Research Article

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Monozygotic twins: distribution of epitopes and multiple autoimmune.

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ABSTRACT

A group of monozygotic male twin patients with autoimmune thyroiditis and vitiligo linked to the HLA-DRB1*04-DQB1*03:02 and HLA-DRB1*03-DQB1*0201 haplotypes are the subject of our clinical, serologic, and immunogenetic investigations. Anti-melanocyte and anti-thyroid autoantibodies were observable in the patients. A critical analysis of the connection between MHC II molecules and the clinical signs of different autoimmune disorders manifested in a single patient, as the twin individuals described here have done.

Keywords : Autoimmunity, Autoimmune diseases, Vitiligo, Thyroiditis, Multiple autoimmunity, Epitope spreading

INTRODUCTION

Numerous variables contribute to the polymorphic pathophysiology of multiple autoimmunity, a clinical and pathological problem that is poorly understood. There have been publications highlighting the genetic predisposition.
connected to specific MHC alleles. For example, HLA-DQB1*04 has been connected with the expression of multiple bullous autoimmune disorders, including pemphigus and/or pemphigoid linked to lupus, in the same patient [1,2]. Another illustration is the common correlation between thyroid disorders and vitiligo, as well as with Addison's illness, atrophic gastritis, pernicious anemia, and other autoimmune pathologies 3–7. The aforementioned conditions typically show a mixed pattern of autoantibodies that are either non-organic or specific to certain organs.
We present clinical, serological, and immunogenetic investigations of two monozygotic twins in our work, who were mostly examined and treated for thyroid autoimmune illness.

MEDICAL SITUATIONS

The cases that were recorded involved twin males aged 23 who were raised in a rural neighborhood; there was no history of twins in the family. Furthermore, there was no history of autoimmune illnesses in the family. The study was authorized by the Bioethics Council of the State of Zacatecas, Mexico, and conducted in compliance with the Helsinki Declaration and the fundamentals of good clinical practice.
When the patients first presented with thyroid enlargement, an 18-year-old was referred for an endocrine evaluation. This clinical picture was classified as a diffuse euthyroid goitre, and for five years the gland enlargement did not cause any symptoms. Following that, one twin (case number 1) developed diffuse, painful thyroid gland discomfort, and the other twin (case number 2) developed it two months later.The symptoms of the twin (case number 2) were comparable. They showed high amounts of anti-thyroid hormone, decreased T3 and T4 hormones, and elevated TSH in both cases antigens.
They were treated with levothyroxine and a mild dosage of prednisone; but, over the course of the following four months, they noticed little areas of skin discoloration that started on the hands and spread to the face and trunk. Figure 1. They now sought the advice of a dermatologist, who identified vitiligo and prescribed laboratory tests to get an accurate autoantibody profile and HLA genotype.

Autoantibody profile specific to an organ

The following autoantibodies were examined using ELISA: anti-parietal cells and anti-smooth muscle in rat stomach (ASMA), anti-glomerular basement membrane in mouse kidney, and anti-cell islets in monkey pancreas. Anti-thyroid, anti-thyroglobulin, and anti-microsomal antibodies were also investigated.
(Euroimmun®), anti-basal membrane and anti-epithelial (desmosome) in the nostrils of cows, anti-sperm cells in rat testicle sections, and anti-interstitial Leyden cells. Human nevi that had been surgically removed for cosmetic purposes and approved for use as antigenic sources for indirect immunofluorescence were used to test anti-melanocytes. The manufacturer's recommendations for diluting serum samples were followed; for tests conducted on human, rat, cow, or murine tissues, the initial serum dilution was 1:20. The slides

were incubated with serum for 30 minutes, then rinsed with PBS and incubated with FITC-polyvalent goat antihuman IgG. PBS washings were examined using an Olympus B-Max 40 fluorescent microscope.

