Immunologic Parameters in Down's Syndrome

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Abstract

Down’s syndrome children are known to have increased susceptibility to respiratory infections. Quantitative or qualitative differences in the various components of the immune system could account for increased susceptibility to infection involving the upper respiratory tract. In an effort to establish certain normal values and to determine if humoral immune abnormalities are associated with the chromosomal anomalies of Down’s syndrome, immunoglobulin levels, certain complement component levels, viral antibodies, hepatitis B surface antigen and milk precipitins from a population of inpatients and outpatients were compared with those of age, sex and race matched control populations.

It does not appear that the upper respiratory infections are associated with abnormally low levels of immunoglobulins or complement, with the possible exception of IgM. Both the inpatient and outpatient Down’s syndrome populations had decreased levels of IgM, indicating a possible relationship with the syndrome itself. In addition, the symptomatology does not seem to be due to IgE mediated atopic sensitivity. Hepatitis B surface antigen was found only in institutionalized Down’s syndrome patients, but it did not seem to be related to the other immune components studied.

Introduction

Children with Down’s syndrome (DS) are known to have an increased incidence of upper respiratory infections and a significant mortality rate owing to pneumonia. It has been postulated that these infections may be associated with an immunodeficiency or immunoincompetency. To determine whether or not a humoral immunodeficiency exists, DS serum immunoglobulins G, M and A have been quantitated by many investigators with somewhat conflicting results1, 5, 8, 9, 10, 17, 20, 23, 25 suggesting that more precisely selected DS and control populations should provide data for more definitive information. Alternatively, few studies have been carried out in which

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immunoglobulins D and E and complement components 3 and 4 have been quantitated. Evaluation of the humoral response in terms of residual antibodies to viral agents commonly responsible for upper respiratory infections is necessary to ascertain if a particular agent or agents are responsible for increased infections. Antibody titers to these agents could also disclose any trend toward immunoincompetency in this population. The present study compares immunoglobulin levels, complement levels and antibodies to certain respiratory agents in DS patients with age, sex and race matched control populations. It also compares the incidence of the hepatitis B surface antigen (HBsAg) and milk precipitins in the same populations since DS patients are reported to have a high incidence of both.

Materials and Methods

Sera were obtained from 26 karyotyped DS patients ranging in age from 8 to 35 years and 26 non-Down's patients with no known chromosomal abnormalities from the South Carolina Coastal Center in Ladson, SC. Sera were also obtained from 20 karyotyped DS children from one to nine years of age, who were outpatients of the Vince Moseley Clinic in Charleston, SC and from 20 children without known chromosomal abnormalities, some of whom had known atopic disease. Each DS patient had an age, race and sex matched control.

Immunoglobulins G, M and A and complement components 3 and 4 were quantitated by radial immunodiffusion utilizing the Mancini endpoint technique*.12 Immunoglobulin D was measured by the Fahey technique†.16 IgE determinations were made by radioimmunoassay.† All of the immunoglobulin and complement determinations were run in duplicate against known standards, and the mean value of the two determinations recorded. Duplicate samples with differences exceeding 10 percent of their mean value were repeated.

Milk precipitin reactions were carried out using the Ouchterlony double diffusion in agar technique. Serum samples were reacted with commercially obtained cow's milk and observed daily for 7 days for precipitation lines. HBsAg determinations were carried out using a modified gel diffusion technique.§ Serum samples were reacted with anti-HBs to detect the presence of the HBsAg. Plates were observed daily for 7 days for precipitation lines. The plates for both determinations were stained with 7.5 percent acetic acid to insure visibility of all precipitation lines.

Antibody titrations were performed on the following antigens¶ commonly associated with respiratory infections in pediatric populations: adenovirus, influenza A and B, parainfluenza 1, 2 and 3, respiratory syncytial, reovirus and Mycoplasma pneumoniae and to cytomegalovirus. All antibody determinations were done using the Laboratory Branch Complement Fixation Method (LBCF).15 Sera were diluted from 1:8 through 1:512 and retitrated using higher dilutions when necessary. A titer equal to or greater than 1:8 was considered seropositive.

The Paired Student t test was used to detect differences in mean values between patient and control groups for all quantitative measurements since each patient was matched with his own control. The Chi Square test was used to find differences in the frequency of response to the respiratory agents and to milk. It

* Behring Tri-Partigen and M-Partigen plates.
† Hyland Immuno Plates.
‡ Pharmacia Phadebas Radioimmunoassay Kit.
§ Abbott Austect Rheophoresis. Quantitation of HBsAg by RIA was not done owing to insufficient serum volume.
¶ Microbiological Associates.
### TABLE I
Serum Immunoglobulin Levels of Down's Syndrome and Control Populations (Based on 26 Inpatients and 20 Outpatients in Each Population)

