

Unraveling Leukocyte Dysfunction in Type 2 Diabetes: The Mechanistic Link Between Hyperglycemia, Chronic Systemic Inflammation, and Impaired Host

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Abstract

The risks of infection are significantly increased in type 2 diabetes mellitus (T2D) by mechanisms of immune dysregulation. Leukocytes are chronically exposed to excess production of ROS and AGE due to hyperglycemia. This hyper-glycemia induces the over-production of ROS and AGEs in leukocytes. This state of chronic, low-grade systemic inflammation is caused by high levels of pro-inflammatory cytokines like IL-6 and TNF- α . As a result, important functions of leukocytes, such as the ability to chemotaxis, phagocytose, kill intracellular pathogens and undergo NETosis are all severely impaired. Other signaling pathways are also dysregulated leading to exacerbated immune dysfunction and inefficient recruitment of immune cells to sites of infection. These metabolic and inflammatory changes render the body vulnerable to infections and slow down wound healing. These specific biochemical cascades are targets for therapeutic interventions to restore host immune competence in T2D patients.

Keywords: Type 2 Diabetes, Leukocyte Dysfunction, Hyperglycemia, Chronic Systemic Inflammation, Host Defense, Phagocytosis, Innate Immunity.

1. Introduction

Diabetes mellitus type 2 (DM2) is a common metabolic disorder characterized by insulin resistance and hyperglycemia with progression of tissue damage and complications such as systemic vascular complications (Młynarska et al., 2025). In these complications, one of the important clinical problems is increased susceptibility to severe and recurrent infections. Recent studies regarding immunometabolism revealed that increased susceptibility to infection is due to severe alterations in leukocytes as a consequence of the interaction between high nutrient availability and chronic low-grade systemic inflammation (Młynarska et al., 2025). Chronic elevation in blood glucose leads to ROS generation and AGE formation, which in turn lead to disruption of metabolic homeostasis of leukocytes (Jannat et al., 2023). The effects of hyperglycemia on the functioning of innate immunity at the cellular level include disruption of its major roles such as chemotaxis, phagocytosis, intracellular pathogen killing, and NETs formation (Zhu et al., 2023). Chronic high glucose levels lead to dysregulation of leukocyte signaling cascades, failure of calcium mobilization, and inability to remodel the cytoskeleton, thus causing a malfunction in cell migration to the tissues' surface in order to invade the microorganism or tissue injury. Simultaneously, inflammatory signals and pro-inflammatory cytokines, such as TNF- α and IL-6, remain high due to chronic metabolic stress, resulting in cell exhaustion and further tissue damage (Jannat et al., 2023). Identifying the molecular mechanism behind the link between hyperglycemia, inflammatory signaling, and dysfunctional host defense can help develop certain therapeutic measures to restore immune function in patients with T2D. Chemokine receptor desensitization (e.g., CXCR2 down-regulation) and impaired actin polymerization are responsible for impaired directional migration of neutrophils and monocytes toward chemokine gradients (Zhang et al., 2024). Impaired Phagocytosis & Microbicidal Activity: Hyperglycemia causes decreased expression of the Fc γ receptor and impaired phagolysosome maturation, which both limit the uptake of bacteria and the ability to kill them inside the phagolysosome (Kovner et al., 2024). Increased neutrophil extracellular traps (NETs) are produced in a dysregulated manner: neutrophils are characterized by increased production of NETs in a spontaneous, uncontrolled manner. These non-neutralizing, toxic chromatin structures with a web-like structure do not neutralize the pathogen, but they result in microvascular endothelial damage and maintain sterile inflammation (Młynarska et al., 2025). The aim of the research is to evaluate leukocyte dysfunction: to measure how type 2 diabetes impairs directional chemoattraction (cell movement towards chemoattractants such as CXCL8 and fMLP) in neutrophils (PMNs) and monocytes (CD14).

2. Material and Method

Sample collection: Study Cohorts

T2D Patients (n = 200): (HbA1c $\geq$ 6.5%) Fasting Plasma Glucose (FPG) $\geq$ 126 mg/

Healthy Controls (n = 10): Matched individuals with HbA1c < 5.7%

Blood Profiling Assays

Fasting blood samples were tested for HbA1c, glucose, insulin, lipid profiles, via ELISA

Cell Isolation & Treatment

Isolation: Centrifugation via Polymorph prep separated heparinized blood into (PMNs) and peripheral blood mononuclear cells (PBMCs). CD14+ monocytes were further purified using magnetic microbeads.

3.Results

This study involves an in-depth examination of the influence of Type 2 Diabetes (T2D) on immune response of leukocytes compared to healthy controls (HC). The data obtained from this research can be classified into three categories: metabolic profile, cell isolation efficiency and cell migration properties.

