GRANULOCYTE FUNCTIONS IN PATIENTS WITH INSULIN DEPENDENT DIABETES MELLITUS

SHUNICHI SHIMIZU, TADATOSHI KURATSUJI, SHIN AMEMIYA
KOTARO ASAYAMA, TADASHI OJIMA and KIYOHIKO KATO*

Department of Pediatrics, School of Medicine, Keio University,
Tokyo, Japan
*Department of Pediatrics, Yamanashi Medical College,
Yamanashi, Japan

(Received for publication October 27, 1982)

ABSTRACT

It is often experienced that when diabetic children suffer from infections, the control of diabetes becomes difficult. It seems important to pay attention to the host defense mechanisms of diabetic patients. Some previous reports showed granulocyte dysfunctions in patients with diabetes. An integrated investigation regarding this problem was carried out.

Chemotactic activity, random mobility, deformability, NBT dye reduction and adherence of granulocytes were measured in 20 patients suffering from insulin dependent diabetes mellitus.

Chemotaxis was impaired significantly (0.66 ± 0.33 to the healthy control). NBT dye reduction and adherence were normal. Random mobility and deformability were also normal, as a whole, but several subjects showed impaired functions.

The fasting blood glucose level, the daily amount of glucosuria, the glycosylated hemoglobin level, the daily insulin requirement and the duration from the onset of the disease were chosen as indices of the clinical state of diabetes in order to see the correlations with the granulocyte functions. As a result, no significant relationship was found between granulocyte functions and these indices.

INTRODUCTION

Pediatricians often experience difficulties in controlling childhood insulin dependent diabetes mellitus (IDDM) when it is complicated with some infections. Therefore it is important to pay attention to infections in caring for diabetic children.
As a matter of course, granulocytes provide man’s first line of defense against bacterial infection. Although there are several reports regarding impaired granulocyte functions of IDDM patients, it is uncertain whether patients suffering from IDDM are more susceptible to infections or not.

Mowat et al. showed a chemotactic defect in granulocyte of patients with diabetes mellitus. Miller also reported poor chemotactic responses in granulocyte of IDDM, and deficiency in the generation of chemotactic activity from plasma of children with diabetes as well. Also demonstrated were the altered bactericidal function and adherence of granulocyte of IDDM patients.

But there are few reports regarding the granulocyte functions of childhood IDDM patients in which several functions of the granulocyte were examined at one time and the data obtained was discussed in comparison with the diabetic state.

The present study was undertaken to see the various aspects of the granulocyte dysfunction in IDDM children and also to determine how the functions of granulocyte are influenced by the diabetic state.

Examined granulocyte functions were chemotaxis, random mobility, deformability, NBT dye reduction and adherence. The generation of the complement-derived chemotactic factor (CDCF) from the sera of IDDM children was also examined. The fasting blood glucose (FBG), the daily urinary glucose loss, the percentage of stable glycosylated hemoglobin against total hemoglobin, the daily insulin requirement and the duration from the onset at the time of examination were chosen as indices for the quality of the control of diabetes.

MATERIALS AND METHODS

Patients:

Twenty insulin-dependent diabetic patients, nine males and eleven females, were studied. The ages of the patients ranged from 1 to 21 years with a mean age of 9.1 years. They were all in good general condition and their diabetic condition was fair to moderately under control except for one case who was mildly ketoacidotic. None had an intercurrent infection at the time of study and no one showed enhanced susceptibility to infections during the course of the disease. Healthy adult volunteers were used as normal controls.

Leukocytes:

Leukocytes were separated from heparinized venous blood by Dextran sedimentation, washed three times and resuspended in Eagle’s minimum essential medium (MEM) at the respective concentrations for each examination.
Granulocyte functions in patients with insulin dependent diabetes mellitus

Chemotaxis, Random Mobility, Deformability and CDCF producibility:

Chemotaxis and random mobility were examined using a modified Boyden's method with or without chemoattractant in the lower chamber. Zymosan-activated plasma was prepared from plasma of a healthy adult volunteer and was used for chemoattractant. In the study of CDCF producibility, zymosan-activated plasma from a diabetic child was used for chemoattractant and granulocytes of a healthy adult were suspended in the upper chamber. Cellular deformability of granulocytes was studied in our previously described original manner. Briefly, it is an application of Boyden's method using two chambers with 1.2 μm and 8.0 μm micropore membrane filters respectively at the same time.

Nitroblue-Tetrazolium (NBT) reduction test:

NBT reduction measurements were done according to a modified Park method. Zymosan-stimulated activity was measured and the percent of formazan positive cells was counted.

Adherence:

Adherence was measured by our original “microplate method” (unpublished, presented at the 30th meeting of the Eastern Chapter of Japanese Association for Infectious Diseases, 1981 at Tokyo), in which 1x10^3 granulocytes suspended in 90% identical plasma and 10% MEM were incubated for 30 minutes in a microplate and then the plate was turned over and the number of adhering granulocytes at the bottom (now ceiling) of the microplate were counted.

