Selective IgM Deficiency: Functional Assessment of Peripheral Blood Lymphocytes in Vitro

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The functional aspects of the peripheral blood lymphocytes from 6 patients with primary selective IgM deficiency (slgMD) were analyzed to elucidate its pathogenesis. The surface IgM positive B cells were present at almost the same percentage as in controls, but the percentage of cluster of differentiation (CD)4+ T cells was higher and that of CD8+ T cells was low. The patient B cells showed a significantly lower proliferative response to Staphylococcus aureus Cowan strain I (SAC) than control B cells and did not produce a significant amount of IgM when co-cultured with control T cells. Interestingly, mitomycin C (MMC)-treated patient T cells induced a greater amount of IgM production by control B cells. In addition, patient B cells treated with SAC and B cell differentiation factors (BCDF) failed to secrete IgM. These results suggest that the pathogenesis of slgMD may be mainly due to an intrinsic defect in B cell maturation.

(Key words: immunodeficiency, helper T cells, B cell proliferation, B cell differentiation)

Introduction

Primary selective IgM deficiency (slgMD) has been reported to be rare, with an incidence of less than 0.03% among the general population (1) and 1% in hospitalized patients (2). But the recent availability of immunological analyses has facilitated further testing which has indicated that this disorder is more common than previously thought. Patients with malignant neoplasias (3, 4), autoimmune diseases (5, 6), infections (7) or those given immunosuppressive agents may develop slgMD; the incidence of slgMD is about 2% (8). Most patients with slgMD typically suffer from severe refractory or recurrent infections, however about 20% of the patients have no symptoms (8). Of these asymptomatic patients, most are adults and their low serum level of IgM is found incidentally during hospitalization for other unrelated diseases. The pathogenetic abnormalities in slgMD have been shown to be in B cell dysfunction (9) or T cell abnormality (10, 11) as analyzed by in vitro immunological examinations.

Here, we report six adult patients with primary slgMD. The surface antigens on peripheral lymphocytes were analyzed and B cell and T cell function was examined in detail. The pathogenesis of this disease is discussed.

Materials and Methods

Patients. All patients (5 males and 1 female; mean age 43 years; range 22–57) had a persistently low level of serum IgM. None of the patients had hematological diseases, autoimmune diseases, renal dysfunction or had received immunosuppressive agents or other drugs such as gold or salazosulfapyridine. Also the cell count of peripheral lymphocytes of each patient was within normal limits. All serum samples were drawn during infection-free periods. Six healthy subjects with a normal level of serum IgM were used as controls in this study.

Measurement of serum immunoglobulins. Serum IgM, IgG, IgA and IgD were measured by a standard radial immunodiffusion method and serum IgE was measured by competitive radioimmunoassay.

Cell preparation. Mononuclear cells (MNC) from heparinized peripheral venous blood of the patients and healthy controls were isolated by centrifugation on Ficoll-Conray (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient. MNC were separated into T cells and non-T cells by a rosetting method with s-(2-aminoethyl)isothiouronium bromide hydrobromide (AET: Sigma Chemical Co., St. Louis, MO)-treated sheep red blood cells (RBC). Then, non-T cells were incubated on plastic
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Proliferative response. T or B cells suspended in 200 μl of culture medium were incubated in 96-well plates at a density of 1 × 10^6 cells/ml with 1% of phytohemagglutinin-P (PHA-P: Difco Laboratories, Detroit, MI), 10 μg/ml of concanavalin A (Con A: Miles Yeda Ltd, Israel) or 0.0025% of Staphylococcus aureus Cowan strain I (SAC: Pansorbin; Calbiochem Behring Diagnostics, La Jolla, CA) for 48 hours at 37°C. And then they were pulsed with 1 μCi of [³H] thymidine (New England Nuclear, Boston, MA) for 18 hours. The cells were harvested on a MASH II automated harvester and their incorporation of [³H] thymidine was measured by standard liquid scintillation counting techniques. The results are shown as the arithmetic mean number of plaque-forming cells (PFC).

Serum Ig levels
As shown in Table 1, all patients had a low level of serum IgM, which was not normalized after they had been clinically improved. The serum levels of IgG and IgA were normal in all patients, but two patients (TN & KT) had a remarkably high level of serum IgE. Four had infections and two had diabetes mellitus and one was asymptomatic (Table 1).