<table>
<thead>
<tr>
<th>Immunoglobulins</th>
<th>In / Out Pts.</th>
<th>Down's Range</th>
<th>Mean</th>
<th>SE*</th>
<th>Control Range</th>
<th>Mean</th>
<th>SE</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA (mg/dl)</td>
<td>In</td>
<td>98-486</td>
<td>258.9</td>
<td>20.98</td>
<td>58-356</td>
<td>197.3</td>
<td>13.66</td>
<td>NS†</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>58-175</td>
<td>93.3</td>
<td>8.18</td>
<td>58-724</td>
<td>121.0</td>
<td>33.54</td>
<td>NS</td>
</tr>
<tr>
<td>IgM (mg/dl)</td>
<td>In</td>
<td>55-258</td>
<td>137.3</td>
<td>10.52</td>
<td>80-288</td>
<td>185.8</td>
<td>12.44</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>46-180</td>
<td>95.4</td>
<td>6.93</td>
<td>72-404</td>
<td>179.6</td>
<td>18.58</td>
<td>NS</td>
</tr>
<tr>
<td>IgG (mg/dl)</td>
<td>In</td>
<td>1120-2040</td>
<td>1534.2</td>
<td>50.53</td>
<td>560-1810</td>
<td>1130.8</td>
<td>55.11</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>63-1400</td>
<td>814.1</td>
<td>75.58</td>
<td>510-1590</td>
<td>821.5</td>
<td>59.37</td>
<td>NS</td>
</tr>
<tr>
<td>IgD (mg/dl)</td>
<td>In</td>
<td>4.1-12.0</td>
<td>7.2</td>
<td>0.44</td>
<td>0.3-10.0</td>
<td>4.3</td>
<td>0.49</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>4.1-12.0</td>
<td>5.3</td>
<td>0.64</td>
<td>0.3-10.0</td>
<td>4.8</td>
<td>1.06</td>
<td>NS</td>
</tr>
<tr>
<td>IgE (IU/ml)</td>
<td>In</td>
<td>20-3000</td>
<td>266.1</td>
<td>116.25</td>
<td>20-2600</td>
<td>597.7</td>
<td>160.88</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>10-250</td>
<td>76.0</td>
<td>17.24</td>
<td>20-860</td>
<td>428.5</td>
<td>65.14</td>
<td>P &lt; 0.005</td>
</tr>
</tbody>
</table>

*Standard error. †Not significant.

was also used to find differences in the frequency of the presence of the HBsAg.

### Results

In table I are shown the mean immunoglobulin levels for the DS patients and their respective controls. The DS inpatients were found to have IgM levels that were significantly lower than their controls; however, IgG and IgD levels were significantly higher than their control group. No significant difference was found between these groups for levels of IgA or IgE. The outpatients with DS were also found to have IgM levels that were significantly lower than their control group. DS outpatient IgE levels were also significantly lower than their controls which included atopic individuals. Levels for IgA, IgG and IgD in DS outpatients did not differ significantly from their control group.

The mean C3 and C4 levels for the DS patients and their matched controls can be seen in table II. DS inpatients were found to have C3 levels that were significantly lower than the control patients, while no difference was found between inpatient groups for levels of C4. No significant difference was found between the outpatient groups for levels of C3 and C4.

### TABLE II
Serum Complement Levels of Down's Syndrome and Control Populations (Based on 26 Inpatients and 20 Outpatients in Each Population)

<table>
<thead>
<tr>
<th>Complement Component</th>
<th>In / Out Pts.</th>
<th>Down's Range</th>
<th>Mean</th>
<th>SE*</th>
<th>Control Range</th>
<th>Mean</th>
<th>SE</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3 (mg/dl)</td>
<td>In</td>
<td>50-111</td>
<td>79.6</td>
<td>3.14</td>
<td>63-202</td>
<td>92.1</td>
<td>5.70</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>56-115</td>
<td>81.0</td>
<td>3.45</td>
<td>50-118</td>
<td>91.0</td>
<td>3.95</td>
<td>NS†</td>
</tr>
<tr>
<td>C4 (mg/dl)</td>
<td>In</td>
<td>14-52</td>
<td>32.3</td>
<td>1.70</td>
<td>15-60</td>
<td>33.0</td>
<td>2.01</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>21-50</td>
<td>34.5</td>
<td>1.64</td>
<td>28-58</td>
<td>38.5</td>
<td>2.14</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Standard error. †Not significant.
The HBsAg was detected in 15.4 percent of the DS inpatient population, while none was found among the matched controls. The HBsAg was not found in any of the DS outpatients or their control group. The incidence of milk precipitins found in the DS inpatients and outpatients was 11.5 percent and 5.0 percent, respectively, while both control populations were negative.

When the seropositivity of the DS patients and the control patients to common respiratory agents and cytomegalovirus was compared, the total control group was seropositive to the parainfluenza 3 virus more often (p < 0.05) than the DS group (39 of 46 compared with 28 of 44, respectively). Respiratory syncytial virus antibodies in the DS inpatients were found significantly more often (p < 0.05) than in the control inpatients (12 of 25 compared with 4 of 26, respectively). No significant differences were found between the groups for any of the other infectious agents. Also, within the four groups, levels of antibody titers to the various agents were not significantly different.

