

IL-1 family cytokines and receptors in IgG4-related disease

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ABSTRACT

Background/aim: The IgG4-related disease (IgG4-RD) is a fibroinflammatory condition that can affect almost any organ, often associated with eosinophilia and increased levels of IgE and IgG4. Overexpression in tissues of Th2-related cytokines but also of IFN- γ has been reported. Given the major role of IL-1 family cytokines in inducing and regulating inflammation, and the paucity of data so far available in IgG-RD, we performed a comprehensive analysis of IL-18, related IL-1 family cytokines and soluble receptors in these patients.

Patients and Methods: Fifteen patients fulfilling the criteria for the diagnosis of IgG4-RD and 80 blood donors as control were recruited. Cytokines of the IL-1 family (IL-1 α , IL-1 β , IL-33, IL-18), soluble receptors (sIL-1R1, sIL-1R2, sIL-1R3, ST2/sIL-1R4) and antagonists (IL-1Ra, IL-18 binding protein -IL-18 BP-) were measured in sera by multiarray ELISA assay. Free IL-18 was calculated as the amount of IL-18 not inhibited by IL-18 BP.

Results: Half of the patients had a multiorgan disease, mainly affecting retroperitoneum, lymph nodes and pancreas. sIL-1R1 ($p = 0.0001$), sIL-1R2 ($p = 0.0024$), ST2/sIL-1R4 ($p = 0.002$) were significantly increased in IgG4-RD sera compared with healthy controls; sIL-1R3 was significantly lower in patients vs controls ($p = 0.0006$).

Conclusions: The increased levels of the soluble forms of the two IL-1 receptors IL-1R1 and IL-1R2 suggest the need to dampen IL-1-mediated inflammation at the tissue level. Elevated circulating ST2/sIL-1R4 levels may represent the marker of an ongoing protective mechanism, but their contribution to organ damage cannot be excluded.

On the whole, the data suggest a tight control of IL-1 family cytokines signalling in IgG4-RD.

1. Introduction

The IgG4-related disease (IgG4-RD) is a rare fibroinflammatory condition that can affect almost any organ, characterized by lymphoplasmacytoid infiltrate, obliterative phlebitis and storiform fibrosis, and often associated with eosinophilia and increased levels of IgE and IgG4 [1,2]. Overexpression in tissues of Th2-related cytokines (IL-4, IL-5 and IL-13) has been reported and related with IgG4 production [3]. T lymphocytes are the most abundant cells in the infiltrates, but conflicting data have been obtained on the T cell profile. By flow cytometry analysis, an expansion of circulating T regulatory, Th2, Th17, and T follicular helper (T_{FH}) cell subsets was detected, together with a higher production of IL-4 and IL-17 [4]. In active untreated IgG4-RD patients, the analysis of T cells by next generation sequencing allowed to identify clonally expanded cytotoxic CD4 T cells producing IL-1 β , TGF- β 1 and IFN- γ in the peripheral blood but also infiltrating affected tissues [5,6]. Gene expression analysis of salivary glands from IgG4-RD patients with

syaladenitis confirmed the overexpression of genes associated with CD4 cytotoxic T lymphocytes (CTLs), including IFN- γ [6].

IL-18 induces activation of Th1 cells and production of IFN- γ by Th1 and NK cells [7]; in fact, both IL-18 and IFN- γ are highly expressed, as proteins and mRNA, in inflammatory infiltrates of IgG4-RD submandibular glands [8]. The inflammatory effects of IL-18 are strongly dependent on the microenvironment, requiring synergism with IL-12 or IL-15 for inducing Th1 polarization and IFN- γ production. In the absence of IL-12 or IL-15, IL-18 may rather induce a Th2 response [9]. The activity of IL-18 is regulated by the IL-18 binding protein (IL-18BP), a soluble molecule that binds mature IL-18 with high affinity and prevents its interaction with cell surface receptors. Only the fraction of IL-18 that is not bound to IL-18BP is actually free of interacting with the membrane receptors on target cells/organs and is biologically active. Measurable concentrations of IL-18 are detectable in sera of normal subjects, while in normal conditions the highly inflammatory cytokine IL-1 β is virtually absent in the circulation. The activity of IL-1 β and of

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the closely related IL-1 α is finely tuned by the differential expression of a group of receptors and soluble inhibitors. IL-1R1 is the signalling receptor, which binds IL-1 and recruits the accessory chain IL-1R3 for initiating signalling, while the IL-1-like soluble antagonist IL-1Ra prevents the association with IL-1R3 and thus signal transduction. The soluble forms of IL-1R1 and IL-1R3 act as decoy inhibitors. Both in its membrane form and as soluble protein, IL-1R2 binds IL-1 β with high affinity and recruits IL-1R3, but is unable to activate intracellular signalling, behaving as a decoy receptor [10].

