

# Immunopathogenesis and detection methods of type 2 diabetes

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**Abstract.** Diabetes is a disease that is increasingly prevalent on a global scale. Type 2 diabetes (T2D) accounts for 90% of all diabetes cases and is a chronic condition primarily influenced by factors such as obesity and genetics. This condition often leads to the development of various cardiovascular diseases. A diverse of different detection methods for the T2D have been developed and achieved good detection and analysis results. In addition, numerous studies have indicated a close connection between T2D and immune inflammation. This research primarily introduces the current status of diabetes, provides a detailed summary of the connection between T2D and the immune system, and presents novel immune biomarkers related to T2D. Some new immunological testing methods will be analyzed in this research. In the future, there is hope that these new biomarkers can be used to design more advantageous therapeutic drugs for diabetes patients and more convenient, sensitive, and low-cost detection methods.

**Keywords:** Type 2 diabetes; Detection method; Mechanism.

## 1. Introduction

According to the data released by the International Diabetes Federation (IDF), it is estimated that there are 537 million adults worldwide who are affected by diabetes, which accounts for 10% of the population in this age group. Additionally, it is predicted that over 6.7 million people will die from diabetes. By 2030, the figure for individuals with diabetes is expected to reach approximately 643 million, indicating a projected growth rate of 45% over the next 20 years. 90% of these patients have type 2 diabetes (T2D). Over the past thirty years, the incidence of T2D has notably increased among adolescents and children. The driving factors include socio-economic conditions, demographics, environmental and genetic factors, urbanization, an aging population, a decrease in physical activity levels, and an increase in overweight and obesity. The pathogenesis of T2D is currently not fully understood. This form of diabetes, which does not depend on insulin, has been shown to be associated with abnormalities in the secretion of insulin from pancreatic beta cells and the inadequate response of insulin-sensitive tissues to insulin. With the advancement of technology, a series of detection methods for T2D have emerged. These range from traditional blood glucose and glycated hemoglobin tests to immune tests for inflammatory factors and cytokines, as well as analyses of genes and miRNAs. There is continuous progress, and more diabetes markers are being discovered [1].

T2D is related to chronic inflammation, highlighting the pivotal role of the immune system in the pathogenesis of T2D. This involvement of the immune system not only provides a new perspective for understanding the complexity of this disease but also offers the possibility for developing new treatment methods. T2D is a chronic, progressive disease and has now become a prevalent chronic disease with an increasing incidence rate annually. T2D is often accompanied by many complications, severely affecting the patients' survival status. These complications include diabetic peripheral neuropathy, cardiovascular complications (myocardial infarction and cerebral infarction), diabetic eye disease (retinopathy), diabetic nephropathy, diabetic foot, etc. Furthermore, wounds of patients with T2D are also prone to recurrent infections. These chronic complications can severely impact the quality of life and life expectancy of diabetes patients.

Metformin is a medication that reduces insulin resistance and improves the body's utilization of insulin. It is widely considered as the primary treatment for T2D in most parts of the world. The primary action of metformin occurs in the liver, where it inhibits gluconeogenesis. This is achieved



through various mechanisms: (1) activation of liver AMPK by liver kinase B1, which reduces the cellular energy load; (2) blockade of adenylate cyclase to decrease the production of cAMP induced by glucagon; (3) inhibition of NADH coenzyme Q oxidoreductase in the electron transport chain, leading to decreased ATP levels and increased AMP/ATP ratio, thereby activating AMPK; (4) inhibition of mitochondrial glycerophosphate dehydrogenase (mG3PDH), which hinders the transfer of NADH from the cytoplasm to the mitochondria, consequently inhibiting lactate-driven gluconeogenesis. Moreover, metformin may also impede cell growth through its interaction with the LKB1 tumor suppressor protein in Peutz-Jeghers syndrome. Nonetheless, metformin can give rise to adverse effects such as decreased appetite, nausea, abdominal discomfort, and diarrhea by altering the gut microbiota composition.

