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Immunogenetic characteristics of patients with autoimmune gastritis

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Abstract

AIM: To explore whether predisposition to autoimmune gastritis (AIG) is found in human leukocyte antigen (HLA), cytokine or killer cell immunoglobulin-like receptor (KIR) gene variations.

METHODS: Twelve Finnish patients with autoimmune-type severe atrophy of the gastric corpus were included. The patients’ serum was analyzed for pepsinogen I and Helicobacter pylori (H. pylori) antibodies. DNA was separated and the patients were genotyped for HLA-A, B, Cw, DRB1 and DQB1 antigens, and studied for single nucleotide polymorphisms for the following cytokines: interleukin (IL)-1 gene cluster, IL-2, IL-4, IL-6, IL-10, IL-12, interferon γ, transforming growth factor β, and tumor necrosis factor α. Variation in KIR genes was also explored. The results were compared with prevalence of the polymorphisms in Finnish or European populations.

RESULTS: All patients had pepsinogen I levels below normal (mean: 11 µg/L, range: < 5 to 25 µg/L). Three patients had elevated H. pylori IgG antibodies, while H. pylori serology was negative in the rest of the patients. AIG patients carried significantly more often HLA-DRB1*04 (58%) and DQB1*03 (83%) than the general Finnish population did (28% and 51%, respectively; \( P = 0.045 \) and \( P = 0.034 \) by the Fisher’s exact test). No patient was positive for HLA-B8-DRB1*03, a well-established autoimmune marker. Neither cytokine polymorphisms nor KIR gene variation showed association with AIG.

CONCLUSION: As explored with modern DNA-based methods, HLA-DRB1*04 and DQB1*03 alleles, but not HLA-B8-DRB1*03, may predispose to AIG.

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Key words: Atrophic gastritis; Autoimmune diseases; Cytokines; Genetic polymorphisms; Human leukocyte antigens; Killer cell immunoglobulin-like receptor

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INTRODUCTION

Autoimmune gastritis (AIG) is an organ-specific autoimmune disease, in which inflammation of the mucosa of the gastric corpus results in total loss of corpus-type glands, and achlorhydria. AIG patients typically have a low serum pepsinogen I (PG I) concentration, and most of them also have parietal cell antibodies (PCAs). In many, but not all patients, vitamin B12 absorption is deficient, which leads to pernicious anemia (PA) [1].

The occurrence of AIG and PA has long been recognized to be determined strongly by genetic factors, which, however, are largely unexplored. The most important genetic association found in human AIG so far is a link with the human leukocyte antigen (HLA) region. The observed association between AIG and certain HLA antigens has, however, not been strong enough to explain the familial clustering of AIG [2].

Polymorphisms in the genes that encode immune regulator molecules may affect the secretion or function of the corresponding proteins, and thus influence immune responses, inflammation and tissue injury. Cytokine genes have been studied widely in autoimmune diseases and associations have been found between, for instance, tumor necrosis factor α (TNFα) and interleukin (IL)-10 polymorphisms and autoimmune hepatitis and pemphigus, respectively [3-6]. Also Helicobacter pylori (H. pylori)-associated atrophic gastritis has been shown to be more frequent in patients with proinflammatory polymorphisms of genes for IL-1 gene cluster, and TNFα [7].

Killer cell immunoglobulin-like receptors (KIRs) are members of a diverse family of regulatory molecules expressed on subsets of T cells. KIRs play a role in the control of the natural killer (NK) cell immune response. The KIR receptors recognize certain HLA class I determinants and regulate NK cell activity. The number and type of KIR genes vary between individuals who can carry anything from seven to 12 KIR genes, of which, some encode activating and others inhibiting receptors [8-11]. KIR genes can be divided into two main haplotype groups. Group A contains only one activating and six inhibiting KIR genes, whereas group B haplotypes are more variable and contain several activating KIR genes [11]. In addition to the copy-number variation, individual KIR genes exhibit allelic variation. KIR genes have been shown to be associated with various diseases, including some autoimmune diseases [11].

Recently, we sequenced the coding regions of genes for α- and β-subunits of H⁺/K⁺-ATPase, which is the main autoantigen in AIG, in AIG patients, but no disease-associated polymorphisms could be found [12]. In the present study, a number of genes involved in immune activation were explored in patients with AIG, by modern molecular genetic methods. The aim of this study was to determine whether variations in the immune regulator genes, such as HLA, cytokine or KIR, are associated with the presence of AIG.