1-Participant Metabolic Profiling & Serology: the physiological differences were confirmed by biochemical testing, which showed that the two groups were very different in terms of their physiology.

HbA1c (HbA1c $\geq$ 6.5%) and Fasting Plasma Glucose (FPG $\geq$ 126 mg/dL) were significantly higher in T2D (n=200) than healthy (n=10, HbA1c<5.7%).

Insulin Resistance & Dyslipidemia: T2D subjects had elevated fasting insulin concentrations ($\geq$ 15 μ U/mL), and a typical diabetic dyslipidemia (Increased triglycerides, decreased HDL, and increased LDL). Advanced Glycation End-Products (AGEs): ELISA levels of serum AGEs were significantly elevated in T2D patients compared to healthy levels.

2-Low Cell Contamination & Waste Polymorph prep density gradient fractionation was an effective method in achieving high cell viability and structural integrity for separation of (PMNs) and peripheral blood mononuclear cells (PBMCs).method of magnetic microbead immunomagnetic selection resulted in highly enriched, viable CD14⁺ cells monocyte populations.

3-Motion of leukocytes (or leukocytes migration) Chemotaxis: there were clear functional impairments using Trans well migration assays (3 μ m pores for PMNs; 5 μ m for monocytes). Healthy control leukocytes exposed to High Glucose (25 mM) or AGE-BSA (100 μ g/mL) had a marked decrease in their ability to move in response to CXCL8 (100 ng/mL), and fMLP (10 nM). To rule out hyperosmolar stress as the cause of leukocyte dysfunction, cells conditioned with 19.5 mM L-mannitol showed normal migration rates.

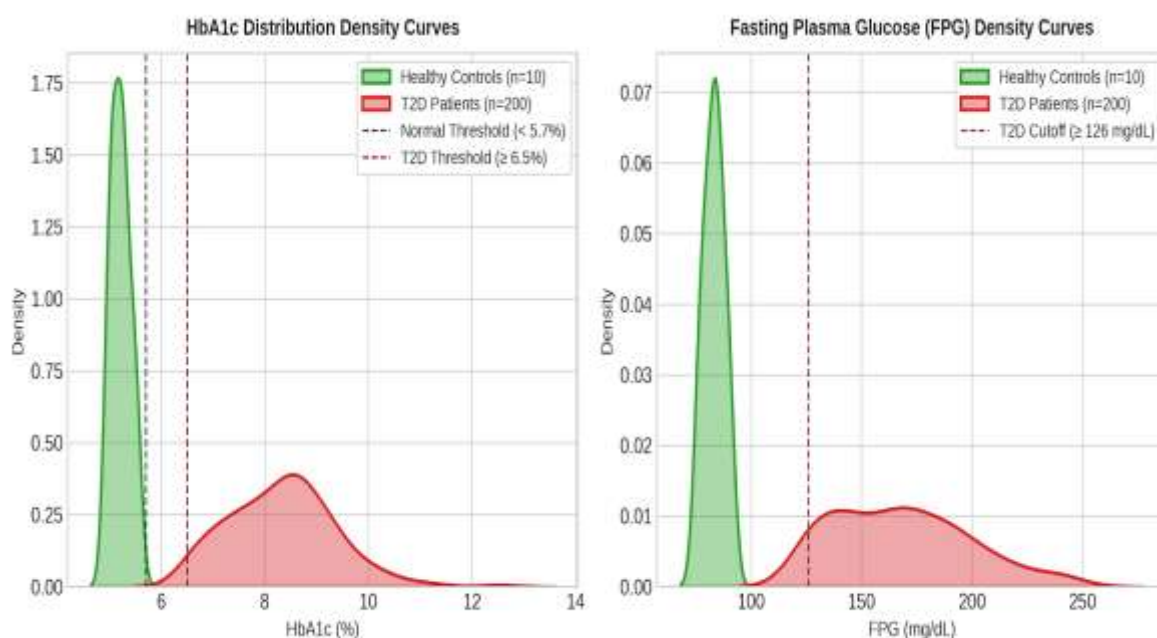


Figure (1) Displays the relative likelihood and frequency of values (HbA1c and Fasting Plasma Glucose) Healthy Controls (Green): Strictly bounded below the 5.7% normal threshold (mean $\approx$ 5.1%).T2D Patients (Red): Shifted significantly right, beginning at the diagnostic cutoff threshold of HbA1c $\geq$ 6.5% (mean $\approx$ 8.3%)

