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Progression of Type 1 Diabetes Mellitus and Lymphocytic Infiltration

Type 1 diabetes is a complex disease in which genetic predisposition plays an important role, and its development begins before symptoms appear or a clinical diagnosis has been established. Its progression is divided into 2 preclinical phases (phase 1 and 2), and 1 clinical phase (phase 3). Although these phases are based on clinical parameters, the main characteristics are explained below, as well as their potential relationship with the presence of immune system cells (lymphocytic infiltration) in the pancreas. The infiltration of immune cells in the pancreatic islets causes chronic inflammation known as insulitis.

Phase 1 is identified by the presence of two or more specific antibodies against beta cell proteins in the pancreas, called autoantibodies. Their detection indicates that the immune system has started to recognize the beta cells as foreign, a process known as autoimmunity. In this phase, immune cells can be found in the pancreatic islets, although blood glucose levels remain normal and no symptoms are evident because the destruction of beta cells is not yet significant.

In **phase 2**, the number of immune cells increases, causing chronic inflammation in the islets, known as insulitis. The autoimmune attack gradually progresses, leading to a progressively greater loss of beta cells. In this phase, islets that have completely lost their beta cells begin to be observed. This reduction in insulin-producing cells results in inadequate regulation of glucose metabolism, leading to abnormal blood glucose levels. This is known as dysglycemia. Despite these changes, symptoms are still not evident. The duration of phases 1 and 2 varies considerably, ranging from a few months up to several years, and tends to be shorter in children, in whom type 1 diabetes mellitus tends to manifest more aggressively.

Phase 3 is marked by the appearance of clinical symptoms and the diagnosis of the disease. In this phase, the significant loss of beta cells can no longer be compensated for, resulting in elevated blood glucose levels (hyperglycemia) and the manifestation of characteristic **symptoms** such as excessive thirst, frequent urination, fatigue, and weight loss. In the pancreas, the infiltration of immune cells intensifies, affecting an increasing number of islets where total beta cell loss can be observed. However, at the time of diagnosis, many individuals still retain a considerable amount of beta cells, although this amount varies significantly from one person to another.

GENETIC PREDISPOSITION

Type 1 diabetes mellitus is associated with a genetic predisposition, where genes related to the human leukocyte antigen (HLA) contribute approximately 30% up to 50% of the genetic risk of developing the disease. In particular, the H**LA-DR3-DQ2 and HLA-DR4-DQ8** alleles are associated with an increased risk, and this risk is even higher in individuals who carry both alleles (DR3/4 heterozygosity).

The remaining genetic risk is attributed to approximately 50 additional genes unrelated to HLA, including genes that encode for insulin and others involved in the regulation of the immune system and beta cell function. Additionally, genetic variation can influence the response to environmental factors, thus determining the initial susceptibility and progression of the disease.

Although genetic predisposition is necessary for the development of type 1 diabetes mellitus, genetic predisposition alone is not enough to trigger the disease. Environmental factors also play a crucial role in its pathogenesis. Aspects such as the maternal and intrauterine environment, the type of neonatal birth, viral infections, the microbiome, antibiotic use, certain foods, and other environmental factors could act as triggers, activating the autoimmune response in individuals with genetic predisposition.

AUTOIMMUNITY AND LYMPHOCYTIC INFILTRATION

In phase 1 of type 1 diabetes mellitus, the immune system begins to recognize certain proteins of beta cells as foreign ***(Figure 1)***. This phenomenon is known as **autoimmunity**, and its exact cause is still not fully understood. During this phase, the immune response targets **autoantigens:** intracellular proteins that are presented on the surface of beta cells via class I HLA molecules (HLA-I). Although in this phase, the infiltration of the islets is not very evident, we can find various cell types:

B lymphocytes produce **autoantibodies** directed against the beta cells, which bind to the autoantigens and form immune complexes that contribute to inflammation and cellular destruction. The 5 autoantibodies identified so far and used as biomarkers for the detection of type 1 diabetes mellitus are: (a) pancreatic islet cell antibodies (ICA), (b) insulin antibodies (IAA), (c) antibodies against the 65 kDa isoform of glutamic acid decarboxylase (GADA), (d) anti-tyrosine phosphatase antibodies (IA-2A), and (e) antibodies against the ZnT8 transporter (ZnT8A). B lymphocytes are also antigen-presenting »

