Hypothesis: Several immunological alterations have been found in patients with multinodular goiter (MG). These alterations, together with the association described between certain autoimmune thyroid diseases and alleles of the major histocompatibility complex (HLA alleles), justify the need for studies of the HLA alleles and MG in an attempt to identify associations.

Design: Case-control study.

Setting: Tertiary referral center.

Patients: Ninety consecutive patients underwent surgical procedures for MG. The control group comprised 100 unrelated, healthy, white subjects.

Intervention: Genotyping for HLA-C alleles was done using the molecular biological technique of polymerase chain reaction using sequence-specific primers and was carried out for all of the patients.

Main Outcome Measures: The analyzed variables included age, sex, family history of thyroid pathological abnormalities, clinical features of the patient, clinical grading of the goiter, intrathoracic thyroid component, goiter weight, associated carcinoma, and the HLA-C gene.

Results: A significant association was observed between the lower incidence of the HLA-Cw4 allele and the appearance of MG (15.5% vs 8.3%, respectively; \( P = .001 \); relative risk [RR] = 0.49). These results suggest that the HLA-Cw4 allele can exert a protective effect against MG. Analysis of the different clinical variables shows the most significant association to be the absence of the HLA-Cw4 allele in patients with goiters with an intrathoracic component (\( P = .001 \); RR = 0.19) and in patients with goiters weighing more than 200 g (\( P = .02 \); RR = 0.17). Associations between the HLA-C alleles and MG were also observed, such as the presence of the HLA-Cw7 allele and a family history of thyroid pathological abnormalities (\( P = .03 \); RR = 3.91) as well as the HLA-Cw1 allele and the presence of goiter-associated thyroid carcinoma (\( P = .02 \); RR = 8.60).

Conclusions: The HLA-Cw4 allele can act as a protector against the development of MG, as it occurs less frequently in the population with MG, and those with this allele develop smaller goiters with no intrathoracic component.

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ter. This occurrence, together with the association described between certain autoimmune thyroid diseases and alleles of the major histocompatibility complex (HLA alleles) justifies the need for studies of HLA and MG in an attempt to identify associations and possible risk factors.

In the past, serological techniques were used to detect HLA antigens, as this is a useful method for detecting class A and B alleles. However, the results are very poor when serological techniques are used for detecting HLA-C alleles. This explains why there are few studies of HLA-C alleles in thyroid pathology. \(^\text{10,11}\) Currently, the development of molecular biological techniques allows for a more reliable detection of these antigens. \(^\text{12}\) However, probably owing to cost and the fact that these techniques have only existed for a short time, there have been only a few studies \(^\text{12-15}\) using these techniques and none to our knowledge using them to study MG.

The aim of this study is to determine whether there are any associations between MG and HLA-C alleles to detect which may be risk factors and which may provide protection from disease.

### METHODS

#### PATIENTS

The study group comprised 90 patients who underwent surgery in our department between January 1997 and December 2000. The mean ± SD patient age was 49 ± 13 years, and most patients (83 patients [92%]) were women, of whom 13 (12%) had a family history of thyroid pathological abnormalities and 21 (23%) lived in goitrogenic areas. The mean evolution time was more than 6 years (mean ± SD evolution time, 82 ± 90 months); 34 cases (38%) were clinically asymptomatic, 24 (27%) had compressive features, and 23 (26%) had hyperthyroidism. On examination, the goiter was grade I (can be touched but not seen) in 6 cases (7%), grade II (can be seen and touched) in 60 (67%), and grade III (affects neighboring structures) in the remaining 24 (27%). On palpation, 79 (88%) of the goiters had an elastic consistency, and 42 (47%) were intrathoracic. Neck and chest radiographic examination showed tracheal deviation or compression in 40 cases (44%), all of them corresponding to intrathoracic goiters. Ultrasonographic examination confirmed the presence of MG, and scintigraphic examination was performed for the 23 patients with hyperthyroidism. Thyroid antibodies were negative in all of the cases.