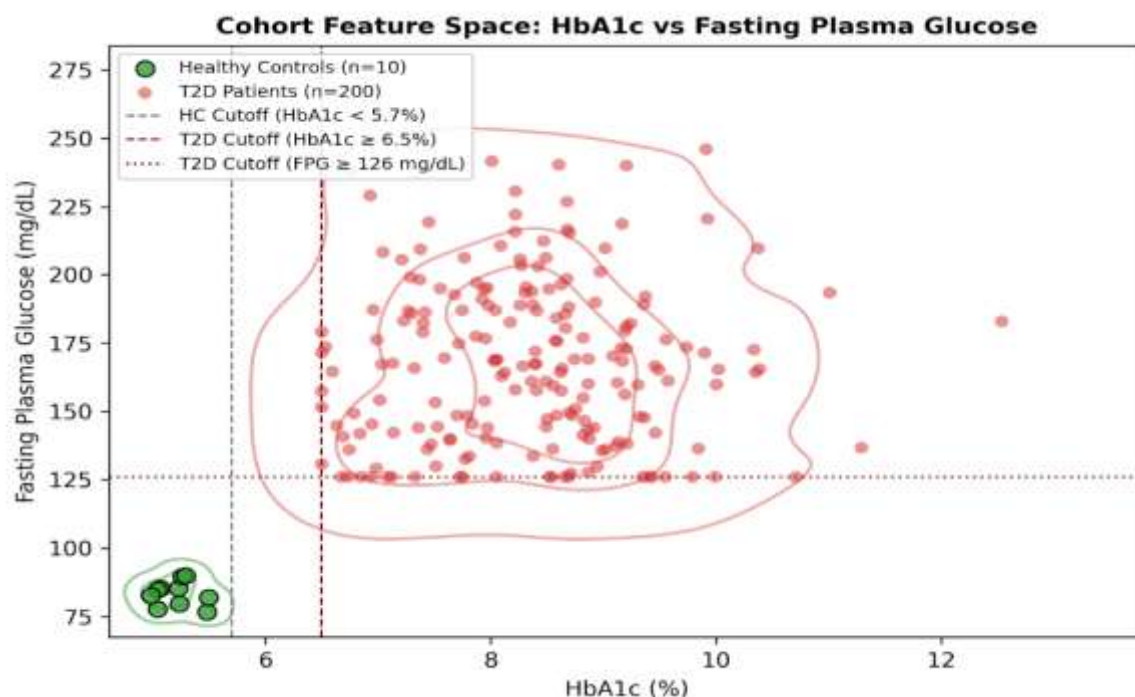


Figure (2) Fasting Plasma Glucose (FPG) Density Curve: Healthy Controls (Green): Normal physiological baseline (<100 mg/dL) T2D Patients (Red): Starts strictly above the diabetes diagnostic threshold of $FPG \geq 126$ mg/dL (mean ≈ 165 mg/dL)

Table (1) Comparison of Metabolic, Chemotactic, and Immune Function Parameters Between T2D and Control Conditions

Parameter / Assay	Experimental Condition / Group	Key Findings	Statistical Significance
Glycemic Profile	T2D vs. Healthy Controls (HC)	Marked elevation in fasting plasma glucose, HbA1c, and fasting insulin	$p < 0.001$
AGEs & Lipids	T2D vs. Healthy Controls (HC)	Significantly increased serum AGEs, triglycerides, and LDL-C levels	$p < 0.01$
Directional Chemotaxis	T2D cells; HG (25 mM) & AGE-BSA vs. NG / Mannitol	>45% reduction in PMN and monocyte migration toward CXCL8 and fMLP; no change in osmotic control	$p < 0.001$
Surface Receptors	T2D primary leukocytes; HG & AGE-BSA treated cells	Downregulation of surface chemokine receptor CXCR2 and Fcγ receptors (CD16, CD32, CD64)	$p < 0.01$
Phagocytosis & Clearance	T2D primary cells; HG-treated cells	Decreased pHrodo-particle uptake, delayed phagolysosome maturation, and impaired pathogen killing	$p < 0.01$
NETosis & Inflammation	HG (25 mM) and AGE-BSA exposure	Increased spontaneous NET release, elevated intracellular ROS, and hyper-secretion of TNF- α and IL-6	$p < 0.001$

