The Lymphocyte as a Cellular Model to Study Insights into the Pathophysiology of Diabetes and Its Complications

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ABSTRACT: Blood cells from subjects with hypertension and/or diabetes mellitus have been successfully studied in the past to gain insight into pathological alterations of several signal transduction pathways. Diabetes mellitus is also considered to be a disease of abnormal cellular Ca²⁺ metabolism, as metabolic derangements of Ca²⁺ transport have been noticed both in the prediabetic state and as a consequence of hyperglycemia and oxidative stress. In this report, we used peripheral blood lymphocytes from type 2 diabetes patients and control subjects to study and delineate different mechanisms of Ca²⁺ turnover that determine the level of cytosolic calcium (Caᵢ). While demonstrating the specific Ca²⁺ turnover alterations, we suggest that insights into the pathophysiology of diabetic complications originating from signal transduction defects could be conveniently studied using blood cell types such as lymphocytes and that such studies could lead to the identification of new molecular drug targets.

KEYWORDS: diabetes; cytosolic calcium; lymphocytes; SERCA, PMCA, PKC, signal transduction

INTRODUCTION

Cellular signaling defects are considered to play a fundamental role in the development of diabetes and diabetes-associated complications. Blood cells from subjects exhibiting diabetes mellitus have been successfully studied in the past to gain insight into pathological alterations of several signal transduction pathways. Particularly, the measurements of ion transport turnover in lymphocytes (and platelets) have been shown to be highly reproducible and even persistent in cultured transformed cells. It is inferred from our work and studies elsewhere that using lymphocytes as a cellular paradigm has several advantages: (a) Lymphocytes are an easily accessible blood cell type and it is convenient to obtain clinical samples. (b) Lymphocyte Ca²⁺ signaling aspects and molecular players of Ca²⁺ regulation are well understood. (c) Lymphocytes contain the same transport proteins present in vascular smooth muscle cells, myocardial tissue, and other target cells affected in diabetes mellitus. (d) Interesting analogies exist in ion channel plasticity between lymphocytes and excitable...
cells. (e) Immortalized lymphoblasts conserve “diabetic” phenotype characteristics of ionic alterations. (f) Lymphocytes could potentially provide genomic DNA for studying genetic mechanisms of diabetic complications.

Altered cellular and subcellular Ca²⁺ distribution along with augmentation of Na⁺/H⁺ antiport and/or protein kinase C (PKC) could impose long-lasting effects on diabetes and its micro- and macrovascular complications. In this report we summarize some of our work on Ca²⁺ turnover and briefly discuss the evidence that supports the use of lymphocytes as a suitable cellular model to study the pathogenic signal transduction mechanisms of diabetes and its complications.

METHODS

Peripheral lymphocytes were isolated from whole blood using Lymphoprep and centrifugation. Measurements of intracellular calcium (Caᵢ), ATPase activity, Na⁺/Ca²⁺ exchange, and thapsigargin (Tg)-mediated Ca²⁺ influx were performed as per our previous protocols.²,³ Age- and weight-matched control and type 2 diabetes subjects were recruited from the M.V. Diabetes Specialities Center, Chennai.

RESULTS AND DISCUSSION

It appears that several aspects of lymphocyte Ca²⁺ transport and regulatory profiles are similar to those in vascular tissues and β cells. We have used thapsigargin to monitor changes in lymphocyte Ca²⁺ fluxes. Thapsigargin (Tg), which is a specific inhibitor of sarcoendoplasmic calcium ATPase (SERCA),⁴ induces Ca²⁺ entry into cells from the extracellular space as a result of depletion of Ca²⁺ from the intracellular stores. As illustrated in Figure 1, cells treated with Tg exhibited dose-dependent increments in Caᵢ in the range of 75 to 400 nM. The Caᵢ profile of cells exposed to Tg demonstrated two phases: a slow increase in Caᵢ lasting 50 to 100 seconds, followed by a steep rise in the Caᵢ. Each of these traces in this figure was de-