AT THE TIME OF DIAGNOSIS OF TYPE 1 DIABETES, MANY PEOPLE STILL HAVE A CONSIDERABLE AMOUNT OF BETA CELLS IN THE ISLETS

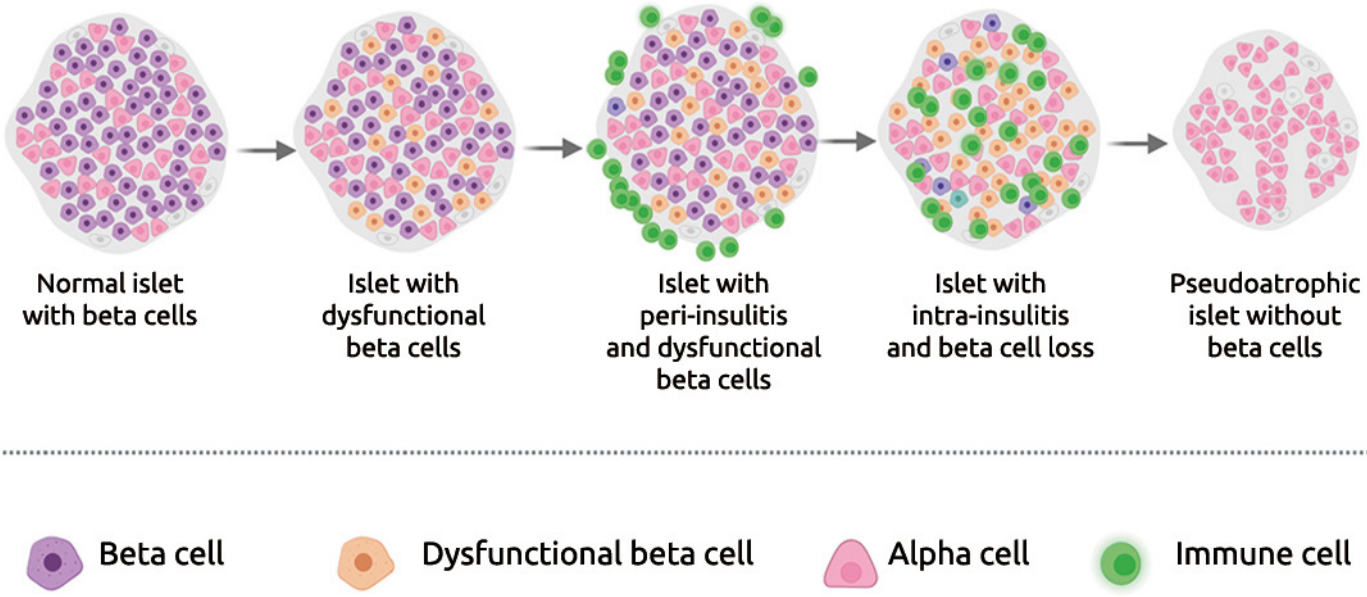

FIGURA 1: Infiltración linfocitaria durante la diabetes tipo 1. Dentro de un islote inicialmente sano, algunas células beta desarrollan alteraciones que dan lugar a una disfunción y a incremento de la presentación de antígenos. Las células inmunitarias son atraídas al islote por factores inflamatorios dando lugar a una infiltración linfocitaria que inicialmente se puede observar alrededor del islote (peri-insulitis) y posteriormente dentro del islote (intra-insulitis). Tras la destrucción de las células beta, las células inmunitarias se retiran, dejando tras de sí un islote pseudoatrófico de forma irregular y sin producción de insulina. En el páncreas de un mismo individuo pueden observarse simultáneamente islotes con distintos niveles de infiltración y destrucción. Diagrama adaptado de Atkinson MA and Mirmira RG. The pathogenic "symphony" in type 1 diabetes: A disorder of the immune system, β cells, and exocrine pancreas. Cell Metab. 2023 Sep 5;35(9):1500-1518. Created with BioRender.com.

