Novel Genetic Association of Wegener's Granulomatosis with the Interleukin 10 Gene

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ABSTRACT. ***Objective.*** Wegener's granulomatosis (WG) is a necrotizing vasculitis characterized by clonal expansions of T cells and production of antibodies against proteinase 3. The disease is associated with expanded dinucleotide repeats in the cytotoxic T lymphocyte antigen 4 (CTLA-4) gene, suggesting that genetic variation(s) in T cell related gene(s) could contribute to the T cell hyperactivity in WG. We investigated the polymorphisms in the genes of 2 cytokines, interleukin 4 (IL-4) and IL-10, which are essential for the polarization of T cells towards Th2 development and for the Ig production by B cells.
Methods. Polymorphisms in the genes coding for IL-10 and IL-4 were analyzed in 32-36 Swedish Caucasian patients and 109 ethnically matched healthy individuals.
Results. There was no association with the IL-4 gene. A CA repeat polymorphism in IL-10 gene, *IL-10.G*, was associated with the disease. This polymorphism has earlier been associated with high autoantibody production.
Conclusion. Our results indicate that the IL-10 gene may influence the disease, perhaps by influencing the production of autoantibodies. (J Rheumatol 2002;29:317–20)

Key Indexing Terms:
WEGENER'S GRANULOMATOSIS
INTERLEUKIN 10
GENE POLYMORPHISM
INTERLEUKIN 4

Wegener's granulomatosis (WG), a disease with necrotizing granulomatous vasculitis affecting small to medium vessels[1–3], is characterized by multiple immune abnormalities such as the clonal expansions of T cells[4-7], and the production of antineutrophil cytoplasmic antibodies (ANCA) directed mainly against proteinase 3 (PR3-ANCA)[8]. Compelling evidence[9] demonstrates that ANCA is a disease marker specific for WG, with a sensitivity of up to 95% in generalized WG and specificity for PR3-ANCA in the range of 80–100%. ANCA is also a useful, although not perfect, tool to assess disease activity in about two-thirds of patients, and there are data that ANCA may play a role in the pathogenesis of WG.

The Th2 signature cytokine, interleukin 4 (IL-4), plays a key role in B cell immunity[10,11]. IL-4 enables the polarization of T cells towards Th2 development, rescues B cells from apoptosis, enhances the antigen-presenting capacity of B cells and the proliferation of normal B cells activated through their antigen receptor or CD40 ligation, and directs switching towards the production of IgE and IgG4. As a potent growth and differentiation factor for activated human B cells[12], IL-10 is critical for the Ig production by B cells from patients with systemic lupus erythematosus (SLE). Peripheral blood mononuclear cells (PBMC) from patients with SLE produce significantly more IgM, IgG, and IgA by the addition of recombinant IL-10 *in vitro*, while *in vivo* administration of anti-IL-10 antibodies significantly reduces the immunoglobulin production by PBMC from patients with SLE in the SCID mice model[13].

There are genetic variations in the genes coding for IL-4 and IL-10. A dinucleotide repeat microsatellite, *4R1*, was found in the second intron of the IL-4 gene situated within the region coding IL-13, IL-5, IL-3, and granulocyte monocyte colony stimulating factor (GM-CSF) on chromosome 5[14]. *4R1* is strongly associated with atopic diseases and serum IgE levels[15]. In the third intron of the IL-4 gene, there is a variable number of tandem repeat (VNTR) polymorphism related to the age of disease onset in multiple sclerosis, implying the capacity of this VNTR polymorphism to shift T cell development towards the Th2 pathway[16]. A CA repeat microsatellite, *IL-10.G*, is located in the promoter region of the IL-10 gene situated on 1q31 and 1q32[17]. It is of interest to note that *IL-10.G* is associated with autoantibody production in SLE[18] and in myasthenia gravis (MG)[19].

An AT repeat polymorphism in the 3′-untranslated region of exon 3 of the gene coding cytotoxic T lymphocyte associated antigen 4 (CTLA-4) is strongly associated with WG, indicating that genetic factor(s) contribute to the T cell hyperactivity in the disease[20]. With respect to the overproduction of autoantibodies in WG, we have conducted a genetic association study on the genes coding Th2 cytokines (IL-4 and IL-10) in Swedish patients with WG and ethnically matched healthy individuals.

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Supported by grants from the Swedish Heart-Lung Foundation, the King Gustaf Vths 80-years Foundation, the Swedish Medical Research Council, the Börje Dahlin, Åke Wiberg, and Magnus Bergwall Foundations.

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Submitted September 7, 1999; revision accepted August 16, 2001.