Discussion

It does not appear that the upper respiratory symptoms so common in DS patients are due to extremely low levels of immunoglobulins, C3 or C4, the inability to respond to certain viral infections or immediate hypersensitivity. However, IgM was decreased for both the inpatient and outpatient DS populations indicating a possible relationship with the syndrome itself. This deficiency could be due to several possibilities including decreased production or half life of the μ chains necessary for IgM synthesis. It is not thought that the IgM produced is defective since these individuals are known to exhibit normal isoagglutinins16 and to produce a primary humoral response to antigenic stimulation7,19 both of which are primarily of the IgM class. The increased levels of IgG found in the institutionalized DS patients support the findings of Sutnick et al.23 who suggested that this increase is a response to persistent infection common to DS institutionalized populations. This increase may also be a compensation for the low levels of IgM as proposed by Stiehm and Fudenberg29.

Serum levels of IgA were found to be normal in both DS populations. However, since these children have chronic respiratory infections, secretory IgA levels could be of more significance. This form of the antibody is one of the primary means of defense in the respiratory and gastrointestinal tracts. Increased levels of IgD were found in the institutionalized DS group which is in agreement with the work of Rundle et al.18 Since little is known about IgD, and its role in the body’s defense has not been firmly established, the significance of this increase remains unknown at present.

It does not seem probable that IgE synthesis is directly related to the chromosomal abnormality since IgE levels were the same or lower than the control populations suggesting that the upper respiratory symptoms were not the result of immediate hypersensitivity. The mean IgE level for the inpatients was not significantly lower than the controls; however, only one DS inpatient had an IgE level greater than 800 IU per ml while the control group had six individuals exceeding this level. If this one DS patient is excluded, the mean value is reduced to 159.3 IU per ml which is significantly lower than the control group (p > 0.05). These findings are in agreement with those of Lopez11 in his study on an institutionalized DS population. All of the immunoglobulins measured, except IgD in the control group, were higher in the inpatient populations further supporting the theory of Sutnick et al.23 of increased immune responses in institutionalized DS populations owing to persistent infections.
Complement should be assessed on patients with recurrent infections, especially if antibodies appear to be normal. C3, which is the complement component present in largest amounts in the serum and generally reflects the total complement activity, is often decreased in immune complex and autoimmune diseases. C4 is present in lower concentrations than C3 and may be used as a more sensitive indicator of complement consumption. C4 is also involved in the classical antigen-antibody activation of the complement pathway but not the properdin or alternate complement pathway. In certain disease states, depression of C4 concentration may precede and is often more marked than depression C3. Agarwal et al.,2 did not find levels of C3 and C4 in DS inpatients to be significantly different from healthy controls.

In the present study C4 levels did not differ significantly from control populations. Although decreased C3 levels were found in the DS inpatients which were significantly different from their controls, these data do not seem to be of critical clinical significance since these levels of C3 approximate the lower limits of the normal range. In this investigation the mean C3 level of the DS inpatients was 79.6 mg per dl while that of Agarwal’s was 74.3 mg per dl. It does not seem, therefore, that these two major components of the complement system are quantitatively involved in the increased susceptibility of DS individuals to upper respiratory infections. The question as to functional capability of complement remains unanswered owing to our inability to freeze the sera before the complement was inactivated.

Precipitating serum antibodies to milk are found in one to two percent of the general population.4 Children with these antibodies generally exhibit several clinical features including chronic respiratory disease. McCrea et al.13 found these antibodies in 34 percent of DS individuals. In the present study, milk precipitins were found in 8.7 percent of the DS patients which is somewhat lower than that reported by McCrea et al.;13 however, these data do support the findings that there is an increased incidence of this antibody in the DS population. Buckley and Dees4 reported a correlation between serum IgA deficiency and an increased incidence of milk precipitins. Serum IgA levels in DS individuals displaying these antibodies, however, were all in the upper range of the accepted normal limits for the appropriate age of the patients indicating no correlation in the DS patients between IgA deficiency and the presence of milk precipitins.

The increased frequency of the HBsAg found in the DS inpatients (15.4 percent) can best be explained by the hypothesis3,21,22,24 that individuals homozygous for the allele Au(1) are more susceptible to the HBsAg while those homozygous for the alternate allele Au and heterozygotes are less or transiently susceptible to this antigen. Since the frequency of finding Au(1) is very low in normal individuals and relatively high in certain types of leukemia, Blumberg et al.3 have suggested that individuals with this trait may be more susceptible to leukemia than those without it. Approximately 10 to 30 percent of DS inpatients exhibit this antigen in their serum, and there have been many reported cases of DS children developing leukemia.3,21,22,28 In this study, the fact that increased frequencies of this antigen were found only in inpatients is probably due to the increased risk of exposure in large institutions.

Since, within the four populations studied, seropositivity to the various respiratory agents was not significantly different, this decreases the likelihood that a single virus or group of viruses is responsible for the increased respiratory infections most often seen in DS children.
However, continuing studies on cell mediated immunity in these populations may shed further light on the relationship of viral infections to DS.

Acknowledgments

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References


