both in vitro and in vivo [62]. The relative proportion of TNF-α to its soluble receptors in biological fluids has been proven to be important in a number of clinical conditions [61–65]. Diminished ratios of sTNFRI/TNF-α and sTNFRII/TNF-α were observed in children with meningococcal septicaemia with a fatal outcome compared with survivors [63]. Increased joint destruction in polyarticular juvenile chronic arthritis may be related to the lower sTNFRI/TNF-α and sTNFRII/TNF-α ratios [61]. Considering the inhibitory effects of the soluble receptors for TNF-α, we wondered whether the ratios of sTNFRs/TNF-α would be different among the disease groups, and be differentially distributed compared with the receptor levels. Indeed, the ratios were significantly higher in patients with granulomatous uveitis associated with sarcoidosis and VKH disease than in patients with acute nongranulomatous uveitis associated with BD and HLA-B27-associated uveitis. These findings suggest that TNF-α would exert more destructive effects in the ocular inflammatory microenvironment of patients with acute nongranulomatous uveitis when compared with granulomatous uveitis and provides an explanation for the different clinical courses of these uveitis subgroups. This may also reflect different pathogenic mechanisms in the two disease groups.

This study has several limitations regarding experimental methods and designs. Firstly, our control group consisted of patients who had undergone elective cataract extraction with no prior history of uveitis. Accordingly, the control patients were older than patients with uveitis. The

relationship between age and human AH cytokine levels was recently investigated. Increased concentration of proinflammatory and proangiogenic cytokines with age was demonstrated [66]. Although the control patients might have a proinflammatory state, the statistical analysis gave clear differences in comparisons with patients with uveitis. Younger controls might have yielded differences with higher statistical significance. Furthermore, analysis of AH samples was performed at one-time point during the course of disease. Therefore, it was not possible to evaluate the effect of the interval between onset of inflammation and AH sampling and intensity of inflammation on the levels of the studied soluble cytokine receptors. Animal models are needed to explore the effect of uveitis activity and to investigate the expression of these soluble cytokine receptors at different time points. Nevertheless, with the use of a multiplex assay, we analyzed AH samples from patients with several specific clinical entities of autoimmune uveitis in parallel allowing measurements of these soluble cytokine receptors simultaneously in the same samples.

In conclusion, our findings suggest that $CD30^+$ T cells might be involved in the regulation of inflammation in granulomatous uveitis, particularly VKH disease and that active autoimmune uveitis might be associated with increased macrophage activation, since we observed elevated sCD163 levels. Granulomatous uveitis is characterized by significantly higher sTNFRs/TNF-α ratios than acute nongranulomatous uveitis. Finally, specific therapeutic targeting of sIL-6R-mediated signalling may represent a novel strategy for the treatment of autoimmune uveitis. To further investigate the relative importance of these soluble cytokine receptors, intervention functional studies in animal models are necessary.

Summary

What was known before

- Cytokines are key regulators of inflammation and are thought to play important roles in the pathophysiology of various autoimmune uveitis entities.
- Previous studies demonstrated upregulation of proinflammatory cytokines, such as IFN-γ, TNF-α, IL-6, IL-15 and IL-17 in the aqueous humour samples from autoimmune uveitis patients.

What this study adds

- $CD30^+$ T cells might be involved in the pathogenesis of granulomatous uveitis, particularly Vogt–Koyanagi–Harada disease.
- Active autoimmune uveitis might be associated with increased macrophage activation, since we observed elevated sCD163 levels.
- Granulomatous uveitis is characterized by significantly higher sTNFRs/TNF-α ratios than acute nongranulomatous uveitis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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