MATERIALS AND METHODS

Patients and healthy controls. Thirty-six unrelated Swedish Caucasian patients and 109 ethnically matched healthy controls were included in the study. All patients fulfilled the described diagnostic criteria[21] and were recruited in the Department of Rheumatology, Karolinska Hospital, Stockholm. The age of the patients ranged from 30 to 81 years (mean 54). Thirty-three patients had PR3-ANCA. Twenty-two patients were male and 14 were female. Nine patients had localized disease involving the upper respiratory tracts and/or eye only, and 27 had a generalized form with renal, lung, heart, and/or central and peripheral nervous system involvement. The study was approved by the ethical committee of the Karolinska Hospital.

DNA extraction and genotyping. DNA was extracted from peripheral blood leukocytes using a standard proteinase K digestion and phenol/chloroform method.

Primers used to amplify the region containing the IL-4 VNTR polymorphism in the third intron of IL-4 gene and the polymerase chain reaction (PCR) variables were designed as described previously[16]. PCR was performed in 20 ml volume per tube in a programmed thermal cycler (Techne, PHC-3 Dri-Block, Cambridge). Products were examined by 2.0% Nusieve GTG agarose gel and electrophoresed at 5 V/cm for 3 h. Alleles were named as B1 with the PCR products of 183 bp in length and, B2, 253 bp.

Fluorescence-based technique was employed to analyze the dinucleotide microsatellite, *4R1* and *IL-10.G*. The primers were commercially obtained from Life Technologies and a fluorescent dye, HEX, was introduced in the 5′ end of the forward primers. The detailed sequence of the primers and the PCR variables were the same as described[15,17], respectively. A mixture of PCR products and internal size standard (Gene Scan 350-TAMRA, Perkin Elmer) was loaded onto 5% denaturing polyacrylamide gel (Long Ranger) in the ABI 377 sequencer. Data were analyzed with the GeneScan 672 (version 1.0) and the Genotyper software (version 2.1, Perkin Elmer). Alleles were named after the length of PCR products[19].

Statistical analysis. The Instat 2.02A software package was used in the statistical analysis. Comparison was done by Fisher's exact test. All p values were two sided. Although the necessity of correcting the p values for multiple statistical significance tests is still being debated[22,23], for cautious interpretation, Bonferroni corrections were made for all p values. Both pc value and p value were given; a pc value was selected to interpret the results.

RESULTS

IL-10.G is associated with WG. Table 1 presents the genotypic distributions of *IL-10.G* in 36 Swedish Caucasian patients and 109 ethnically matched healthy individuals. A significantly higher percentage of patients heterozygous for *IL-10.G* allele 134 and 136 were observed compared with healthy individuals. There was also a significant difference with respect to some of the genotypes that were absent in patients but present in the healthy group. The prevalence of the WG-associated genotype (*IL-10.G* 134/146) in the ANCA positive patients (38%, 14/33) was significantly higher than that in the healthy individuals (8%, 9/109, p <0.0001, odds ratio: 8.19, 95% confidence interval 3.10–21.6). Due to the small number of patients who lacked ANCA (n = 3), it is difficult to draw any statistical conclusion in ANCA negative WG.

The allelic frequencies of *IL-10.G* in patients with WG and healthy individuals are shown in Table 2. The percentage of *IL-10.G* allele 134 was significantly increased in WG patients, thus supporting the results from the genotypic analysis. There was no relation of *IL-10.G* to the sex of the patients, the age of disease onset or the organ involvement in the patients (data not shown).

Table 1. IL–10.G genotypes in Swedish Caucasian patients with Wegener's granulomatosis and healthy controls.

Genotype	Patients n = 36 (%)	Controls n = 109 (%)
130/140	NP	1 (0.9)
132/132	NP	1 (0.9)
132/134	NP	1 (0.9)
132/136	1 (2.8)	3 (2.8)
132/138	NP	1 (0.9)
132/140	NP	1 (0.9)
132/144	NP	3 (2.8)
134/136	15 (41.7)*	9 (8.3)
134/138	NP	1 (0.9)
134/142	NP	1 (0.9)
134/144	NP	4 (3.7)
134/146	NP	1 (0.9)
136/136	NP	6 (5.5)
136/138	2 (5.6)	3 (2.8)
136/140	NP	8 (7.3)
136/142	NP	5 (4.6)
136/144	6 (16.7)	12 (11.0)
136/146	1 (2.8)	9 (8.3)
136/148	NP	2 (1.8)
138/138	NP	2 (1.8)
138/140	4 (11.1)	5 (4.6)
138/142	NP	1 (0.9)
138/144	NP	6 (5.5)
138/146	NP	3 (2.8)
140/142	NP	1 (0.9)
140/144	2 (5.6)	5 (4.6)
140/146	1 (2.8)	NP
140/152	1 (2.8)	NP
142/142	NP	3 (2.8)
142/144	2 (5.6)	5 (4.6)
144/144	NP	5 (4.6)
144/146	1 (2.8)	1 (0.9)

NP: not present.
* p < 0.0001; pc < 0.0032 vs healthy controls; OR 7.94; 95% confidence interval: 3.07–20.5.