Sulfonylureas represent a collection of medications that enhance the production of insulin in the pancreas. Several examples of such drugs encompass glipizide, glyburide, glimepiride, tolbutamide, and glibenclamide. The primary mechanism of action for these medications involves the stimulation of pancreatic  $\beta$ -cells, prompting them to release more insulin. This process involves the regulation of ATP-sensitive potassium channels (referred to as KATP potassium channels) on the membrane of pancreatic  $\beta$ -cells. Although sulfonylureas and glinides differ in their receptor binding sites, both of them lead to channel closure and cell depolarization, consequently resulting in an elevation in intracellular calcium concentration. As a result, insulin secretion increases. Therefore, this research will introduce the relationship between T2D and immune inflammation, and will discuss the detection methods for T2D.

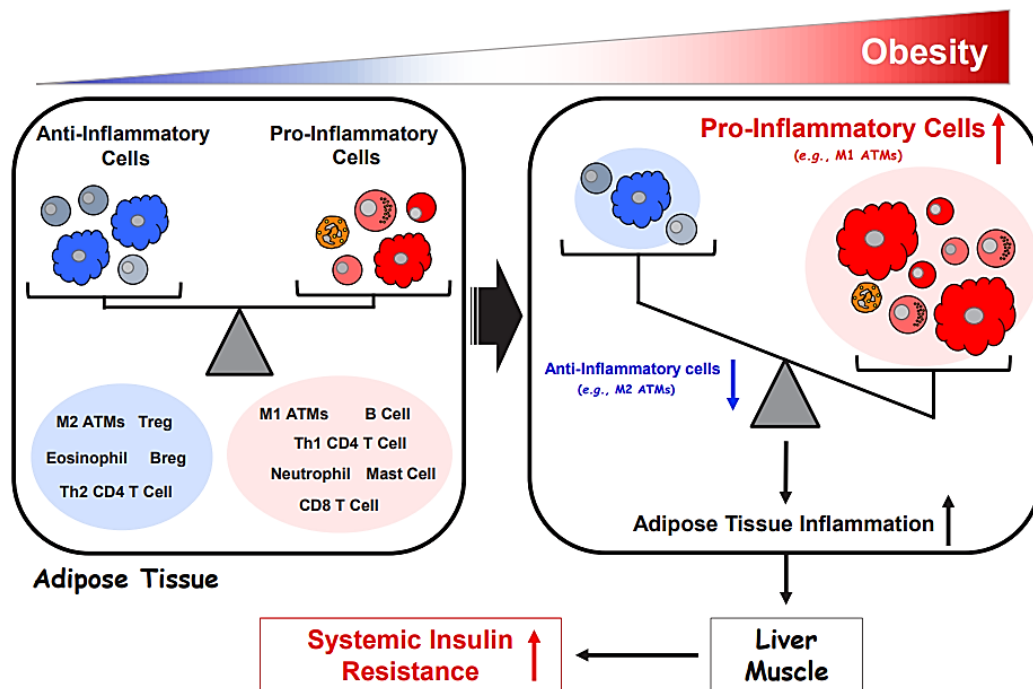
## **2. Relationship and immunological mechanisms between T2D and immune inflammation**

In fact, both categories of drugs interfere with inflammasomes or exhibit anti-inflammatory effects when treating diabetes. A low degree of islet inflammation is considered an important part of the etiology of T2D. An increase in immune cells can be observed within the islets of individuals with T2D. By comparing the islet cells from tissue sections of healthy individuals and T2D patients, an increase in macrophages marked by CD68+ cells within and around the islets can be noticed.

Currently, an increasing number of scientific frontiers are focusing on the relationship between diabetes and immune inflammation. Unlike typical acute inflammation, diabetic inflammation primarily manifests as cellular infiltration and systemic chronic inflammation. Changes in pro-inflammatory cytokines and chemokines in various organs, a decrease in the mass and function of islet  $\beta$ -cells, and an increase in innate immune cells and pro-inflammatory mediators are also observed. With technologies such as whole-genome linkage scans and candidate gene studies, it has been verified that T2D is greatly influenced by gene-environment interactions. Genetic variations in transcription factor 7-like 2 (TCF7L2) significantly elevate the risk of developing T2D.