By using ELISA, the following autoantibodies were found: anti-thyroid anti-gliadin (Biosource® MBS700052), anti-transglutaminase (Biosource® MBS074348), anti-GBM, anti-Dsg 1 and 3, anti-BP180, and BP230, peroxidase (Abcam® ab178632), anti-thyroid microsome antibody (LABio® LS-F10286), anti-thyrosinase (Antibodies® A103622), and etc.

antigens (Lübeck, Germany: Euroimmun AG®). The serum used in the aforementioned experiments was diluted 1:100 and incubated for two hours. Following a fresh incubation with goat-anti-human IgG HRP-labelled antibody (horseradish peroxidase-labelled antibody), the plates were cleaned. Lastly, The color reaction was triggered by TMB/H2O2, and Thermo Scientific's Multiskan FC was used to measure the optical density (OD) at 450 nm.

Other antibodies against

Indirect immunofluorescence was used to identify anti-nuclear antibodies (ANA) on HEp-2 cells (Antibodies Incorporated®), AMA in rat kidney tissue, anti-DNA in Crithidia luciliae, and ANCA in human leukocytes (Euroimmun®). Anti-Ro/SSA, anti-La/SSB, anti-Sm, anti-RNP, anti-Scl70 (the previous 5 by Euroimmun®), anti-Jo1 (The Binding Site®), anti-beta 2 glycoprotein 1, antiphospholipids, and anti-CCP were among the other organ nonspecific autoantibodies identified by ELISA.

Nepthelometry was used to identify the rheumatoid factor. Adverse Every autoantibody test included controls who were in good health.

Examination of HLA

Low-resolution sequence specific priming (SSP) was used to evaluate MHC class II molecules following the PCR amplification of the HLA-DRB1 and HLA-DQB1 genes. The assay was carried out at Mexico City's Instituto Nacional de Ciencias Médicas y Nutrici´ on, Salvador Zubir¨an.

Information Gathering

The software GraphPad Prism version 9.2 (GraphPad, San Diego, CA, USA) was used to conduct statistical analyses. The significance of the presence or lack of autoantibodies was assessed using a Fisher's exact test; a value of $p < 0.05$ was regarded as statistically significant.

FINDINGS

The instances discussed here were first diagnosed as goitre but later reevaluated as thyroid conditions. A few years later, anti-thyroglobulin, anti-thyroid peroxidase, and anti-microsomal autoantibodies that were positive for thyroiditis developed clinical symptoms. which ultimately caused hypothyroidism. Following the first thyroiditis, vitiligo-like skin lesions with decreased pigmentation appeared. As of the now, comprehensive A variety of autoantibodies were found by autoantibody screening (Fig. 1 and Table 1).

The fact that twin number 1 showed a distinct increase in the titre of some autoantibodies was an intriguing discovery. Only one of the twins tested positive for thyrosinase, while both had anti-melanocyte antibodies. Vitiligo is characterized by these antibodies. Furthermore, without any clinical indication of another autoimmune disease, both patients had positive rheumatoid factor, anti-smooth muscle antibodies, anti-BP230, anti-epithelial antibodies, anti-beta pancreatic cells, anti-sperm cells, anti-parietal cells of stomach autoantibodies, and positive rheumatoid factor (Fig. 2 and Table 1). The haplotypes HLA-DRB1*04-DQB1*03:02 and HLA-DRB1*03-DQB1*0201 were revealed by HLA typing in both individuals.

DISCUSSION

The twins in question are monozygotic, and the current article describes their clinical symptoms of thyroiditis coupled with vitiligo. They also possessed many organ-specific autoantibodies associated with the haplotype HLA-DRB1*04-DQB1*03:02. This observation has been documented by other writers before [8]. Nevertheless, none of the other autoimmune diseases that our patients exhibited clinical manifestations of were pernicious anemia, autoimmune blistering disease, Sjogren's syndrome, rheumatoid arthritis, or lupus.

The twins' HLA-DRBI*04-DQB1*03:02 and DRB1*03-DQB1*02 haplotypes, which have been associated with vitiligo and thyroiditis, were different, as were their autoantibody patterns and titres.

The presence of many autoimmune diseases in a single patient is a complex clinicopathological process known as multiple autoimmunity [9,10]. This clinical entity is often linked to the existence of different autoantibodies. instead of autoreactive cells [4,5]. This intriguing immunological anomaly hardly ever happens. Here, we describe two instances of immunological responses in monozygotic twins.

against melanocytic cell antigens and thyroid dominant autoantigens.

