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Autoantigens in Type 1 Diabetes:
Looking for New Suspects

ANTIGENS: JUST STRANGE MOLECULES?

A few years ago, the word antigen was not part of our everyday vocabulary. However, the pandemic caused by the SARS-CoV-2 virus in 2020 made it a commonly used term in our daily lives. Antigen tests became essential, although not everyone may have understood the meaning of the word "antigen."

The term antigen was first coined in 1899 by L. Deutsch as a contraction of "antisomato-gen" ("that induces the production of antibodies"). The term antibody appears for the first time in 1891 in the second of what are known as "Experimental Studies on Immunity" by Paul Ehrlich (1).

An antigen is any substance capable of being recognized by components of the immune system. Traditionally, we think of antigens as molecules foreign to us. This is so because the main function of the immune system is to distinguish between self and non-self, recognizing antigens from microorganisms, fungi, and parasites, and destroying these foreign organisms, thereby maintaining our integrity.

For immune cells, specifically T and B lymphocytes, to successfully carry out this distinction, it is necessary to generate lymphocytes that recognize foreign molecules. For this, during the evolution of vertebrates, mechanisms of somatic recombination and central tolerance arose: during the develop- »

ment of T and B lymphocytes (in the thymus and bone marrow, respectively), hypervariable antigen receptors are generated through random combinations of genomic segments. The problem arises when selecting from the myriad of receptors generated those that would recognize foreign antigens: how to choose which receptors are best at identifying microbial antigens when (thankfully!) neither the thymus nor the bone marrow houses all terrestrial microorganisms, fungi, and parasites? The solution is very practical: let's eliminate all cells whose receptors recognize self-antigens ("autoantigens"), and by default, all others will recognize antigens from outside my body, i.e., foreign ones. This is what is called **central tolerance,** a process in which lymphocytes undergo stages of positive and negative selection, eliminating those that recognize autoantigens with too much affinity.

VERY INTERESTING, BUT WHAT RELATIONSHIP DO ANTIGENS HAVE WITH DIABETES?

An **autoantigen** is a self-molecule, normally present in the body, that is recognized by the immune system. Ideally, this recognition of self should only occur in the thymus and bone marrow, during the development of lymphocytes, and those who recognize them should be eliminated. But, as with everything in life, nothing is perfect. For reasons that are still not entirely clear, the process of central tolerance allows some autoreactive lymphocytes (those that recognize autoantigens) to survive. Fortunately, we have additional tolerance mechanisms throughout the body, such as **regulatory T lymphocytes** or the induction of non-functional states in **autoreactive lymphocytes,** which help control these autoantigen recognizers.

The problem arises if central or peripheral tolerance fails; in that case, autoantigens are recognized as if they were foreign antigens, leading to the destruction of self-cells. This triggers so-called autoimmune diseases, in which components of the immune system specifically destroy our own cells. Depending on which autoantigens the autoreactive lymphocytes recognize, different autoimmune diseases will develop.

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease with metabolic consequences, in which autoreactive lymphocytes recognize autoantigens from beta cells as foreign, causing their death. This results in a decrease in insulin production and the development of hyperglycemia due to the destruction of beta cells (2). It is the most common form of diabetes in children and young people, and by the time a patient is diagnosed with T1DM, most beta cells have already been destroyed. Its prevalence and incidence increase annually (3). This latter fact supports the hypothesis that the onset of T1DM is determined not only by genetic factors but also by the presence of certain environmental factors. In fact, the concordance among monozygotic twins is approximately 50% (4).

The onset of T1DM could be determined not only by genetic factors but also by the presence of certain environmental factors.

How is the destruction of pancreatic beta cells produced? The current model indicates that before the clinical progression of the disease, several immunological events occur, including processing and presentation of the autoantigen(s) by antigen-presenting cells, activation of lymphocytes, migration of activated autoreactive lymphocytes to the islets of Langerhans ("insulitis"), and the destruction of beta cells. This process begins years before the clinical diagnosis but goes unnoticed until the number of beta cells falls below a critical threshold, at which point the disease is diagnosed (3). The key, then, lies in early identification of which individuals are experiencing this silent autoimmune attack and against which autoantigens this response is occurring.

The main **autoantigens** identified so far in T1DM are (pre)(pro)insulin, 65 kDa glutamic acid decarboxylase (GAD65), tyrosine phosphatase 2 (IA-2), and zinc transporter 8 (ZNT8), among others. Against these 4 main autoantigens, autoantibodies are produced during the autoimmune response (5); these autoantibodies are not pathogenic in T1DM, unlike what happens in other autoimmune diseases like lupus, and rather serve as an indicator of the activation of autoreactive T lymphocytes in T1DM. These autoantibodies can be detected and quantified in the laboratory. Thus, they are highly useful not only for the diagnosis of T1DM vs other types of diabetes but also for identifying individuals »

IN OUR LABORATORY, WE INVESTIGATE WHETHER ENDOGENOUS METABOLITES PRODUCED BY BETA CELLS IN PATIENTS WITH TYPE 1 DIABETES MELLITUS ARE RECOGNIZED BY MUCOSAL–ASSOCIATED INVARIANT T CELLS (MAIT CELLS)

THE ONSET OF TYPE 1 DIABETES MELLITUS MAY BE DETERMINED NOT ONLY BY GENETIC FACTORS, BUT ALSO BY THE PRESENCE OF CERTAIN ENVIRONMENTAL FACTORS

» at risk of developing the disease, serving as **biomarkers** of the disease.