IL-33 is another member of IL-1 family, highly homologous to IL-18, which contains a nuclear localization signal and acts as transcription regulating factor. IL-33 can be released extracellularly as full length protein through cell death pathways and cleaved to shorter forms of increased cytokine activity. Acting as a cytokine and binding to its receptor IL-1R4 (previously known as T1/ST2), IL-33 drives type 2-dependent inflammation and also tissue repair [11,12]. The soluble form of the receptor, ST2/sIL-1R4, is a natural inhibitor of IL-33.

Given the major role of these pleiotropic cytokines in inducing and regulating inflammation, and the paucity of data so far available in IgG-RD, we performed a comprehensive analysis of IL-18, related IL-1 family cytokines and soluble receptors in these patients.

2. Patients and Methods

2.1. Patient selection

Fifteen patients fulfilling the proposed criteria for the diagnosis of IgG4-RD were recruited [13]. Eighty sex and age-matched blood donors formed the control group. IgG4-Related Disease Responder Index was calculated according to Carruthers et al. [14] and the Disease Activity Index according to Ebbo et al. [15].

The study was approved by the local Ethics Committee, and patients gave their written informed consent.

2.2. Cytokine detection

Cytokines of the IL-1 family (IL-1 α , IL-1 β , IL-33, IL-18), soluble receptors (sIL-1R1, sIL-1R2, sIL-1R3, sIL-1R4) and antagonists (IL-1Ra, IL-18 binding protein -IL-18BP-) were measured in sera by a custom-made multiarray ELISA assay (Quansys Biosciences, Logan, UT).

After measurement of the concentrations of both IL-18 and IL-18BP in each sample, the law of mass action has been applied to calculate free IL-18 (i.e., the fraction of cytokine not bound to its inhibitor IL-18BP), using a K_d of 0.4 nM [16], as previously described [17,18].

2.3. Statistical analysis

Cytokine levels in patients and controls were compared by means of the analysis of variance with Bonferroni *post hoc* test and with Mann Whitney test. Correlation was determined by means of the Spearman rank correlation test. $p < 0.05$ was considered significant.

3. Results

Clinical and serological features of the patients are summarized in Table 1.

Eight patients had a multiorgan disease and 7 had involvement of a single organ. The disease affected retroperitoneum (6 patients), lymph nodes (5 patients), pancreas (5 patients), salivary glands (4 patients), biliary ducts (3 patients), orbit (1 patient), skin (1 patient), lung (1 patient), thyroid (1 patient), and hypophysis (1 patient).

IL-18, IL-18BP and free IL-18 levels were not significantly different in patients and controls.

IL-1 α and IL-1 β were both undetectable in control or patient sera, with the exception of patient 3. Similarly, IL-33 was undetectable in control or patient sera, again with the exception of patient 3. In this

Table 1

Demographic and clinical features of IgG4-RD patients.

	Median	IQR
Age (years)	56	51–74
Sex (M/F)	6/9	
Disease duration (months)	7	1–134
IgG (mg/dL)	1557	1220–1745
IgG4 (mg/dL)	215	137–352
IgG/IgG4	14,36	13,39–20,87
IgE (UI/ml)	46	24–86
ESR (mm/h)	35,5	25,5–70,5
CRP (mg/dL)	0,65	0,18–2,62
Eosinophils (N/ μ L)	200	100–360
C3 (mg/dL)	123,5	122–132
C4 (mg/dL)	26,5	21,7–30,6
Single organ/Multi organ	7/8	
IgG4-RD responder index	8	4–10
Disease activity index	5	2–6

patient, low but measurable amounts of IL-1 α , IL-1 β and IL-33 were detected; in a second serum sample obtained after one year of immunosuppressive therapy, lower but measurable amounts of IL-1 α , IL-1 β and IL-33 were still detectable.

The soluble receptors sIL-1R1 ($p = 0.0001$), sIL-1R2 ($p = 0.0024$), ST2/sIL-1R4 ($p = 0.002$) were all significantly increased in IgG4-RD sera compared with healthy controls (Fig. 1). sIL-1R3 was significantly lower in patients vs controls ($p = 0.0006$) (Fig. 1). After adjustment for multiple comparisons with Bonferroni *post hoc* test, significant differences were confirmed.