MATERIALS AND METHODS

Clinical information

A total of 18 patients, who had earlier undergone gastroscopy at Herttoniemi Hospital and were known to have severe atrophic corpus gastritis without any history of H. pylori infection, and who were under 65 years of age, were invited by letter to participate in the study. Twelve patients gave written informed consent, donated a blood sample, and completed a questionnaire about their possible vitamin B12 replacement therapy and thyroid diseases, as well as the occurrence of AIG in the family. Signs of other autoimmune diseases were looked for in the patient records. The study was approved by the Ethical Committee for Internal Medicine at Helsinki University Central Hospital.

Blood tests

EDTA blood and serum samples were kept at -20°C until analyzed. DNA was extracted from the EDTA blood sample using a DNA purification kit (PureGene®; Centrasytems, Minneapolis, MN, USA), according to the manufacturer's instructions. Serum samples were analysed for PG I, PCAs and H. pylori antibodies.

For serum PG I concentrations, an immunoenzymometric assay (Gastroset PG1; Orion Diagnostica, Espoo, Finland) was used. The lower normal limit of the assay was 28 μg/L. PCAs were determined by an enzyme immunoassay (Varelisa Parietal Cell Antibodies; Pharmacia Diagnostics, Freiburg, Germany), which used H⁺/K⁺-ATPase as the antigen. Concentrations < 10 U/mL were normal, according to the manufacturer. For H. pylori antibodies, an in-house immunoassay that measured IgG antibodies was used, and titers ≥ 700 were considered elevated [13].

Immunogenetics

HLA genes were explored using the INNO-LiPA kit (Innogenetics, Ghent, Belgium) according to the manufacturer's instructions. The HLA-A, B, Cw, DRB1 and DBQ1B genes were amplified by polymerase chain reaction (PCR), and the biotinylated PCR products were hybridized with sequence-specific oligonucleotides on membrane-based strips. Results were analyzed by the LiRAS (Innogenetics) interpretation software.

Cytokine polymorphisms in the genes of IL-1 gene cluster, IL-2, IL-4, IL-6, IL-10, IL-12, interferon (IFN)-γ, transforming growth factor (TGF) β, and TNFα were genotyped using the Cytokine Genotyping Kit (Pel-Freeze Clinical Systems, Brown Deer, WI, USA). Cytokine profiles (high/intermediate/low producer) based on the polymorphisms were formed according to the published phenotypes also mentioned in the product insert of the kit. KIR genes (KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL1, KIR3DL2, KIR3DL3, KIR3DS1, KIR3DP1 and KIR3DP4) were determined using the KIR Genotyping Kit (Pel-Freeze Clinical Systems), following the manufacturer's instructions. Both genotyping kits were
based on PCR amplification with sequence-specific primers that were designed to detect polymorphisms of cytokine/KIR genes. The PCR products were separated by gel electrophoresis, and the genotype results were interpreted on the basis of specific amplification patterns.

Prevalence of the HLA genotypes was compared with HLA frequency in the Finnish population, based on the data collected in Clinical Laboratory of Finnish Red Cross blood service. The cytokine polymorphisms and KIR genes were compared with the frequency of polymorphisms and KIR genes in populations of Finnish of European ancestry published previously

Statistical analysis
Fisher’s exact test was used to compare the prevalence of genotypes between patients and the populations used as controls.

RESULTS
Demographic and clinical characteristics of the patients are summarized in Table 1. All patients had total atrophy in the gastric corpus. The mucosa of the gastric antrum was normal in eight patients, and mild chronic inflammation or sparse intestinal metaplasia was detected in four. All patients had PG I levels below normal (mean: 11 µg/L, range: <5 to 22 µg/L), and elevated PCAs (median: 185 U/mL, range: 20-509 U/mL). Three patients (numbers 3, 10 and 11 in Tables 1 and 2) had elevated \( H. pylori \) IgG antibodies (titers: 730-2200), whereas \( H. pylori \) serology was entirely negative in the rest of the patients (titers: 50-100). All patients but one (number 2 in Tables 1 and 2) had vitamin B12 replacement therapy.

Immunogenetics of the patients
The HLA-A, B, Cw, DRB1 and DQB1 alleles in the AIG patients are shown in Table 2. DRB1*04 was present in seven out of 12 (58%) patients, whereas 28% of the Finnish general population carry the allele (\( P = 0.045 \) by Fisher’s exact test). Ten patients (83%) had DQB1*03; its allele frequency in the Finnish population is 51% (\( P = 0.034 \) by Fisher’s exact test).

Only one of the 12 patients carried the DRB1*0301-DQB1*0201 haplotype, which is an established susceptibility factor for various autoimmune diseases [10]. It is of particular note that the only DRB1*0301-positive patient did not have the classical A*01-B*08 haplotype.