The indications for surgery were the presence of compression symptoms (24 cases [27%]), suggestion of malignancy (23 cases [26%]), asymptomatic intrathoracic goiter (14 cases [16%]), hyperthyroidism (12 cases [13%]), a progressive increase in size (7 cases [8%]), the patient's own request (5 cases [5%]), and radiological tracheal compression (5 cases [5%]). Eighty-six patients (96%) received a total thyroidectomy, and the other 4 (4%) received a hemithyroidectomy for a unilateral MG. The mean ± SD weight of the excised specimen was 83 ± 67 g, with 16 cases (18%) having specimens weighing more than 200 g. The histological study confirmed the presence of multinodular hyperplasia in all of the cases, and 11 cases (12%) were associated with thyroid carcinoma.

#### HLA-C TYPING

The molecular biological technique of polymerase chain reaction using sequence-specific primers was used for typing the HLA-C genes. \(^\text{12-14}\) For this, a peripheral blood extraction was taken in a tube containing EDTA. The steps for this technique include the Higuchi method of rapid nucleic acid extraction and DNA quantification with a spectrophotometer (Amer- sham Pharmacia Biotech, Piscataway, NJ). Subsequently, the FasttypeTM SSP System (Bio-Synthesis, Inc, Lewisville, Tex; supplied by Diagnostica Longwood SA, Zaragoza, Spain) was used for typing following the manufacturer's recommendations. When the amplification reaction was finished, the tubes were left to cool at room temperature and the amplification was viewed in agarose gel using a UV-light transilluminator. The presence of amplification was recorded using a Polaroid 440 camera (Pol- laroid [France] SA, Montigny le Bretonneux, France). The entire HLA-typing process was carried out by 1 of us (M.R.M.).

### CONTROL GROUP

The method described earlier was also used to study HLA-C alleles in a control sample of 100 unrelated, healthy, white subjects. This control group corresponded to subjects representative of the healthy population randomly selected from unrelated, healthy, Spanish, white blood donors. We previously ruled out thyroid pathological abnormalities in these subjects by clinical examination.

### STATISTICAL ANALYSIS

The variables analyzed for detecting associations between certain alleles and certain subgroups of patients with goiter included age, sex, family history of thyroid pathological abnormalities, residence in goitrogenic areas, being clinically asymptomatic, presence of hyperthyroidism, compression symptoms, goiter grading based on physical examination, presence of an intrathoracic component according to the criterion of Da- han et al \(^\text{16}\) (goiter that, in an operative position, has its lower edge at least 3 cm below the sternal manubrium), excised goiter weight of 200 g or more, and presence of goiter-associated thyroid carcinoma.

The HLA-C allele frequencies were estimated by direct counts, and they represent the percentage of individuals who are positive for a particular allele. To compare the differences between the frequencies in the control and MG groups, a \(\chi^2\) contingency table analysis was done using the Pearson \(\chi^2\) test and the Mantel-Haenszel test, with the Fisher exact test when the expected value for an HLA marker was less than 5. All of the analyses were performed using the Epi Info version 3.01 software package (Centers for Disease Control and Prevention, Atlanta, Ga). The relative risk (RR) was calculated according to the Woolf method. The \(P\) values were corrected by multiplying them by the number of alleles tested (Bonferroni correction). \(^\text{17}\) The association between the clinical variables and HLA-C alleles was made using the Pearson \(\chi^2\) test, with the Fisher exact test when the expected value for an HLA marker was less than 5. Only \(P\) values of less than .05 were considered statistically significant.

### RESULTS

#### GENERAL ANALYSIS

The most common alleles in MG were HLA-Cw7 (37 alleles [20.6%]), followed by HLA-Cw16 (24 alleles [13.3%]), and HLA-Cw5 (19 alleles [10.6%]). Table 1 shows that the distribution of the HLA-Cw4 allele in MG is significantly less than in the control group (15 alleles [8.3%]; \(P=.001\); Bonferroni-corrected \(P=.02, \text{RR}=0.49\)). Thus, the HLA-Cw4 allele has an incidence of 15.5% in...
the control group whereas it occurs in just 8.3% in the MG group. These results suggest that the HLA-Cw4 allele can provide a protective effect against MG.

