December 2008

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Tracking Down Immune Markers from Alternative System Pathway Factors in a Diabetic Population

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Hyperglycemia associated with type 1 diabetes (T1D) alters the host immune system, resulting in a predisposition to infectious diseases. The high risk of infection in the diabetic population may lead to life-threatening situations. The early proteins of the alternative complement system pathway, constituting factors P, B, and D, have been shown to play an important role in preventing infection because they form a membrane attack complex (MAC)-C5-9, which debilitates the target microbes and/or molecules via cytotoxic and cytolytic reactions. Patients who are devoid of or contain low levels of these proteins may be susceptible to developing chronic infections. We have observed striking differences in partially fractionated serum proteins in diabetic patients (type 2) relative to controls, through single and two-dimensional gel electrophoresis. Our data, obtained from 50 diabetic patients in the age group of 25–45 years, who had the disease for fewer than 5 years, indicated patterns in low- and high-molecular-weight proteins, which could be grouped into five different categories with minor differences in their respective levels of protein expression. Immunoblot assay could barely detect the presence of properdin expression in diabetic patients. Quantization by ELISA in 99 patients indicated low levels of properdin expression in 70% of 50 diabetic patients (6.5 ± 3 μg/mL) when compared to nondiabetic controls (19.5 ± 8.5 μg/mL). This study concluded that patients with low expression of properdin should be advised to take extensive preventive measures and seek early management with appropriate treatments against infection.

Key words: type 1 diabetes; hyperglycemia; properdin

Introduction

Type 1 diabetes (T1D) is a condition that makes it hard for the body to control the level of glucose in the blood. The resulting hyperglycemic condition can cause a number of problems and allows pathogens to grow and infections to develop more quickly.1 These infections may include foot and leg infections, including diabetic foot ulcers, which are caused by poor circulation and nerve damage (known as diabetic neuropathy), vaginal yeast infections in women, ringworm and athlete’s foot, styes, boils, carbuncles, nail infections, urinary tract infections, gingivitis, and lung and sinus infections.2 These infections linked to diabetes may be spread to others, depending on their cause. The long-term effects of the conditions may include kidney damage from frequent infections, leading to end-stage renal disease, dental problems like periodontitis, permanent skin damage, and amputation for progressive infection of the feet or legs. Normally the immune system of the

body is geared to protect the body from foreign invaders. The hyperglycemic condition alters the immune responses, and the normal host defenses against diseases become less efficient. The complement system is one of the complex biochemical cascades of the immune system. It can mark pathogens for phagocytosis and activation of the membrane attack complex (MAC) to initiate cell lysis and inflammation. It is thought that the complement system might play a role in diabetes as in many other diseases with an immune component. The complement system influences the activity of numerous cells and tissues and the physiological mechanisms of the body. Target cells for membrane attack complex action may be heterologous erythrocytes, nucleated cells (autologous or foreign), bacteria (Gram-negative, susceptible to serum), microscopic fungi, viruses with a surface envelope, and virus-infected cells. The early components of the alternative pathway are known as factors and each molecule is named by a letter as in factors B, D, and P. Factors B and D combine with C3, forming an unstable compound, C3bBb, which binds with C5 convertase and becomes stable after binding with factor P (properdin) C3bBb3b and initiates the terminal pathway of complement activation. Properdin prolongs the half-life of surface-bound C3bBb from 1 1/2 minutes to about 18 minutes. Deficiency or malfunction of the molecule may lead to severe impairment of alternative pathway activation, depending on the precise nature of the defect. One parameter of functional defect in the presence of measurable levels of protein is an impaired generation of the more active tetramer and trimer forms. We are interested in using a proteomics approach to search for a biomarker as an early indicator of associated disorders of diabetes before the appearance of clinical symptoms. Moreover, we are interested in determining the expression levels of properdin in diabetic patients to find the efficacy of alternative system pathway factors for eradication of the infections.

Methods

Blood samples were collected from 100 persons with clinically proven type 2 diabetes according to the WHO criteria (fasting blood sugar >5.5 mm/L). Samples were also collected by venipuncture in healthy, age- and gender-matched controls (n = 50). The control group was also confirmed as nondiabetic by testing fasting blood sugar levels (<5.5 mm/L) and monitoring glucose levels 2 hours after oral administration of 75 grams of glucose. The duration of disease in subjects was from 6 months to fewer than 5 years, and they were all between 25 and 45 years of age. Serum and plasma were separated according to standard laboratory techniques. Ethical approval for this study was obtained from the Aga Khan University, and consent was received from the subjects.