Obesity is also one of the significant precursors. Children who develop diabetes have a Body Mass Index significantly higher than their non-diabetic counterparts, and this obesity could be influenced by environmental factors. Currently, there are no clinical means available on the market that can cure or prevent the onset and progression of diabetes. Presently, treatments are primarily symptomatic, focusing on obesity and insulin resistance. Obesity-associated pro-inflammatory factors stimulate a state of systemic low-grade inflammation in individuals diagnosed with T2D, thus leading to metabolic inflammation. It is worth noting that IL-1 contributes to the immune response of pancreatic  $\beta$ -cells by impeding their function, activating the transcription factor Nuclear Factor-kappa B (NF- $\kappa$ B), diminishing tissue sensitivity to insulin, and promoting  $\beta$ -cell dysfunction and apoptosis [2]. This phenomenon could potentially represent an immune origin of T2D. This phenomenon could potentially represent an immune origin of T2D. Using IA-2 and GAD-65 autoantibody assays, it has been discovered that obese adolescents with T2D might also experience insulin deficiency due to autoimmune attacks on the islets, similar to type 1 diabetes (T1D). The gradual decline in  $\beta$ -cell function and mass is not only characteristic of T1D but also of T2D. As a result, the line between T2D and T1D is becoming increasingly blurred.

Patients with T2DM caused by obesity have a large number of immunological features as follows [3]. With the emergence of chronic inflammation in large adipose tissues (AT), there is an increase in the expression of tumor TNF- $\alpha$ , interleukins-6, IL-1 $\beta$ , anti-insulin agents, and leptin, while anti-cytokines such as adiponectin, IL-4 and IL-1Ra are reduced in expression. In AT, macrophages are highly expressed with a majority transitioning from the M2 type to the M1 type, the former expressing anti-inflammatory cytokines, and the latter expressing pro-inflammatory cytokines (which hinder the ability of insulin receptors to transmit signals in insulin-sensitive tissues). A decrease in the frequency of DC dendritic cells in AT has also been observed. During high-fat diet (HFD) intake in animals, pro-inflammatory  $\gamma\delta$ T, Th1, and CD8+ T cells increase, accompanied by high expression of anti-inflammatory NK, Th2, and Treg cells, as shown in Figure 1. In severely obese patients, there is a selective increase in Th CD4+ cells [4].



**Figure 1.** Regulation of adipose tissue inflammation in obese patients [4].

In the bodies of T2D patients, both neutrophils and a majority of lymphocytes exhibit infiltration: In the islets of amyloid-positive T2D patients, the quantity of CD68+ macrophages increase. Pro-inflammatory M1 polarized macrophages (cells positive for both CD68 and iNOS) are more than tissue-repairing M2 polarized macrophages (cells positive for both CD163 and CD204). In the pancreas of T2D patients, CD8+ T cells infiltrate more in the exocrine tissues and periphery of the islets, but no significant change is observed inside the islets. There are also more resident leukocytes in the T2D islets, including CD11b+/CD11c+ myeloid cells. The quantities of CD45+ cells and CD20+ B cells are also slightly increased. These changes all indicate that there has been significant immune cell infiltration and activation in the islet tissues of T2D patients, associated with chronic inflammatory responses and a decline in islet  $\beta$ -cell function.

Apart from interleukins and immune cells, IgG antibody glycosylation is also closely related to T2D [5]. Through MALDI-TOF-MS spectrometry, meta-analysis, and the use of clinical features, IgG glycan, and their combinations for 10-fold cross-validation calculation of the AUC of ROC curves, a reduction in alpha2,3-linked sialylation in T2D patients is discovered reflecting a pro-inflammatory and biological aging state, and glycosylation features are significantly related to T2D [6]. IgG antibody glycosylation can also reflect the genetic body in diabetes and cardiovascular diseases.

Pancreatic islets, in response to variations in blood glucose, produce IL-1 $\beta$ , leading to a compromised insulin secretion [7]. This assertion is supported by a majority of clinical treatment outcomes [8]. The most potent evidence suggests that the use of IL-1 $\beta$  antibodies, such as canakinumab, can

significantly lower HbA1c levels by 0.9%. IL-1 blockade therapy has the potential to improve insulin secretion and glucose control [9], offering an effective new strategy and perspective for the short-term treatment of diabetes [8]. In the CANTOS trial, 90% of patients, screened based on CRP levels and cardiovascular diseases, exhibited a decrease in HbA1c levels within 6-9 months of using IL-1 $\beta$  antagonists [10], alongside a reduction in the mortality rate associated with heart failure induced by diabetes [11]. However, this approach has its limitations; it does not significantly reduce blood glucose levels and does not maintain efficacy over long-term usage [8]. Moreover, IL-6 can change insulin resistance and enhance GLP-1 mediated insulin secretion. During obesity, the inflammation-induced insulin resistance and  $\beta$ -cell functional impairment are exacerbated [12].