» cells that can engulf small fragments of proteins, process them intracellularly, and present them bound to class II HLA molecules (HLA-II) on the surface of the B lymphocyte. These antigens can be recognized by T lymphocytes, initiating a cellular immune response.

Dendritic cells and **macrophages** also infiltrate the pancreatic islets, where they act as **antigen-presenting cells (APCs)**. Unlike B lymphocytes, whose main role is to produce antibodies, APCs are specialized cells that capture, process, and present protein fragments to other immune cells. In type 1 diabetes mellitus, APCs present beta cell antigens to **T lymphocytes** in nearby lymph nodes, activating them. Cytotoxic T lymphocytes (CD8+) migrate from the lymph nodes to the pancreas, where they recognize the autoantigens and destroy the beta cells by direct contact, releasing toxic substances or specific proteins such as perforin and granzyme. Helper T lymphocytes (CD4+) coordinate the immune response by releasing inflammatory cytokines, proteins responsible for regulating the immune response and recruiting and activating other immune cells. Cytokines such as interferon-gamma, interleukin-1, and tumor necrosis factor-alpha not only damage the beta cells but also stimulate the production of more cytokines, creating a continuous cycle of inflammation and cellular destruction. When they mistakenly recognize the body's own beta cells, both CD8+ and CD4+ T lymphocytes are known as **autoreactive** T lymphocytes.

As type 1 diabetes mellitus progresses, the proportion of infiltrated islets and the number of T lymphocytes increases, spreading throughout the pancreas (Figure 1). Cytotoxic T lymphocytes (CD8+) are the most abundant white blood cells in the infiltrated islets. The pathological diagnosis of insulitis is based on the detection of, at least, 3 pancreatic islets infiltrated by 15 or more CD45+ cells per islet. The CD45 marker is present in all types of immune cells, including T lymphocytes, B lymphocytes, monocytes, and granulocytes. If the infiltration occurs around the islets, it is called peri-insulitis, while if it occurs inside the islets, it is called intra-insulitis.

Histopathological studies (of tissues under the microscope) in pancreases from human donors have revealed that individuals with multiple autoantibodies before diagnosis (phase 2) and those recently diagnosed (phase 3) show significant heterogeneity in islet infiltration. In the pancreas of an individual, "healthy" islets without lymphocytic infiltration can coexist with islets with intense infiltration, as well as with pseudoatrophic islets in which all beta cells have been destroyed and are generally no longer infiltrated ***(Figure 1)***. The only cells that persist in these pseudoatrophic islets are the other types of endocrine cells of the islet: alpha, gamma, epsilon, and pancreatic polypeptide (PP) cells.

COMMUNICATION BETWEEN BETA CELLS AND THE IMMUNE SYSTEM

The process of beta cell destruction is »

» not solely due to the immune system. In fact, beta cells are believed to play an active role. Various alterations in these cells precede the onset of the disease and may contribute to autoimmunity. A crucial factor in this process is the increased expression of antigens via HLA-I molecules, a phenomenon known as **HLA-I hyperexpression**. This increase in expression enhances the interactions between HLA-I and T lymphocytes, facilitating antigen presentation and promoting the activation of autoreactive CD8+ T lymphocytes.

HLA-I hyperexpression in pancreatic islets has been identified as a distinguishing feature of type 1 diabetes mellitus. However, the factors that trigger this hyperexpression are still unknown. One possible explanation is that under stress conditions, beta cells release inflammatory cytokines that induce HLA-I hyperexpression, not only in the affected cell but also in adjacent cells within the islet. These cytokines could act as signals attracting immune cells that damage the islets and promote further HLA-I expression in cells that would otherwise not be as visible to the immune system.

Additionally, it is possible that damaged or stressed beta cells process and present cellular antigens abnormally. This alteration in processing could also generate new antigens, known as neoantigens, which the immune system recognizes as foreign proteins. The presentation of these neoantigens via HLA-I could lead autoreactive CD8+ T lymphocytes to destroy the beta cells.