Stable glycosylated hemoglobin:

Stable glycosylated hemoglobin was measured by high performance liquid chromatography which has been reported recently.

In each examination of granulocyte function, the result was expressed as the ratio to the value of normal control which was examined at the same time except for NBT reduction in which real numbers of percent of reduced cells were compared. Normal ranges (defined as mean ± 1 SD range here) were estimated according to more than 70 normal control value in our laboratory for each examination.

RESULTS

Table 1 shows the compilation of the routine laboratory data of the patients and the duration of their disease. Fig. 1 gives the value of chemotaxis, deformability indices, random mobility and CDCF generation from plasma of diabetic children, each in comparison with the simultaneously examined respective
Table 1
Clinical and laboratory data of 20 children with IDDM

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs.)</td>
<td>20</td>
<td>9.1</td>
<td>1-21</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>20</td>
<td>166.1</td>
<td>65-416</td>
</tr>
<tr>
<td>St-GHb (%)</td>
<td>14</td>
<td>6.91</td>
<td>4.9-10.3</td>
</tr>
<tr>
<td>Urine Glucose (g/day)</td>
<td>19</td>
<td>31.5</td>
<td>2-108</td>
</tr>
<tr>
<td>Insulin Requirement</td>
<td>20</td>
<td>0.81</td>
<td>0.38-1.42</td>
</tr>
<tr>
<td>Duration Since Onset</td>
<td>20</td>
<td>44.2</td>
<td>1-218</td>
</tr>
</tbody>
</table>

FBG: fasting blood glucose, St-GHb: stable glycosylated hemoglobin.

Fig. 1 Chemotaxis (CTX), deformability index (D.I.), random mobility (RM), generation of complement derived chemotactic factor (CDCF) in patients with IDDM.
(1.0 represents the value of normal control examined at the same time and the shaded area represents the estimated normal range).
control value which is expressed as a value of 1.0. The shaded area represents the estimated normal range and brackets, ± SD of the results.

In the study of chemotaxis, granulocytes from patients with IDDM showed significant deficiency, 0.66 ± 0.33 (M ± SD) of the control value (p<0.05).

Deformability indices showed 0.84 ± 0.89 (M ± SD) of the control, scattered in a rather wide range and no apparent trend. It is worth noticing that twelve out of 19 cases examined showed less than −1 SD value of the normal controls.

Also in the study of random mobility, though the result showed 0.81 ± 0.98 (M ± SD) of the control as a whole, ten out of seventeen cases examined gave results less than −1 SD of the controls.

The generation of CDCF from plasma of IDDM patients showed no significant difference from that of the control.

Fig. 2 shows the results of the NBT dye reduction test and adherence respectively. The shaded area represents the normal range. In both the NBT test and adherence study, the results remained almost within the normal range.

The relationship between the granulocyte functions and some clinical data of the IDDM was analyzed. The fasting blood glucose level, the percentage of

![Fig. 2 Results of NBT dye reduction test and adherence.](image-url)
Table 2

Coefficient of correlation between granulocyte functions and clinical data

<table>
<thead>
<tr>
<th></th>
<th>FBG mg/dl</th>
<th>St-GHb %</th>
<th>U-Glu g/day</th>
<th>Insulin unit/kg</th>
<th>Duration months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotaxis</td>
<td>r = -0.21</td>
<td>r = -0.40</td>
<td>r = -0.24</td>
<td>r = 0.01</td>
<td>r = -0.04</td>
</tr>
<tr>
<td></td>
<td>n = 16</td>
<td>n = 9</td>
<td>n = 15</td>
<td>n = 16</td>
<td>n = 16</td>
</tr>
<tr>
<td>Deformability</td>
<td>r = -0.25</td>
<td>r = -0.37</td>
<td>r = -0.41</td>
<td>r = 0.10</td>
<td>r = -0.11</td>
</tr>
<tr>
<td></td>
<td>n = 16</td>
<td>n = 9</td>
<td>n = 15</td>
<td>n = 13</td>
<td>n = 16</td>
</tr>
<tr>
<td>Random Mobility</td>
<td>r = -0.23</td>
<td>r = -0.33</td>
<td>r = -0.24</td>
<td>r = 0.38</td>
<td>r = -0.17</td>
</tr>
<tr>
<td></td>
<td>n = 13</td>
<td>n = 8</td>
<td>n = 12</td>
<td>n = 13</td>
<td>n = 13</td>
</tr>
<tr>
<td>CDCF Production</td>
<td>r = -0.01</td>
<td>r = -0.39</td>
<td>r = 0.06</td>
<td>r = -0.10</td>
<td>r = 0.21</td>
</tr>
<tr>
<td></td>
<td>n = 15</td>
<td>n = 8</td>
<td>n = 14</td>
<td>n = 15</td>
<td>n = 15</td>
</tr>
<tr>
<td>NBT Reduction</td>
<td>r = 0.19</td>
<td>r = -0.16</td>
<td>r = 0.02</td>
<td>r = -0.22</td>
<td>r = -0.31</td>
</tr>
<tr>
<td></td>
<td>n = 17</td>
<td>n = 13</td>
<td>n = 15</td>
<td>n = 17</td>
<td>n = 18</td>
</tr>
<tr>
<td>Adherence</td>
<td>r = 0.12</td>
<td>r = 0.14</td>
<td>r = 0.06</td>
<td>r = -0.11</td>
<td>r = -0.09</td>
</tr>
<tr>
<td></td>
<td>n = 18</td>
<td>n = 14</td>
<td>n = 17</td>
<td>n = 19</td>
<td>n = 20</td>
</tr>
</tbody>
</table>

