THE STUDY OF OPSONIZATION ACTIVITY OF
THE PHAGOCYTOSIS PROCESS IN MENINGITIS,
SEPSIS, AND RECURRENT INFECTION IN
CHILDREN

S. NEZAM DIBA, MSPH, AND A. FARHOUDI, M.D.

From the Department of Immunology, the Children's Hospital Medical Center, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran.

ABSTRACT

In this survey, opsonization activity of sera in 110 patients with meningitis, sepsis and recurrent infection; 12 patients after healing; and 50 normal adults has been studied. Opsonization activity was measured by an assay depending on electronic particle counting to measure yeast uptake (alternative pathway activator) which was modified in our laboratory.

Three classes of immunoglobulins (IgG, IgM, IgA) and C3 levels were also studied depending on single radial immunodiffusion by using Behring kit in our patients.

54 pathological sera with defective opsonization showed low C3 levels, two with lower than normal opsonization activity showed low C3 levels, 11 defective opsonization cases showed normal C3 levels, 3 of which were related to diabetic patients.

22 instances of decreased IgG, 14 cases of decreased IgM and 18 cases of decreased IgA were observed in 110 patients.

The opsonization activity, C3 and immunoglobulin levels in 12 patients have become normal after healing.

INTRODUCTION

Phagocytosis is one of the first lines of defense against infection. The phagocytic process is composed of four interrelated phases: chemotaxis, opsonization, ingestion and digestion. Most organisms, especially extracellular organisms have surface factors which enable them to resist against phagocytosis. Opsonization is the important process which enhances phagocytosis of these organisms. The function of serum opsonins is to react with microorganisms and to increase hydrophobicity, thereby reducing the charge repulsion between microorganisms and phagocytic cells and make them more susceptible to ingestion by phagocytes. Opsonization of bacteria may occur by at least one of the mechanisms as noted below:

1) specific antibody alone (IgG1, IgG2, IgG3, IgG4).
2) specific antibody (IgG, IgM) acting in concert with complement by activating C3 via the classic pathway of C1, C4 and C2.
3) opsonization can be non-specific which is done via activation of alternative pathway and generation of C3b, fibronectin, tuftsin, C4b, C5 and C.R.P.

C3b acts via CR1, CR3, IgG via FC-receptors on mononuclears, PMNs and eosinophils; fibronectin acts via receptors on mononuclears and neutrophils, tuftsin acts via receptors on neutrophils and C3b acts via CR1 receptor on phagocytic cells.

Opsonization defects may be primary (genetic) or secondary after some diseases such as
Phagocytosis Process in Meningitis

Diagram (1): Number of samples of each disease.

malnutrition, chronic liver diseases, diabetes, nephrotic syndrome, partial lipodystrophy, uremia, splenectomy, septicemia, immune complex disease, excessive immune suppression, bacterial, viral and fungal infections, after some cases such as surgery (cardiopulmonary bypass), burns, trauma, and after using some drugs and antibiotics or it may occur after inhibition of opsonins or their receptor function. Opsonization defect may occur after inhibition of phagocytic cell function because of existence of inhibitors.

Causes of opsonization defects are low synthesis, excessive loss, excessive catabolism, excessive stimulation of complement system and excessive immunosuppression.

SUBJECTS

In this survey opsonization activity and three classes of immunoglobulin (IgG, IgM, IgA) and C3 levels of 110 patients (aged 1 month-13 years), 44 female and 66 male, with meningitis (12 cases, 10.91%), sepsis (13 cases, 11.82%), recurrent infection (29 cases, 26.36%), nephrotic syndrome (12 cases, 10.91%), malnutrition, and chronic diarrhea (6 cases, 5.15%), liver cirrhosis (3 cases, 2.73%), diabetes (4 cases, 3.64%), primary immunodeficiency (19 cases, 17.27%), and thalassemia (12 cases, 10.91%) (diagram 1, Table V), and 12 patients with meningitis, sepsis and recurrent infection after healing were studied. Opsonization activity in 50 healthy adults (aged 17-60 years) (26 female, 24 male) were also studied.

MATERIALS

1- Balanced salt solution.
   a- Hank's
   b-RPMI-1640
2- Live-baker yeast (Saccharomyces cerevisiae) was suspended in 0.85% saline and inactivated at 100°C for 30 min in water bath, after washing for 2-3 times with saline resuspended to 100×10^6 particles /ml.
3- Sera:
   Sera were separated from blood as soon as possible and stored at −70°C until analysis.
4- Normal PMN were separated from heparinized blood (20 unit heparin for 1 ml blood) by dextral sedimentation of red cells. After 45 min at room temperature, washed 3-5 times in Hank's and resuspended to 10×10^6/ml.