The HLA-Cw16 allele has a lower frequency in the control group (15 alleles [7.5%]) than in the MG group (24 alleles [13.3%]). However, these initially significant differences ($P = .0496; RR=1.92$) are lost when we apply the Bonferroni correction (Bonferroni-corrected $P = .89$).

**CLINICAL VARIABLES ANALYSIS**

On analyzing the associations between the different clinical variables of the patients with MG and HLA-C alleles, we see that there is no significant association with regard to age, sex, residence in potentially goitrogenic areas, patients’ symptoms (asymptomatic status, compression symptoms, or hyperthyroidism), or goiter grading (all $P > .05$) (Table 2).

By contrast, 4 statistically significant associations were revealed between HLA-C alleles and clinical variables (Table 2). There were 3 alleles, HLA-Cw1, HLA-Cw4, and HLA-Cw7, with different distributions. As shown in Table 2, the most important association was the low representation of the HLA-Cw4 allele in patients with goiters with an intrathoracic component ($P = .001; RR=0.19$) and in patients with goiters weighing more than 200 g ($P = .02; RR=0.17$). A relationship between HLA-C alleles and MG was also observed for 2 other variables. One
is between the presence of the HLA-Cw7 allele and a family history of thyroid pathological abnormalities (P = .03; RR = 3.91), and the other is between the HLA-Cw1 allele and the presence of goiter-associated thyroid carcinoma (P = .02; RR = 8.60).

On application of the Bonferroni correction, the association between the presence of an intrathoracic component and the low frequency of the HLA-Cw4 allele persisted (Bonferroni-corrected P = .02; RR = 0.19), indicating that this allele could protect against the development of goiter with an intrathoracic component.

Molecules of HLA antigens are membrane glycoproteins with the biological role of presenting peptides to T lymphocytes, and they are characterized by their extraordinary polymorphism. Both class I and class II HLA alleles continually provide an updated sample of potential antigens from cytosolic and extracellular compartments to T lymphocytes. A relationship has been found between autoimmune benign thyroid pathological abnormalities and certain HLA alleles. Susceptibility to developing these autoimmune diseases is primarily related to certain specific peptides presented by HLA molecules, as has been shown previously in type 1 diabetes mellitus. These types of associations have been described principally in Graves disease and Hashimoto thyroiditis, as well as in other diseases such as Meniere disease, schizophrenia, osteoarthritis, and others, among which are tumoral diseases such as leukemia. However, it has not been described as a protective factor against MG. For the first time to our knowledge, we describe a similar association between the HLA-Cw4 allele and the presence of goiters with an intrathoracic component. The HLA-Cw4 allele has been associated with susceptibility to diseases such as Meniere disease, schizophrenia, osteoarthritis, and MG, as they have been limited so far to thyroid pathological abnormalities of a clear autoimmune origin. In the available articles that we consulted, we only found the Russian study by Rasovskii et al in which 101 nodular goiters were reviewed and associations with the HLA-Aw19, HLA-A28, and HLA-B18 alleles were found. Paipastorides et al studied HLA alleles in hypothyroidism, especially in Graves disease, and observed that no HLA-A or HLA-B alleles were associated with cases of toxic MG in their series.