Three states in T1DM have been defined: state 1, preclinical, characterized by the presence of autoantibodies and normoglycemia; state 2, characterized by the presence of autoantibodies and hyperglycemia; and state 3, characterized by the presence of clinical symptoms (known as symptomatic T1DM)3. Many individuals with a single antibody do not go on to develop clinical T1DM. However, **the presence of > 2 antibodies is usually indicative of progression to a clinical state.**

CAN WE APPLY TO CLINICAL PRACTICE WHAT WE ALREADY KNOW ABOUT AUTOANTIGENS IN T1DM?

As a matter of fact, yes. The development of a specific autoantigen therapy that selectively eliminates autoreactive T lymphocytes would be the safest approach, as it would only eliminate the lymphocytes causing the disease, not all T lymphocytes in general. Many clinical trials, past and present, are based in various ways on administering autoantigens to restore tolerance to them.

Many clinical trials for the treatment of T1DM are based on administering autoantigens to restore tolerance to them. This approach has shown promising results in preclinical and clinical trials (3, 6). However, so far, no antigen-specific therapy has ever reached clinical use. This may be due to different patient subgroups responding differently to the same therapy, which is why it is important to categorize based on the present autoantibodies and specific genetic markers. Additionally, optimization of autoantigen administration protocols regarding dose, frequency, and route of administration is necessary (7).

Currently, positive results are beginning to emerge from drugs that eliminate all T lymphocytes. However, ideally, we should aim to develop antigen-specific immunotherapies, either alone or in combination with other immunoregulatory agents, to make treatment more specific. For example, **a Phase II clinical trial** (NCT05742243) is currently starting to assess the efficacy of oral insulin administration alongside **abatacept,** a recombinant protein formed by the extracellular domain of human CTLA4 and a fragment of the Fc domain of human immunoglobulin G1. In this context, oral insulin would act as a specific antigen therapy, while abatacept would help by inhibiting the immune response.

MANY CLINICAL TRIALS FOR THE TREATMENT OF TYPE 1 DIABETES MELLITUS ARE BASED ON ADMINISTERING AUTOANTIGENS TO RESTORE TOLERANCE TO THEM

» NEW AUTOANTIGENS: MAYBE THEY ARE NOT ALL PROTEINS...

One of the dogmas of immunology is that the antigens recognized by T lymphocytes are peptides derived from foreign or self-proteins. However, there are significant exceptions. For example, **NKT lymphocytes** recognize lipids, and γδ T lymphocytes primarily recognize lipids and phosphoantigens. More recently, **mucosal-associated invariant T cells (MAIT cells)** have been identified, which recognize low molecular weight bacterial metabolites, such as those derived from bacterial metabolism of vitamin B (8).

They are a common cell type (about 5% of T lymphocytes in the blood and 35% in the liver), and their activation triggers the secretion of pro-inflammatory cytokines, cytotoxic effector function, migration, and proliferative expansion, inducing the death of target cells (9).

They can also recognize cells presenting endogenous metabolites, although the identity of these metabolites has not yet been elucidated. In fact, these cells are implicated in autoimmune diseases; in the case of T1DM alterations in the frequencies of MAIT cells were observed, along with a greater cytotoxic phenotype, making them capable of destroying beta cells (10). However, none of the studies conducted identified the autoantigens that these cells were recognizing.

In our laboratory, we investigate whether endogenous metabolites produced by beta cells in patients with T1DM are recognized by MAIT cells and whether this activation could be initiating and/or maintaining autoreactivity and damage to beta cells. To do this, we will identify autoantigens that activate MAIT cells and then analyze the characteristics of these autoreactive MAIT cells in samples from patients with T1DM, unaffected relatives,

and healthy donors. Finally, we will examine their frequencies and phenotypic characteristics. Thanks to the support of the *Juvenile Diabetes Research Foundation* (JDRF), which funds this project, we will shed light for the first time on the autoantigens recognized by MAIT cells in T1DM, as well as the role of endogenous metabolites in the onset and/or maintenance of autoreactivity in this disease, paving the way for the development of new antigen-specific immunotherapies.

In conclusion, advancements in immunological techniques and knowledge of the immune system open doors to the identification of new relevant antigens in T1DM. Thus, we broaden our range of potential therapeutic targets for the treatment of this and other autoimmune diseases. **D**

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