Protein concentration was measured by the method of Bradford. One-dimensional gel electrophoresis of proteins from 50 patients was performed by denaturing (SDS) discontinuous gel electrophoresis using the Laemmli gel method. Two-dimensional gel electrophoresis of same proteins was based on the protocol of O’Farrell, using a mini cell system (BioRad). For quantification and detection of properdin, the ELISA assay was performed on 99 patient samples and 50 control samples according to supplier’s instructions (Antibody Shop PP-0401CE). ELISA assay included the patients’ samples, which were used for two-dimensional gel electrophoresis analyses. In this assay micro-wells coated with a monoclonal antibody directed against human properdin were used. Bound properdin was detected with the same monoclonal antibody label as that of biotin. Horseradish peroxidase (HRP)-conjugated streptavidin was then allowed to bind to the biotinylated detection
antibody and incubated with a chromogenic peroxidase substrate.

Results and Discussion

Serum protein profiles of diabetic patients showed striking differences on SDS polyacrylamide gel electrophoresis. Expression patterns on the gel showed either the faint bands or the undetectable quantity of the 22-kDa and 52-kDa proteins. On the basis of their apparent molecular weights as well as their low abundance, the 22-kDa and 52-kDa polypeptides might represent the properdin family of proteins (factor P). On the basis of similarity in protein profiles of patients’ samples, these were grouped into eight different categories from P1-P8. The P8 group contained the 50 samples that showed profiles very much like those of the nondiabetic control group, indicating a uniform protein profile (data not shown).

Further concentration of the properdin protein was determined in all groups of patients and in the nondiabetic control group through ELISA with 99.9% sensitivity and 99.9% specificity. Analysis indicated the presence of properdin in the range of 5.6–9.8 μg/mL in groups P1, P4, P5, P6, and P7 (Table 1), which is below the normal range of properdin (11.1–31.47 μg/mL) in healthy individuals (control group). Concentration of properdin in groups P2 and P3 was found in the normal range in spite of indicating different electrophoretic patterns of the proteins. Properdin concentration in group P8 of the diabetic patients, which showed protein profiles similar to those of nondiabetics, also indicated a normal range. The decrease in concentration of properdin in groups P1, P4, P5, P6, and P7 was not consistent with the duration of diabetes. Lower concentrations of properdin in diabetic patients generally refer to type 2 properdin deficiency, which is found in 50% of our diabetic population, as represented in groups P1, P4, P5, P6, and P7. The other 50% diabetic population of group P8 showed the normal range, which needs to be checked as to whether it is functionally active. Further structure–function analysis can be performed in the future.

Factor P has been shown to stabilize the labile C3 convertase (C3bBb). The complement system comprises a group of more than 30 different proteins that play a role in the immune response.7 These proteins normally circulate in an inactive or “precursor” form. During an infection, complement proteins are broken down into active fragments that can perform a variety of functions (http://www.emedicine.com/med/topic). The interaction between the players of the immune system and infections ultimately determines the health of the person.8 This deficiency of properdin may be involved in leading to recurrent bacterial and viral infections, making the patients susceptible to meningococci. This work gives a better understanding of what is going on behind the scenes during the production of an immune response in type 2 diabetes, but many mysteries remain. Low levels of properdin factors have not been reported in diabetic patients in detail. Further progress

<table>
<thead>
<tr>
<th>Group identification (n)</th>
<th>Average properdin concentration (μg/mL)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 (1)</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td>P2 (5)</td>
<td>18.93</td>
<td>6.8</td>
</tr>
<tr>
<td>P3 (7)</td>
<td>15.22</td>
<td>4.06</td>
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<td>P4 (10)</td>
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<td>7</td>
<td>0.08</td>
</tr>
<tr>
<td>P6 (8)</td>
<td>7.4</td>
<td>1.3</td>
</tr>
<tr>
<td>P7 (11)</td>
<td>6.5</td>
<td>2.3</td>
</tr>
<tr>
<td>P8 (50)</td>
<td>22</td>
<td>5.7</td>
</tr>
<tr>
<td>Control, nondiabetic (50)</td>
<td>11.1–31.47</td>
<td></td>
</tr>
</tbody>
</table>

ELISA was performed for the in vitro determination of properdin in human serum or plasma according to supplier’s instructions (Antibody Shop PP-0401CE). Group identification was done on the basis of protein similarity obtained in PAGE. n represent the number of samples in each group.

TABLE 1. Determination of Properdin Concentration by ELISA
in this field promises to inform the design of new and improved immune-based therapies to delay the infection-related complications. Although it is clear that the observation made by our work is preliminary and does not fully explain the heterogeneity in general susceptibility to infection, it does, however, focus attention on the need to identify additional risk factors in properdin-deficient persons to improve risk prediction and avoidance strategies. Bioinformatics and traditional molecular biology tools will provide a wealth of information in the near future.

**Conclusion**

Properdin deficiency needs to be checked in diabetic patients in different global populations, and properdin-deficient individuals should be considered for additional tests, such as determination of IgG and Fcγ receptor allotypes. These patients should also be considered for vaccination against infections as a part of early management.

**Acknowledgments**

We thank Dr. Anwar Ali Siddiqui and his research group for helpful discussions in our conduct of proteomics work. We are also thankful to Dr. Philippe Frossard and the Department of BBS for the financial support provided for this work.

**Conflicts of Interest**

The authors declare no conflicts of interest.

**References**