In both instances, thyroid epitopes linked to thyroiditis set off the initial autoimmune reaction. Subsequently, the clinical picture changed to show vitiligo as a second autoimmune illness co-occurring with autoantibodies against melanocytes. Following that, other autoantibodies manifested themselves, most likely as an epiphenomenon, without causing any

clinical problems. In terms of the genetic relationships between HLA alleles, it has been documented that individuals with autoimmune thyroid illness who also have another autoimmune pathology, such as type I diabetes, vitiligo, alopecia areata, or other [11]. Multiple autoimmunity plagues the mestizo population, as evidenced by the allelic connection between DRB1*04 and thyroid illness and vitiligo found in a prior study conducted in Mexico [8]. The current report adds credence to these findings.

Although vitiligo is not an uncommon condition in twins, as it may affect up to 23% of monozygotic twins [12], our patients have two autoimmune diseases with multiple autoantibodies connected to the HLA-DRB1*04-HLA-DQB1*03:02 and HLA-DRB1*03-HLA-DQB1*0201 haplotypes. Considering the above described elements, we questioned why these haplotypes were connected to various antigens linked to distinct autoimmune disorders. We provide an explanation for this result using various theoretical angles.

First, it has been reported that the surface of MHC proteins can handle epitopes of distinct antigens under diverse stereochemical circumstances. very rare instances of bullous autoimmune disorders linked to lupus erythematosus. In this case, HLA-DQB1*0301 or DRB1*0402 may be able to address various unrelated protein epitopes simultaneously.

such as ribonucleoproteins, which are autoantigens of lupus or similar disorders, and desmogleins, or BP proteins, of bullous diseases. This is made possible by the fact that the β chain interacts with each epitope using a distinct residue [2].

Second, an alternative explanation is that the invariant chain (Ii) CLIP domain temporarily binds the MHC II protein's cleft, and during its passage through the endosome complex, it inhibits the non-specific loading of unrelated peptides into the cleft of developing MHC II protein. The remaining "Ii" chain is then cleaved by enzymes, and CLIP is eventually removed from the cleft surface. permitting a particular antigenic peptide to bind. Three structural domains of CLIP are able to momentarily bind the MHC II cleft: CLIP1, the first domain, is the canonical includes residues 83–101; the second section, known as CLIP2, is a noncanonical domain situated between residues 92–107; the third region, known as the noncanonical CLIP 3, is situated between residues 93–111. The bulk of human alleles correlate with CLIP1. Nevertheless, some MHC II proteins, such DQ2.5, can bind to CLIP2 or CLIP3, and these domains are connected to certain autoimmune illnesses like type 1 diabetes and/or celiac disease [13] for unclear reasons. Additionally dependent on MHC polymorphisms for the interaction between CLIP and the MHC II interface is a single β chain residue, like glycine, that changes (HLA-DPβ84gly) is important in the establishment of immunity and may influence how "Ii" associates with its corresponding antigenic peptide [14].

Why our patients reacted differently to several self-antigens at different times is another fascinating question. The thyroid and melanocytic immunodominant epitopes in the cases presented here initiated two kinds of organ-specific autoantibodies linked to vitiligo and thyroiditis; subsequently, months later, a second wave of autoantibodies against clinically insignificant epitopes developed. Empirical data indicates that the initial self-antigenic challenge can result in autoimmune tissue damage, which can then be followed by protein modification leading to intramolecular epitope spreading or, in the cases reported here, a form of intermolecular protein spreading. The current research tracing autoantibody families provides an intriguing timeline on epitope dominance and spreading in

CONCLUSION

In summary, the twin patients in our study had the haplotypes HLA-DRB1*04-DQB1*03:02 and HLA-DRB1*03-DQBI*02 demonstrated clinical evidence of multiple autoimmunity, offering an incredible chance to investigate the stereochemical processes by which MHC II molecules may bind to and process distinct epitopes.

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