### 3. Classic detection methods for T2D

The detection methods for T2D mainly include the following:

**Fasting blood glucose test (FPG):** FPG is a common method, requiring patients to fast for at least 8 hours. The venous blood is then drawn to test the blood glucose level. If the blood glucose level exceeds the normal range (fasting blood glucose  $\geq 7.0$  mmol/L), the patient may be diagnosed with T2D.

**Oral glucose tolerance test (OGTT):** OGTT requires patients to drink a certain amount of glucose solution while fasting, followed by a series of blood glucose tests. If the test results show that the blood glucose level exceeds the normal range (blood glucose  $\geq 11.1$  mmol/L) two hours later, the patient may be diagnosed with T2D.

**Random blood glucose test:** This test can be conducted at any time. If the blood glucose level is above the normal range and accompanied by symptoms of diabetes (random blood glucose  $\geq 11.1$  mmol/L), the patient may be diagnosed with T2D.

**Hemoglobin HbA1c assessment:** The HbA1c assessment evaluates the mean level of glucose in the bloodstream for the preceding two to three months. Individuals with HbA1c levels above the normal range ( $\geq 6.5\%$ ) may be considered to have T2D. However, these testing methods can only provide a preliminary judgment on the possibility of T2D, and the final diagnosis needs to be confirmed by combining medical history, symptoms, and other related examination results. If T2D is suspected, it is advised to seek medical attention for further examination and diagnosis promptly.

There are advantages and disadvantages to each of the existing diagnostic methods for T2DM. Comparing FPG and OGTT to the HbA1c test, the latter is more convenient in a clinical workflow as it does not necessitate fasting. Samples can be collected at any time, and it is a superior predictor of long-term complications. However, there may be situations where the clinical sensitivity of HbA1c is relatively low. Factors such as age, race, ethnicity, and any clinical condition that can alter the lifespan of red blood cells or hemoglobin levels may impact HbA1c levels, regardless of glucose concentration. Furthermore, the availability and cost of the HbA1c test are restricted in certain regions. Based on the National Health and Nutrition Examination Survey, the number of T2D cases diagnosed via the HbA1c method with a set diagnostic threshold is relatively limited in the United States. Additionally, the correlation between HbA1c and fasting blood glucose, insulin resistance, and insulin secretion is lower as well. Currently, the gold standard for diagnosing T2D is still the fasting plasma glucose test. This is primarily due to its widespread availability, affordability, and compatibility with automated clinical chemistry analyzers. Despite requiring at least 8 hours of fasting, being constrained by time and effort, and only reflecting blood glucose levels at one instance, it remains one of the most widely accepted testing methods.

### 4. Emerging immunodetection technologies

The identification and evaluation of novel circulating biomarkers, such as miRNA, present valuable methods for improving individual stratification, prognosis, and treatment selection for T2D [13]. Recent research has elucidated that MicroRNA (regulatory RNA with a length of approximately 22 nucleotides) is closely associated with T2D. The dysregulation of miRNA in T2DM has been

extensively validated, with an increase noted in miR-9, miR-150, miR-146a, miR-27a, and others. In contrast, certain miRNAs, such as miR-126, miR-15a, miR-145, miR-375, and miR-223, have exhibited reductions. It is worth noting that the expression level of miR-146a experiences a significant decrease in individuals with T2DM [14], highlighting its crucial role in regulating inflammation and insulin resistance in patients. Furthermore, there is a correlation between miR-126 and fasting blood glucose levels in humans, suggesting that plasma miR-126 holds promise as a non-invasive biomarker for the prediction and diagnosis of T2DM.

The most effective method to analyze circulating micro RNA is through the combination of qPCR and RT-PCR technologies, measuring a small number of identified candidate biomarkers in a large number of cases. Nevertheless, the lack of reproducibility in measuring circulating miRNAs remains problematic as it stems from various issues encompassing sample collection, preparation, as well as RNA separation and quantification. As a consequence, this presents a formidable obstacle when employing MicroRNA as a diagnostic tool for diabetes detection.