With regard to benign thyroid disease, there are still few studies on HLA-C alleles, and only Ozaki et al have described the relationship between 1 HLA-C allele and a thyroid pathological abnormality (HLA-Cw7 allele and differentiated thyroid carcinoma). Our study shows that there is a significant association between the lower incidence of the HLA-Cw4 allele and the appearance of MG. In fact, on analyzing the different clinical variables in the MG subgroup, we observed an association between the absence of the HLA-Cw4 allele and the presence of goiters with an intrathoracic component. The HLA-Cw4 allele has been associated with susceptibility to diseases such as Meniere disease, schizophrenia, osteoarthritis, and others, among which are tumoral diseases such as leukemia, however, it has not been described as a protective factor against MG. For the first time to our knowledge, we describe a similar association between the HLA-Cw4 allele and the presence of goiters with an intrathoracic component. The HLA-Cw4 allele has been associated with susceptibility to diseases such as Meniere disease, schizophrenia, osteoarthritis, and MG, as they have been limited so far to thyroid pathological abnormalities of a clear autoimmune origin. In the available articles that we consulted, we only found the Russian study by Rasovskii et al in which 101 nodular goiters were reviewed and associations with the HLA-Aw19, HLA-A28, and HLA-B18 alleles were found. Paipastorides et al studied HLA alleles in hypothyroidism, especially in Graves disease, and observed that no HLA-A or HLA-B alleles were associated with cases of toxic MG in their series.

When analyzing the risk of developing a goiter among individuals bearing the HLA-Cw4 allele, we observed that it drops to half of the risk that the rest of the population has (RR = 0.49). In other words, the degree of protection against goiters in these patients is 2 times higher than in the control population. In patients with MG and the HLA-Cw4 allele, the degree of protection against the development of an intrathoracic component is more than 5 times higher (RR = 0.19) than in patients with MG without the HLA-Cw4 allele. Additionally, the degree of protection by the HLA-Cw4 allele against the development of a large goiter weighing more than 200 g was higher still (RR = 0.17), ie, the incidence of these large goiters was 6 times higher in cases in which the HLA-Cw4 allele was absent. Lastly, despite the high degree of protection, the relationship between the HLA-Cw4 allele and goiters weighing more than 200 g is not statistically significant on application of the Bonferroni correction, possibly owing to the small size of this subgroup of patients in our series.

As for the general distribution of the HLA-C alleles, we also observed a tendency at the limit of statistical significance between goiters and the HLA-Cw16 allele. This allele can only be detected by molecular biological tech-
niques, and some of its subtypes have been described in recent years. This justifies the few articles in which it has been described, but among the few associations described for this allele are those that link it with Behcet disease, acquired immunodeficiency syndrome, and melanoma. Therefore, further studies of goiter could be of wider interest.

On the other hand, our study shows that the HLA-Cw7 allele is associated with MGs that have thyroid carcinoma, although statistical significance is lost when the Bonferroni correction is applied. This allele seems to constitute a risk factor for the development of goiter at high risk of becoming malignant, and therefore, it could be useful for defining a poor prognosis of this disease. The HLA-Cw7 allele has also been considered in some studies as a regulator of the immune response in neoplastic processes. Although the HLA-Cw7 allele might be regarded as a risk factor for the development of thyroid carcinoma when associated with MG, considering the small number of cases of cancer in our series, this association must be regarded with some reservation.

Lastly, we found that the HLA-Cw7 allele could be associated with a family history of thyroid pathological abnormalities, although owing to the small number of case studies, this observation should be considered with some caution. Nonetheless, this finding is supported by previous studies showing that the HLA-Cw7 allele can condition a poor immune response in patients with neoplasms and that this HLA-C allele is associated with differentiated thyroid carcinoma. Therefore, in people with a family history of thyroid pathological abnormalities, there is greater risk of developing thyroid carcinoma. Our finding of an association between the HLA-Cw7 allele and such family history indicates that patients with both characteristics could be at major risk of malignization. Our previous results in patients with differentiated thyroid carcinoma revealed a relationship between the HLA-Cw7 allele and this carcinoma.

To summarize, we describe for the first time, to our knowledge, an association between HLA-C alleles and goiter. The HLA-Cw4 could have a protective effect against this disease, and it especially prevents the development of an intrathoracic component. By contrast, the HLA-Cw1 allele could be related to a major risk of developing cancer, and the HLA-Cw7 allele could be related to a family history of goiters. These observations lead to a new way of detecting those patients in which surgical intervention could be justified, but further and wider series must be carried out to substantiate these results.

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