The levels of IL-1Ra, on the contrary, were similar in patients and controls.

No correlation was found between soluble receptors' levels and C reactive protein, IgG4 or IgE levels, disease activity index or responder index.

4. Discussion

The data obtained in this paper show that the levels of most soluble and decoy receptors of the IL-1 family are increased in IgG4-RD, suggesting a tight control of the cytokine signalling.

As reported in most normal or pathological conditions, IL-1 α and IL-1 β , very potent and tightly regulated cytokines, are undetectable. However, higher levels of the soluble forms of the two IL-1 receptors IL-1R1 and IL-1R2 are observed, suggesting an ongoing need of dampening inflammatory IL-1 signalling at the tissue level. The increased presence of sIL-1R1 and sIL-1R2 is in fact a sign of IL-1-mediated inflammation at the tissue level and of the activation of the long-range IL-1 control mechanisms, i.e., activation of proteases that cleave the membrane IL-1R1 and IL-1R2 receptors and release the IL-1-capturing soluble receptor forms [19]. The control of IL-1 β signalling (IL-1 α is an alarmin released at very low levels) is likely obtained mostly through an increased release of sIL-1R2. In fact, sIL-1R1 is present at lower levels compared to sIL-1R2, and in addition its ability to capture IL-1 β is probably hampered by the presence of IL-1Ra, which binds to sIL-1R1 with the same affinity as IL-1.

The inhibitory role of sIL-1R3 is not well understood, although it has been proposed that it could form inactive receptor complexes with membrane IL-1R1 bound to IL-1, and higher affinity inhibitory complexes with sIL-1R1. It should be noted that sIL-1R3 may form complexes also with sIL-1R4 in capturing IL-33, although also in this case clear evidence is not available [19]. However, in IgG4-RD patients the levels of sIL-1R3 are decreased, suggesting a lower efficacy of sIL-1R3 in controlling the activation of either IL-1 or IL-33. The IL-1 receptor antagonist IL-1Ra is in the normal range, further suggesting that control of IL-1-mediated inflammation takes place essentially through the action of sIL-1R2. In other inflammatory conditions, such as chronic