METHODS

In this survey we have used ZF Coulter counter with dual size threshold instead of ZB model. By using this system, both yeast particles and phagocytes are counted. Then the opsonization activity was reported as percent uptake of yeast, and also because in diabetic patients at 30 min (the usual time of incubation) more than 90% of phagocytes ingest yeast. Then there will be no difference between normal and diabetic patients. After using different times of incubation, the best time

<table>
<thead>
<tr>
<th>Description</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunodeficiency</td>
<td>Thalassemia</td>
</tr>
<tr>
<td>Liver Cirrhosis</td>
<td>Diabetes</td>
</tr>
</tbody>
</table>

Table I. Number of sample in each disease

<table>
<thead>
<tr>
<th>Malnutrition</th>
<th>Nephrotic Synd.</th>
<th>Recurrent Infection</th>
<th>Sepsis</th>
<th>Meningitis</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent</td>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
</tr>
<tr>
<td>5.15</td>
<td>6</td>
<td>10.91</td>
<td>12</td>
<td>26.36</td>
<td>29</td>
</tr>
</tbody>
</table>

126
was 5 min, thus the defect of these patients was obvious.

C3 opsonization activity is studied in this method because yeast is an activator of the alternative pathway of complement.20 IgG and C3 are not effective in this method. Immunoglobulin (IgG, IgM, IgA) and C3 levels were measured depending on single radial immunodiffusion by using Behring kit in this survey.

RESULTS

51 pathological sera with defective opsonization showed low C3 levels, two cases of lower than normal opsonization activity showed low C3 levels, 11 defective opsonization cases showed normal C3 levels—this case has been reported to be a relatively common immunodeficiency, three of 11 cases of defective opsonization with normal C3 levels were related to diabetic patients (Table II).

Opsonization activity in 110 patients, 112 normal children, and 50 normal adults is shown in diagram 2.

22 instances of decreased IgG, 14 decreased IgM, and 18 decreased IgA were observed in 110 patients (Table III).

The mean percent uptake activity in 110 patients aged one month-13 years (41 female, 66 male) was 47.2±34.86; 2 S.D. range: 0-80.

The mean percent uptake in 50 healthy adults aged 17-60 years (26 female, 24 male) was 64±18; 2 S.D. range: 46-82.

The mean percent uptake in 112 children aged 11-12 years (47 female, 65 male) was 55±27; 2 S.D. range: 16-83.

The opsonization activity in patients in comparison with normal adult group (T = 11.26, P<0.001) and in comparison with normal children group (T = 7.78, P<0.001) was lower than control group (Table IV, V).

The results in this study have been compared with mean values from opsonization activity in 112 normal...
Phagocytosis Process in Meningitis

Table III. Immunoglobulin deficiency in 110 patients

<table>
<thead>
<tr>
<th>Diseases</th>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
</tr>
<tr>
<td>Meningitis</td>
<td>3</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Septicemia</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Recurrent Infection</td>
<td>3</td>
<td>10.34</td>
<td>1</td>
</tr>
<tr>
<td>Nephrotic Syndrome</td>
<td>5</td>
<td>41.67</td>
<td>2</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>2</td>
<td>33.33</td>
<td>1</td>
</tr>
<tr>
<td>Diabetes</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hepatic Cirrhosis</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Thalassemia</td>
<td>1</td>
<td>8.33</td>
<td>1</td>
</tr>
<tr>
<td>Immunodeficiency</td>
<td>8</td>
<td>42.10</td>
<td>8</td>
</tr>
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</table>

Table IV. Comparison between results of 110 patients and normal adults

<table>
<thead>
<tr>
<th>Number</th>
<th>Cases</th>
<th>Age</th>
<th>Female</th>
<th>Male</th>
<th>Opsonization activity range</th>
<th>Mean ± 2 SD</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>Patients</td>
<td>1 Month-13 Years</td>
<td>44</td>
<td>66</td>
<td>0-80</td>
<td>47.2± 34.86</td>
<td>64± 18</td>
<td>11.26</td>
</tr>
<tr>
<td>50</td>
<td>Normal Adult</td>
<td>17-60 Years</td>
<td>26</td>
<td>24</td>
<td>46-82</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table V. Comparison between results of 110 patients and 112 normal children

<table>
<thead>
<tr>
<th>Number</th>
<th>Cases</th>
<th>Age</th>
<th>Female</th>
<th>Male</th>
<th>Opsonization activity range</th>
<th>Mean ± 2 SD</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>Patients</td>
<td>1 Month-13 Years</td>
<td>44</td>
<td>66</td>
<td>0-80</td>
<td>47.2± 34.86</td>
<td>55± 27</td>
<td>7.78</td>
</tr>
<tr>
<td>112</td>
<td>Children</td>
<td>11-12 Years</td>
<td>47</td>
<td>65</td>
<td>16-83</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Children. Bacteria which were isolated from blood, CSF and infectious sites were as follows: *S. aureus*, Pneumococci, *Haemophilus influenzae* type-b, *E-coli*, Klebsiella, Enterobacter, *Neisseria meningitidis*, *Salmonella para-A*.

**DISCUSSION**

Based on the results of the present investigation and other studies, opsonization is an important line of defense against extra-cellular organisms.

In this study, lower than normal opsonization activity with low C₃ levels was observed.

Based on other investigations (Kerr, et al., 1983) this is related to fibronectin. Defective opsonization in individuals with normal C₃ levels were observed also. This case has been shown to be a relatively common immunodeficiency (Soothill and Harvey, 1976; Johnson, 1980 a, b).

This hereditary deficiency occurs with a frequency of about one in 20 (5%) of normal population. Defective opsonization with normal C₃ levels were shown in diabetes; the cause of this defect is the reaction between glucose and opsonins (C₃b, FC of IgG) and their receptors on phagocytes.

**REFERENCES**