Future prospects include the utilization of coulter counters to detect intracellular microRNA (miRNA) to determine the presence of T2D. This involves hybridizing target microRNA with probes and enriching the probe-microRNA hybrid double strands by binding to viral protein p19. Subsequently, nanopores are used for quantitative analysis of the abundance of this double strand. This method can measure the resistance change produced when the current passes through the electrolyte solution and calculate the quantity and size characteristics of the small particles suspended in the solution. Specifically, when suspended particles pass through a narrow electrolyte channel, it causes a momentary change in resistance; thus, different sized molecules produce different current drops. This resistance change can be measured to obtain information about the number and size of the particles. Hence, nanopores can be employed as a novel detection method for T2D, showing significant speed and sensitivity advantages compared to other widely used RT-RCT diagnostic technologies for measuring miRNA.

The latest technology involves the use of carbon-based nanobiological sensors to detect circulating MicroRNA found outside cells. The remarkable durability of circulating miRNA, which maintains stability in the circulatory system and exhibits resistance to enzymatic degradation by RNases, extreme pH variations, high thermal conditions, long-term preservation, and multiple freeze-thaw cycles, in addition to its relatively straightforward detection approach, position it as an optimal instrument for prompt, non-invasive, and real-time diagnostics.

Carbon-based nanobiological sensors are well-suited because they can demonstrate exceptional sensitivity and identify distinct signals even in situations with minimal levels of concentration. Carbon nanodots (CND) display nanosized dimensions, either in a flat or spherical form, remarkable water solubility, wide absorption in the ultraviolet-visible spectrum, and robust fluorescence. These nanodots hold promise for the synthesis of a variety of carbonaceous nanostructures, including graphene, diamond nanoparticles, and carbon nanotubes. These materials have found extensive applications in sensing applications [15]. In recent years, CNDs being environmentally friendly, biocompatible, and cost-effective materials, have gained increasing popularity. They can be produced using plants as renewable sources in green CND, as they are easy to synthesize, time-saving, non-polluting, and inexpensive. CNDs exhibit excellent biocompatibility, bioselectivity, minimal toxicity, and enhanced quantum size. They play a significant role in analyzing small biomolecules related to diseases and can be combined with fluorescence sensors, electrochemical sensors, and photoelectric sensors to detect specific circulating markers, offering the advantages of simplicity, cost-effectiveness, and rapid detection.

CND sensors can be combined with fluorescence for miRNA recognition. Single-stranded DNA with a FAM label can be affixed to CNDs, utilizing the fluorescence resonance energy transfer between CND and FAM, leading to CND fluorescence quenching. In addition, the combined utilization of gold nanoparticles (AuNP), CNDs, and self-assembled tetrahedral DNA nanostructures at the nanoscale can enable the prediction of miRNA and telomerase functions via dual fluorescence

pathways. To facilitate the transportation of these structures within live cells, the iRGD peptide sequence is subsequently introduced. Additionally, employing the nanoparticle tracking analysis technique allows for the identification of non-communicable diseases. This technique serves as a potent means to amplify the detection signal of analytes, even when they are present in limited concentrations. Compared to Northern blotting, qRT-PCR, and gene microarray technologies, CNDs exhibit outstanding multifunctionality, label-free monitoring, low cost, and high sensitivity. Although circulating miRNAs have been extensively studied as biological markers for T2D, how they mediate intercellular communication remains an unanswered question. Future research objectives should focus on tracking post-transcriptional miRNAs.

## 5. Conclusions and prospects

This study systematically analyzed the research status of diabetes, discussed the relationship between T2D and the immune system, and proposed new immune biomarkers related to T2D. In the treatment of T2D, IL-37 emerges as a highly auspicious medication that operates as both an anti-inflammatory substance and a diagnostic indicator for diabetes. As evaluated through the steady-state model assessment of insulin resistance, individuals with elevated IL-37 levels exhibited enhanced insulin sensitivity. Furthermore, among those with diminished levels, beta cells manifested escalated compensatory insulin secretion to combat insulin resistance. The level of IL-37 expression also mirrors the obesity condition of a person.

The outlook for future diabetes treatment primarily revolves around three aspects: Firstly, enhancing insulin sensitivity in various cells of the body; secondly, improving the function of pancreatic beta cells to normalize insulin secretion; and thirdly, researching and developing new antidiabetic drugs targeting novel therapeutic sites. As long as future medications make significant advancements in these three areas, minimize adverse reactions, and continually improve drug safety, individuals with diabetes will experience a better quality of life. Ultimately, this chronic disease, diabetes, will be conquered by humanity.

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