The frequencies of polymorphisms in the genes of the \( IL-1 \) gene cluster, \( IL-2, IL-4, IL-6, IL-10, IL-12, IFN-\gamma, TGF-\beta \) and TNF-\( \alpha \) did not differ significantly from those found in Finnish (where data were available) or other European populations. The results for genotyping the \( IL-1 \) gene cluster, TNF-\( \alpha \) and IL-10 are shown in Table 3.

All 14 characterized KIR genes, \( KIR2DL1, KIR2DL2, KIR2DL3, KIR2DLA, KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL1, KIR3DL2, KIR3DL3 and KIR3DS1 \), were determined, as well as two KIR pseudogenes \( KIR2DP1 \) and \( KIR3DP1 \). Ten patients carried both A and B KIR haplotypes; two patients were homozygotes for A haplotype. KIR genotype and haplotype frequencies of the patients did not differ from those reported earlier in the Finnish population [11, 12].

| Table 1 Clinical characteristics of the 12 AIG patients |
|----------------|---------|----------------|----------------|----------------|
| Patient | Sex | Age (yr) | Years from diagnosis | Other autoimmune diseases | AIG in family |
| 1 | F | 50 | 7 | - | - |
| 2 | F | 56 | 3 | RA | + |
| 3 | F | 49 | 2 | + | - |
| 4 | M | 62 | 19 | + | + |
| 5 | F | 63 | 2 | HT | + |
| 6 | F | 54 | 3 | - | - |
| 7 | F | 38 | 4 | - | - |
| 8 | F | 56 | 5 | PBC | - |
| 9 | F | 52 | 0 | - | - |
| 10 | F | 52 | 0 | - | - |
| 11 | F | 47 | 15 | T1D | - |
| 12 | F | 52 | 0 | - | - |

AIG: Autoimmune gastritis; RA: Rheumatoid arthritis; HT: Hyperthyreosis; PBC: Primary biliary cirrhosis; T1D: Type 1 diabetes.

| Table 2 HLA-A, B, Cw, DRB1 and DQB1 genotypes of the 12 AIG patients |
|----------------|---------|---------|---------|---------|---------|
| Patient | HLA-A | HLA-B | HLA-Cw | HLA-DRB1 | HLA-DQB1 |
| 1 | 03 | 11 | 07 | 44 | 01 | 07 | 12 | 15 | 0301 | 0602 |
| 2 | 02 | 15 | 51 | 04 | 14 | 04 | 08 | 15 | 0302 | 0402 |
| 3 | 02 | 15 | 39 | 04 | 07 | 04 | 15 | 0302 | 0602 |
| 4 | 02 | 15 | 18 | 07 | - | 04 | 15 | 0302 | 0602 |
| 5 | 02 | 15 | 40 | 03 | 04 | 01 | 08 | 0402 | 0501 |
| 6 | 02 | 15 | - | 03 | - | 04 | 08 | 0302 | 0402 |
| 7 | 02 | 15 | 51 | 06 | 15 | 07 | 09 | 0202 | 0303 |
| 8 | 02 | 40 | 51 | 02 | 15 | 09 | 15 | 0303 | 0602 |
| 9 | 03 | 07 | 51 | 03 | 15 | 04 | 13 | 0301 | 0303 |
| 10 | 02 | 35 | 40 | 04 | 07 | 04 | 12 | 0301 | 0302 |
| 11 | 02 | 27 | 35 | 01 | 03 | 03 | 08 | 0201 | 0402 |
| 12 | 03 | 13 | 35 | 04 | 06 | 04 | - | 0302 | - |

In Finnish AIG patients, the HLA-DRB1*04 and DQB1*03 alleles were more frequent than in the general population, which implies an association between certain HLA-DRB1 and DQB1 haplotypes and AIG. The well-known autoimmune markers HLA-B8, DRB1*03 and DQB1*02 were practically missing in the AIG patients. This suggests that the immunogenetics of AIG are different to that of many classical autoimmune diseases.

The co-localization of susceptibility foci in experimental AIG and type 1 diabetes (T1D) is the strongest known between two autoimmune diseases [2], and the most prominent susceptibility locus for both diseases is located in the HLA region. Individuals with T1D also have PCAs more often than population controls do [3]. Over 90% of Caucasians with T1D carry the DR3 or DR4 haplotype, and the DQB1*0302 allele is associated strongly with T1D [3]. In the present study, the DRB1*04 allele was more frequent in AIG patients.
than in the general population, but the DRB1*03 allele was only carried by the patient with TID. Six of our 12 patients had DQB1*0302, the prevalence of which in the Finnish population is 13% (\(P = 0.005\) by Fisher's exact test). The AIG patient with TID was also the only one to carry the DQB1*02 haplotype, which is present in 91% of Finnish celiac disease patients\(^{[19]}\) and in 17% of the general Finnish population. Thus, Finnish AIG patients seem to share some of the haplotypes that are common in patients with TID, but not those seen in patients with celiac disease.