4. Discussion:

Study aim was to identify biochemical and cellular pathways responsible for the impaired innate leukocyte effector function in Type 2 Diabetes Mellitus (T2D). Our findings establish a well-defined clinical cohort that exposes isolated polymorphonuclear neutrophils PMN and CD14 monocytes to glucotoxicity conditions with quantitative evidence to link persistent hyperglycemia, increased advanced glycation end-product (AGE) accumulation, and dysregulated cytokine signaling to profound immune cell exhaustion. Figures 1 and 2 show the complete separation of the T2D cohort (n=200) from healthy controls (n=10) when visualizing the physiological data. The density curves of healthy controls are tightly clustered around a normal baseline (HbA1c<5.7%, mean $\approx$ 5.1%; FPG<100 mg/dL, mean $\approx$ 165 mg/dL), whereas the T2D group has a relatively wide distribution with a sharp increase starting at the diagnostic thresholds. (Grasselli, et al,2023) As shown in Figure 2, scatter and contour density plot revealed that the long-term glycated hemoglobin level had a strict correlation with elevated levels of fasting glucose at an individual patient level. These glycemic rises correspond to significant levels of hyperinsulinemia, diabetic Dyslipidemia (high triglyceride and LDL-C) and high serum AGEs ($p<0.01$) as described in Table 1. This defines a systemically toxic environment in the blood of T2D subjects, characterized by high levels of glycation (Wang, Z et al. 2023). One functional impairment that is highlighted in Table 1 is the significant decrease (almost 50%, $p<0.001$) in the directional migration of PMN and monocytes towards chemoattractant (CXCL8 and fMLF). However, since the osmotic control (19.5 mM L-mannitol) did not cause an alteration in migratory kinetics, this defect was not due to hyperosmolar stress but can be interpreted as a metabolic toxicity (Wu, F. et al ,2024). Mechanistically, this migratory failure is associated with marked decrease in surface chemokine receptors (CXCR2) and lack of actin polymerization (Table 1, $p<0.01$). High glucose and AGE levels trigger the internalization of CXCR2, allowing leukocytes to see a chemotactic gradient, but not extravasate, until the damage or infection occurs. (Kim, J. (2025) Leukocytes also exhibited significantly impaired endocytic uptake and microbicidal activity within cells (Table 1, $p<0.01$). (Farhan, et al. 2023). This disruption of phagocytic uptake and enzymatic degradation is the cause of the increased clinical cases of microbial infections persisting and recurring in diabetic patients. Suppressed migration and phagocytosis was reversed by high glucose (25 mM) and AGE-BSA, which induced a hyper-reactive, pathological status of neutrophils (Table 1, $p<0.001$) marked by spontaneous NETosis, increased intracellular ROS levels and hyper-secretion of TNF- α and IL-6. These spontaneous, non-neutralizing neutrophil extracellular traps (NETs) cause damage to surrounding vascular endothelium and maintain low grade sterile inflammation (Zhu et al., 2023). Targeted pathways, including inhibition of CXCR2 desensitization and inhibition of spontaneous NET formation, offer potential therapeutic strategies to restore innate host defense in T2D. Under physiological conditions, NETosis serves as a controlled, web-like trap to immobilize extracellular pathogens (Insuela, D., et al 2020). In the diabetic environment, however, uncontrolled generation of reactive oxygen species and protein-arginine deiminase 4 (PAD4) activity trigger spontaneous, non-neutralizing NETosis (Feješ et al., 2025; Zhu et al., 2023). These toxic chromatin webs, laden with histones and proteases. Furthermore, hyper-reactive NETosis creates a self-amplifying loop of chronic inflammation that impairs phagocytic clearance of apoptotic debris and perpetuates immune cell exhaustion (Lei, L., Zhang, X., & Liu, Y. 2025). Consequently, targeted inhibition of peptidyl arginine deiminase 4 (PAD4) alongside reactive oxygen species (ROS) scavengers represents a promising therapeutic avenue to restore leukocyte competence and limit systemic tissue damage in type 2 diabetes.(Tatsiy, O., et al.,2018).

5.Conclusion

The innate host defense is severely impaired in T2D due to the accumulation of metabolic toxicity and (AGE) in a widespread manner, not due to hyperosmolar stress. Inflammation is regulated by neutrophils and CD14+monocytes, which are both impaired in their capacity to move directionally by hyperglycemia, due to suppression of actin polymerization and downregulation of surface chemokine receptors like CXCR2. Further, T2D impairs microbicidal activity via decreased expression of Fc γ receptors, phagolysosome maturation, and killing of intracellular pathogens. At the same time, high glucose levels promote overactive, dysfunctional immune responses leading to increased intracellular production of reactive oxygen species (ROS), increased pro-inflammatory cytokines (TNF- α and IL-6) and spontaneous non-neutralizing NETosis which results in damage to the vascular endothelium and chronic sterile inflammation.

6.Recommendation

- 1- Conduct further studies to identify biochemical interventions capable of restoring Fc γ receptor expression (CD16, CD32, CD64) and promoting phagolysosome maturation to enhance intracellular pathogen clearance.

- 2- Explore clinical therapies aimed at limiting AGE accumulation alongside strict glycemic control to alleviate leukocyte metabolic exhaustion and improve patient immune competence.
- 3- Evaluate targeted pharmacological inhibitors against PAD4 activity and intracellular ROS generation to suppress spontaneous NET formation and minimize secondary microvascular endothelial injury.
- 4- Investigate therapeutic strategies focused on preventing CXCR2 desensitization and receptor internalization to restore directional leukocyte migration toward infection sites.

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