WHAT THERAPIES EXIST TO PROTECT BETA CELLS IN TYPE 1 DIABETES MELLITUS?

In recent decades, various strategies have been developed with the goal of protecting beta cells from autoim- »

IN THE PAST DECADES, VARIOUS STRATEGIES HAVE BEEN DEVELOPED TO PROTECT BETA CELLS FROM AUTOIMMUNE ATTACK AND HALTING OR PREVENTING TYPE 1 DIABETES IN AT-RISK INDIVIDUALS

»mune attack and halting or preventing type 1 diabetes mellitus in individuals at risk. Among these immunotherapies is the antibody **Teplizumab**, which binds to the CD3 protein on the surface of T lymphocytes, modulating their activity without completely suppressing the immune system. Approved in the United States back in November 2022, it is the first drug approved to delay the onset of type 1 diabetes in adults and children over 8 years old in phase 2 of the disease. Clinical trials have shown that a 14-day regimen of teplizumab can delay the progression of the disease to phase 3 by approximately 2 years and help preserve the functionality of remaining beta cells. Further studies have also shown that two 12-day regimens can preserve beta cell function in children and adolescents with newly diagnosed type 1 diabetes mellitus.

Similarly, antigen-based immune therapies involve administering specific beta cell proteins to teach the immune system not to recognize them as foreign. An example is the *"Primary Oral Insulin Trial"* **(POInT)** study, where oral insulin is used to induce immune tolerance to insulin and prevent beta cell destruction. Unlike injectable insulin, powdered insulin is administered daily with a meal and does not affect blood glucose levels.

Current research is also focusing on protecting beta cells through early detection of the disease. In Germany, studies like **Fr1da** aim to identify children at high risk of developing type 1 diabetes mellitus by detecting autoantibodies, allowing for optimal treatment from the start, thus preventing severe metabolic imbalances. On the other hand, the study *"Antiviral Action against Type 1 Diabetes Autoimmunity"* **(AVAnT1A)** explores the possible prevention of autoantibody »

» development through COVID-19 vaccination in babies with high genetic risk. This study is based on observations during the pandemic that indicated that children at higher risk for type 1 diabetes mellitus were more likely to develop autoantibodies after contracting a SARS-CoV-2 infection. In the **AVAnT1A** study, saliva and stool samples are also monitored to identify the viruses with which children have been in contact, aiming to clarify the connections between early childhood viral infections and type 1 diabetes mellitus. Lastly, the study *"European Action for the Diagnosis of Early Non-clinical Type 1 Diabetes for Disease Interception"* **(EDENT1FI)** aims to detect the disease early in 200,000 children across Europe, as well as assess the psychosocial, medical, and economic impact of this detection in various healthcare systems and European populations. This will enable the development of innovative and more personalized therapeutic strategies for the prevention and effective management of type 1 diabetes mellitus. D

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CONCLUSIONS

Genetic predisposition, along with various environmental factors, plays a crucial role in the development of the disease. It is known that the autoimmune attack begins well before the clinical diagnosis of the disease, resulting in the loss of beta cells. These cells are attacked by the immune system due to the abnormal recognition of autoantigens. This erroneous recognition triggers an autoimmune response that leads to the infiltration of pancreatic islets by various immune cells. Among these cells, B lymphocytes produce autoantibodies, which amplify the autoimmune response and serve as disease markers detectable in blood. Cytotoxic T lymphocytes (CD8+) destroy the beta cells, while helper T lymphocytes (CD4+) coordinate the autoimmune attack and recruit more immune cells. Additionally, beta cells also play an active role in the progression of the disease by producing abnormal proteins and increasing the expression of HLA-I molecules, thus increasing the visibility of the beta cells to the immune system. The latest advancements in research focus on prevention during the early phases of the disease through the use of immunomodulators, with the goal of avoiding or halting the progression of the autoimmune attack and preserving beta cells.

This article has been reviewed by a Certified Associate of INPACT. INPACT is INNODIA People living with Type 1 Diabetes Community, and its certification program ensures that the perspectives of people with lived experience and their expert opinions are an integral part of the relevant aspects of research and drug development.

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