FBG: fasting blood glucose, St-GHb: stable glycosylated hemoglobin, U-Glu: daily urinary glucose.

stable glycosylated hemoglobin against total hemoglobin, amount of daily urinary glucose, daily insulin requirement at the time of examination and the duration of the disease since its onset were chosen as indices of the clinical state of the disease. Table 2 summarizes the coefficient of correlation between these data. Relations between fasting blood glucose levels and chemotaxis or deformability, and the amount of stable glycosylated hemoglobins and chemotaxis or deformability are shown in Fig. 3.

DISCUSSION

Granulocyte dysfunctions have been reported diversely in patients with IDDM, and we have also demonstrated certain granulocyte dysfunction here. Though the metabolic base for the granulocyte dysfunctions remains to be clarified, carbohydrate metabolism is disturbed in patients with IDDM, and granulocyte functions are highly dependent on carbohydrate metabolism. Hence, it is suggested that granulocyte dysfunctions are likely due to altered carbohydrate metabolism in IDDM. There are several reports regarding impaired chemotactic activity of granulocytes in diabetic patients as is also the case in this study. We could trace no report regarding random mobility of the granulocyte from the IDDM patient. As to the deformability Miller et al. have documented its impairment by different methodology. In this study, random
mobility or deformability were defective in some subjects. But, as a whole, they were not significantly impaired. In the study of adherence, no abnormalities were found in our study, though previous reports show impaired granulocyte adherence in patients with poorly controlled diabetes or in diabetic patients other than IDDM.16 Regarding the NBT reduction test there are opposite opinions, impaired17 and normal,3 and in our study the results were within normal limits. In the report that showed impaired NBT reduction in diabetics, the subjects studied were all adults, including many non-IDDM.

Some clinical data which may represent the status of the disease control in each aspect were chosen to investigate the relationship between the clinical state of diabetes and the granulocyte functions. Fasting blood glucose level was chosen as a conventional index of blood glucose control in diabetes mellitus. The amount of urine glucose represents the daylong control of the disease or relatively short term blood glucose control, and the percentage of stable glycosylated hemoglobins is an index of long term blood glucose control.10 However no significant correlation among them was found as a result. Mowat and Baum1 demonstrated no correlation between chemotactic activities and plasma glucose levels. On the other hand, Zielinski and coworkers18 showed that in insulin dependent diabetic patients who excrete urine glucose of more than 60 g a day, chemotactic activity was poor and Bagdade et al.5 demonstrated the correlation between the ability of granulocyte adherence and plasma glucose concentrations in patients with
poorly controlled diabetes.

We found no apparent impairment of granulocyte functions except for the chemotactic impairment, or correlation between granulocyte functions and diabetic state contrary to some reports hitherto made. In this study, none of the subjected patients was very poorly controlled, so it is likely that granulocyte functions of IDDM patients under ordinary control show little abnormalities regardless of the blood glucose control or the duration of the disease. But it is speculated that there are possibilities of other granulocyte dysfunctions or relationships between granulocyte dysfunction and diabetic state if further investigations were made with an additional number of more poorly controlled subjects in whom some yet unrevealed factor(s) might exist and affect the granulocyte functions. For example in IDDM patients under seriously ill control such as in a ketoacidotic state, in addition to poor control of blood glucose, plasma levels of catecholamines or cortisol are altered.10,20 This may influence cyclic 3' 5' adenosine monophosphate (c-AMP) or cyclic 3' 5' guanosine monophosphate (c-GMP) levels of granulocytes and affect the granulocyte functions.21

SUMMARY

Impairment of granulocyte chemotaxis was demonstrated in patients with IDDM. Other granulocyte functions (random mobility, deformability, NBT dye reduction and adherence) were revealed to be within normal limits in this investigation. Though there are several reports regarding impaired granulocyte functions other than chemotaxis in patients with diabetes, it is likely that granulocyte functions are little affected in IDDM patients who are not so poorly controlled like those studied here. The disturbed functions hitherto reported in diabetes, not fitting to the present report, might be due to the problems of subjects including those other than IDDM patients, or the difference of the controlled state of the patients studied.

ACKNOWLEDGMENTS

We wish to thank Professor Mitsuru Osano for his advice, and Miss Kyoko Shinohara for her secretarial aid.

This work was supported in part by a grant from the Japanese Ministry of Health and Welfare, Immunodeficiency Research Project.

This work was presented at the 85th annual meeting of the Japanese Pediatric Society.

REFERENCES