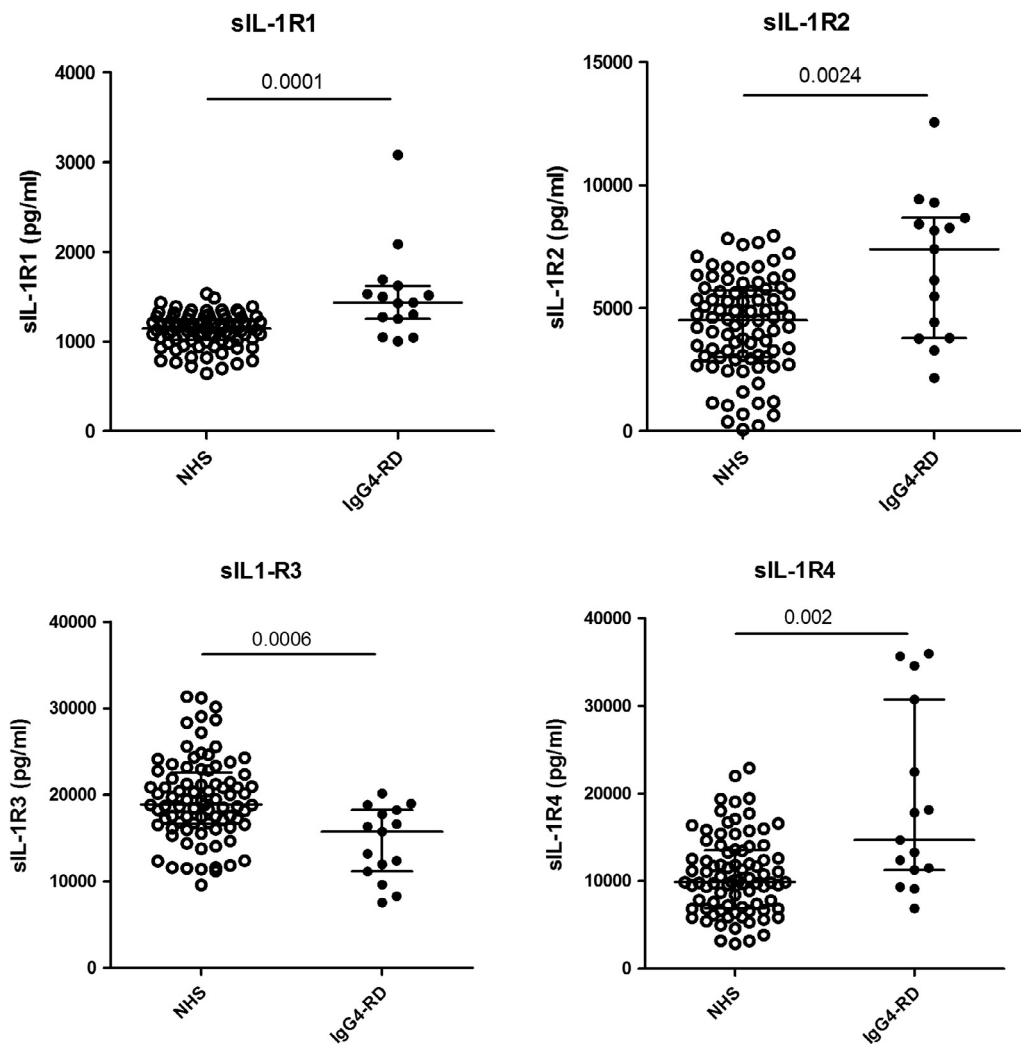


Fig. 1. Levels of soluble cytokines and receptors of the IL-1 family in IgG4-RD vs. normal healthy controls. sIL-1R1, sIL-1R2, sIL-1R3 and IL-1R4 were measured in serum of 80 normal healthy subjects (NHS) and 15 IgG4-RD patients. Data are expressed as pg/ml serum and compared, between IgG4-RD patients and controls, by means of the non-parametric Mann Whitney test (median, IQR and p values are given in each panel).

arthritis or sepsis, an overproduction of IL-1Ra is observed, most likely as an additional or alternative attempt of controlling the increased tissue levels of IL-1.

Similarly, the alarmin IL-33 is undetectable in the circulation (except in one patient), in the presence of high amounts of its decoy receptor ST2/sIL-1R4. IL-33 has the potential to affect several pathways possibly involved in the pathogenesis of IgG4-RD. By promoting the production of type 2 cytokines by Th2 cells, ILC2, mast cells and basophils, IL-33 may contribute to eosinophilia and IgE hyperproduction. By supporting macrophage transition to the M2 phenotype, IL-33 is critical for tissue repair, but it is also potentially involved in the induction of fibrosis [20]. In pulmonary and hepatic fibrosis, IL-33 is abnormally expressed and drives extracellular matrix deposition. A similar pro-fibrotic role has been suggested for skin, pancreas and renal fibrosis, while in the heart IL-33, induced by mechanical stress in cardiac fibroblasts, limits cardiomyocyte hypertrophy and fibrosis [21]. An increased expression of IL-33 and ST2/IL-1R4 has been recently observed in salivary glands from IgG4-RD patients; by double immunofluorescence, M2 macrophages have been identified as main cellular source of IL-33, while no data are available on ST2/IL-1R4 expression in the tissue [22].

In this context, increased levels of sIL-1R4 are viewed in most cases as protective, while representing a biomarker of cardiomyocyte stress and fibrosis in cardiovascular disorders.

However, sIL-1R4 activities are more complex than inhibition of IL-33 signalling, and only partially clarified. In fact, it has been shown in a mouse model of obesity that sIL-1R4 not only regulates IL-33

production and signalling, but it also induces the production of collagen type I and fibronectin in vascular smooth muscle cells [21]. In the organs affected by IgG4-RD, local IL-33 production may contribute to fibrosis, and therefore elevated circulating sIL-1R4 levels may represent the marker of an ongoing protective mechanism. However, it still needs to be investigated whether also in a human pathology such as IgG4-RD sIL-1R4 may act on vascular smooth muscle cells or on other target cells by enhancing the production of extracellular matrix components. In this scenario, elevated sIL-1R4 levels may in fact contribute to further organ damage. Follow-up studies of IgG4-RD patients are necessary, in order to evaluate whether the levels of soluble receptors of the IL-1 family have any predictive value on the disease course or response to therapy. Further studies addressing the expression of IL-1 family receptors on circulating cells and affected tissues may shed more light on the mechanisms leading to fibrosis in IgG4-RD.

5. Conclusions

IL-1 family cytokines signalling is tightly controlled in IgG4-RD; the contribution of ST2/sIL-1R4 to further organ damage cannot be excluded.

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Conflicts of interest

The authors report no conflicts of interests.

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