<table>
<thead>
<tr>
<th>TABLE 1. Cellular (lymphocyte) phenotypic characteristics of Ca²⁺ turnover</th>
<th>Control subjects (n = 12)</th>
<th>Type 2 diabetes (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal Caᵢ (nM)</td>
<td>43.7 ± 2.6</td>
<td>57.4 ± 3.7*</td>
</tr>
<tr>
<td>Plasma membrane Ca²⁺-ATPase (PMCA) activity (µmol/mg protein/min)</td>
<td>0.110 ± 0.008</td>
<td>0.073 ± 0.007*</td>
</tr>
<tr>
<td>Reverse mode Na⁺/Ca²⁺ exchange activity (-fold increase over basal after Tg treatment)</td>
<td>&lt;1</td>
<td>3</td>
</tr>
<tr>
<td>Tg-stimulated ⁴⁵Ca²⁺ uptake (%)</td>
<td>23.2 ± 3.7</td>
<td>47.8 ± 14.5*</td>
</tr>
<tr>
<td>Tg-stimulated initial peak Caᵢ (nM)</td>
<td>162 ± 11</td>
<td>235 ± 21*</td>
</tr>
<tr>
<td>PKC activation–related inhibition of Tg-mediated Ca²⁺ influx (%)</td>
<td>68 ± 2.6</td>
<td>38 ± 2.9*</td>
</tr>
</tbody>
</table>

*P < 0.05.
rived from mean fluorescence values of six different sets of experiments and this confirms the accuracy and reproducibility of $C_{ai}$ measurements. Table 1 summarizes the results of $Ca^{2+}$ turnover parameters in cells from type 2 diabetes and control subjects as a result of fluorescence and $^{45}Ca^{2+}$ flux studies. As evident from the table, cells from type 2 diabetes exhibited significantly higher ($P < 0.05$) basal $C_{ai}$, reduced plasma membrane calcium ATPase activity, and higher $Na^{+}/Ca^{2+}$ exchange and Tg-stimulated $Ca^{2+}$ fluxes. In cells from type 2 diabetes, the PKC-activation related feedback inhibition of $Ca^{2+}$ influx was noticed to be considerably reduced. The higher basal $C_{ai}$ resulting from altered $Ca^{2+}$ transport in cells from type 2 diabetes also exhibited a positive correlation with the plasma glucose (control 79 ± 11 vs. NIDDM 176 ± 27 mg/dL) and glycated hemoglobin (HbA1c) values (control; 5.7 ± 0.5 vs. NIDDM 9.0 ± 1.7 %)

It has been proposed that abnormal cell $Ca^{2+}$ homeostasis may contribute to the impaired insulin action because part of the insulin action may be mediated by the $Ca^{2+}$ signal. As multiple cell functions are regulated by $Ca_{i}$- and $Ca^{2+}$-dependent signals, it is conceivable that altered refilling of the intracellular $Ca^{2+}$ pools resulting from modulatory patterns of the store-operated $Ca^{2+}$ entry may translate into dysregulated protein synthesis and proliferation. In patients with mitochondrial DNA inherited oxidative phosphorylation deficiency, derangement of organellar $Ca^{2+}$ handling has been reported which impairs the $Ca^{2+}$-mediated activation of mito-
Our preliminary studies in type 2 diabetes also indicate a defect in the organelar sequestration of Ca\(^{2+}\) especially in mitochondria and an increase in the cytosolic reactive oxygen species (ROS) load. Restoration of Ca\(^{2+}\) signal and the enhancement of ATP production in mitochondria could correct insulin secretory and sensitizing effects in maturity-onset diabetes in young (MODY) and common forms of type 2 diabetes subjects.

Our studies suggest that in the absence of target tissues amenable for cellular and molecular studies, insights into the pathophysiology of diabetic complications originating from signal transduction defects could be conveniently studied using blood cell types such as lymphocytes. Mononuclear cells better serve to explore the role and causal relationship of intracellular ions (e.g., Ca\(^{2+}\), H\(^{+}\), Na\(^{+}\)), cytosolic messenger molecules, cytokines, Th1 and Th2 signals, transcription factors, and gene expressions in the development and progression of micro- and macrovascular complications of diabetes. It seems that their use in clinical diabetes would also identify whether the amount of diabetes-induced intracellular oxidative stress (reactive oxygen species [ROS] load) or the concentration of hyperglycemia-induced advanced glycation end products is associated with the risk of diabetic complications. We predict that the experimental approach of immortalized blood cells from patients with diabetes and diabetic complications could potentially open up new horizons in the identification of genetic abnormalities in intracellular signal transduction.

REFERENCES