Table 2. IL-10.G allelic frequencies in Swedish Caucasian patients with Wegener's granulomatosis and healthy controls.

Allele	Patients n = 72 (%)	Controls n = 218 (%)
130	NP	1 (0.3)
132	1 (1.4)	11 (5.0)
134	15 (20.8)*	17 (7.8)
136	25 (34.7)	63 (28.9)
138	6 (8.3)	24 (11.0)
140	8 (11.1)	21 (9.6)
142	2 (2.8)	19 (8.7)
144	11 (15.3)	46 (21.1)
146	3 (4.2)	14 (6.4)
148	NP	2 (0.9)
152	1 (1.4)	NP

NP: not present.
* p < 0.01; pc < 0.05; OR 3.11; 95% confidence interval 1.46–6.61, vs healthy controls.

No association of IL-4 gene with WG. The VNTR polymorphism in the IL-4 gene was not different in allelic (data not shown) and genotypic frequencies (Table 3) between WG patients (n = 32) and healthy individuals. Also the dinucleotide repeat polymorphism *4R1* showed the same genotypic (Table 3) and allelic (data not shown) frequencies in WG patients and healthy individuals. There was no relation of IL-4 VNTR and 4R1 to the sex of the patients, the age of disease onset, the involvement of organs, or presence of circulating ANCA in the patients (data not shown).

DISCUSSION

Our study documents for the first time that WG is associated with *IL-10.G* in the IL-10 gene. The association is relatively strong. Together with the previous report that WG is associated with the AT repeat microsatellite polymorphism in the CTLA-4 gene, our results further advocate the notion that genetic factors contribute to the disease etiology. Further, our results also demonstrate that WG is a polygenetic disease, similar to other autoimmune disorders such as insulin dependent diabetes mellitus and multiple sclerosis.

Both IL-4 and IL-10 are Th2 cytokines and of importance in B cell immunity. The fact that WG is associated with IL-10 but not IL-4 gene suggests that IL-4 and IL-10 may have different influences on the initiation and development of the disease. *IL-10.G* genotype 134/136 and allele 134 are associated both with the concentration of anti-acetylcholine receptor antibodies in MG[19] and with levels of autoantibodies in SLE[18], although it is difficult to know exactly whether the same genotype and allele was committed for MG and SLE due to the different methods used[18,19]. The *IL-10.G* genotype 134/136 and/or allele 134 might influence the production of autoantibodies and finally the expression of the disease. Recent data support influence of *IL-10.G* on the secretion of IL-10[24]. In this study, the allele *IL-10.G* 136 was associated with increased production of IL-10 in the supernatant of lipopolysaccharide-stimulated PBMC from patients with multiple myeloma.

IL-10.G may serve as a genetic marker for a disease gene that is closely located to the IL-10 gene. The important location of *IL-10.G* in the promoter region of the IL-10 gene where many potential transcriptional factor binding sites are situated[25] and, the functional properties of IL-10 in heightening Ig production strongly suggest that *IL-10.G* itself might contribute to the pathogenesis of WG. Further functional studies of *IL-10.G* are necessary to elucidate the mechanisms behind the association of WG with the IL-10 gene.

Table 3. IL-4 gene polymorphisms in Swedish Caucasian patients with Wegener's granulomatosis and healthy controls.

	Patients (%)	Controls (%)
VNTR polymorphism		
B1/B1	1 (3.1)	6 (5.6)
B1/B2	14 (43.8)	34 (31.8)
B2/B2	17 (53.1)	67 (62.6)
Total	32 (100)	107 (100)
4R1 microsatellite		
152/154	2 (6.3)	2 (1.9)
152/158	NP	1 (1.0)
154/156	27 (84.4)	89 (85.6)
154/160	NP	1 (1.0)
156/156	NP	1 (1.0)
156/158	3 (9.4)	9 (8.7)
156/160	NP	1 (1.0)
Total	32 (100)	104 (100)

VNTR: variable number of tandem repeat; NP: not present.

ACKNOWLEDGMENT

We thank Drs. Elisabeth Svennungsson and Martha Christensson for providing WG samples.

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