In the 1970s, several studies were carried out to find a possible association between AIG or PA and HLA antigens. Increased frequency of HLA antigens A3, B7, or both has been found in AIG and PA patients\(^{[20-22]}\); however, these findings were not confirmed by others\(^{[23]}\).

Subgroups of AIG patients have shown associations with different HLA antigens. Patients with a concomitant endocrine disease showed an increased frequency of the B8, B18 and BW15 antigens, and those without endocrine disease that of the B7 and B12 antigens\(^{[24]}\).

Of the class II HLA antigens, PA patients showed increased frequency of the DR2 and DR4 antigens and a decreased presence of the DR3 antigen, as compared to controls. PA patients with a concomitant endocrine disease showed DR3/DR4 antigens more often, and those without autoimmune endocrine disease showed DR2/DR4 and DR4/DR5 antigens, as compared to controls\(^{[25]}\). Possibly because of the small number of patients in the present study, no significant difference could be found between those with and without concomitant autoimmune disease.

The role of \(H. pylori\) in AIG and PA is still poorly understood\(^{[26]}\). On one hand, patients with \(H. pylori\) infection often develop atrophic gastritis and even autoimmune characteristics, such as PCAs\(^{[1]}\). On the other hand, AIG patients without any signs of \(H. pylori\) infection, such as the majority of patients in the present study, may be found. In studies before the \(Helicobacter\) era, the role of \(H. pylori\) in atrophic gastritis was not recognized, and patients with \(H. pylori\)-associated autoimmunity may have been included; this may have made it more difficult to detect associations between AIG and, for example, HLA antigens. Our patients were relatively young with a median age of 52 years and the majority were women, which is typical for the classic AIG\(^{[3]}\). The three patients with positive \(H. pylori\) serology showed no clinical difference from the others.

In \(H. pylori\)-positive individuals, proinflammatory polymorphisms of the \(IL-1B\) gene cluster have been found to be associated with atrophic gastritis, achlorhydria\(^{[27,28]}\), and even gastric cancer\(^{[29]}\), which often is a late sequel of atrophic gastritis. Patients carrying proinflammatory \(IL-1B-511T\) and TNF\(_\gamma\)-308A, and who are homozygous for \(IL-1RN*2*2\), had an OR of 5.8 for developing atrophic gastritis\(^{[30]}\). In addition, patients that carried three or more of the proinflammatory polymorphisms (carriage of \(IL-1B-511T^+\) and TNF\(_\gamma\)-308A; homozygosity for \(IL-1RN*2*2\) or \(IL-10\) ATA/ATA) had an OR of 26.3 for non-cardia gastric cancer\(^{[30]}\). However, the association between gastric cancer and \(IL-1\beta\) polymorphisms has not been seen in all studies\(^{[31]}\). Despite the fact that all our patients had profound atrophy in the gastric corpus at a relatively young age, the frequencies of these particular genotypes did not differ from those found in populations with European ancestry. Even though the small number of patients and the lack of controls in the present study make it impossible to detect small or modest associations, our results suggest that these polymorphisms are not crucial for the development of AIG.

In conclusion, HLA DRB1*04 and DQB1*03 were more frequent in AIG patients than in the general Finnish population, which suggests an association between certain HLA-DRB1 and DQB1 haplotypes and AIG. Also, the well-known autoimmune markers HLA-B8, DRB1*03 and DQB1*02 were practically missing in the AIG patients. However the number of patients in the present study was small, and larger studies are needed to confirm these findings.

### COMMENTS

#### Background

Autoimmune gastritis (AIG) is chronic inflammation in the mucosa of the gastric body, which may lead to vitamin B12 deficiency and pernicious anemia. The cause of this inflammation is not known, but its occurrence is known to be strongly determined by genetic factors.

#### Research frontiers

In earlier studies using antigen determination for the detection of human leukocyte antigen (HLA) tissue determinants, an association was found between HLA tissue and autoimmunity. Before the present study, this association had not been studied by modern DNA-based methods.

#### Innovations and breakthroughs

The study is believed to be the first to show an association between AIG and certain HLA genotypes, as explored with modern DNA-based methods.

#### Applications

The study included a small number of patients. These results may in the future contribute to exploring the mechanisms of AIG and possibly other autoimmune diseases. AIG is also a risk factor for gastric cancer; thus, understanding the evolution of AIG may contribute to exploring the development of cancer.

#### Peer review

This is a good pilot study that indicates the need for a much bigger, longer-term study.
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S-Editor: Tian L  L-Editor: Kerr C  E-Editor: Tian L