Cell surface markers on lymphocytes
Analysis of surface antigens on peripheral lymphocytes revealed no difference in the distribution of CD20 and CD2 positive cells between patients and controls. The peripheral blood of patients showed a significant increase in the percentage of CD20 positive cells (910 ± 147 cells/ml, p<0.01) and a reduction of the percentage of CD8 positive cells (293 ± 53 cells/ml, p<0.05) in the peripheral blood of patients compared with that of controls (Table 2).

Proliferative response
The proliferative response of peripheral T and B cells stimulated with mitogens is shown in Fig. 1. Treatment with PHA-P or Con A induced proliferation of patient T cells at almost the same level as that of controls, but the proliferative response of patient B cells to SAC was markedly lower (Fig. 1) than that of controls (10,978 ± 1,249 cpm; mean ± SD).

T cell-dependent Ig production
We examined the capacity of Ig production by patient B cells in the presence of PWM (1%). These B cells produced only one-third to one-tenth of the control level of IgM when cultured with autologous T cells (Fig. 2, open column of R of each patient), and one-fourth to two-thirds of the control level of IgM when cultured with normal T cells (Fig. 2, open column of Q 1.0 - 1.2 mg/ml) which were treated with or without MMC for 30 minutes in the presence of 1% of pokeweed mitogen (PWM: GIBCO, Grand Island, NY). They were incubated in 96-well microtiter plates for 6 days, and immunoglobulin (Ig)-producing cells were enumerated by a reverse plaque assay with protein A (Sigma Chemical Co., St. Louis, MO) as previously reported (15). Briefly, after 6 days an appropriate dilution of cultured cells was added in duplicate to a reaction mixture containing a 10% suspension of protein A-coupled sheep RBC and 0.6% agarose. The mixture was spread onto an agarose-coated glass slide and these slides were flooded with a 1:100 dilution of rabbit anti-human IgM, IgG or IgA antiserum and then incubated at 37°C. This was followed by flooding with a 1:25 dilution of guinea pig complement and further incubation. The results are shown as the arithmetic mean number of plaque-forming cells (PFC).
Table 1. Patient Profile

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>IgM</th>
<th>IgG</th>
<th>IgA</th>
<th>IgD</th>
<th>IgE</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 TN</td>
<td>50</td>
<td>M</td>
<td>18</td>
<td>1,534</td>
<td>283</td>
<td>≤1.0</td>
<td>2,000</td>
<td>cholangitis, gout, liver abscess, dermatitis</td>
</tr>
<tr>
<td>2 MU</td>
<td>57</td>
<td>M</td>
<td>6</td>
<td>1,413</td>
<td>288</td>
<td>0</td>
<td>59</td>
<td>diabetes mellitus</td>
</tr>
<tr>
<td>3 TS</td>
<td>22</td>
<td>M</td>
<td>32</td>
<td>2,127</td>
<td>245</td>
<td>2.5</td>
<td>394</td>
<td>Streptococcal infection, psoriasis pustulosa, chronic tonsillitis, bronchitis</td>
</tr>
<tr>
<td>4 TF</td>
<td>34</td>
<td>M</td>
<td>1</td>
<td>1,314</td>
<td>168</td>
<td>ND</td>
<td>ND</td>
<td>diabetes mellitus, polyarthritis</td>
</tr>
<tr>
<td>5 KT</td>
<td>57</td>
<td>M</td>
<td>0.4</td>
<td>1,747</td>
<td>462</td>
<td>2.5</td>
<td>2,000</td>
<td>asymptomatic (healthy)</td>
</tr>
<tr>
<td>6 MT</td>
<td>37</td>
<td>F</td>
<td>34</td>
<td>1,446</td>
<td>340</td>
<td>ND</td>
<td>ND</td>
<td>ND: not done.</td>
</tr>
</tbody>
</table>

Normal ranges: IgM: 60–280 mg/dl, IgG: 800–1800 mg/dl, IgA: 90–450 mg/dl, IgD: 0–15 mg/dl, IgE: <400 μ/ml.

Table 2. Analysis of Surface Markers on Peripheral Lymphocytes of Patients

<table>
<thead>
<tr>
<th></th>
<th>TN</th>
<th>MU</th>
<th>TS</th>
<th>TF</th>
<th>KT</th>
<th>MT</th>
<th>mean ±SD</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD20 cell (%)</td>
<td>15.0</td>
<td>19.7</td>
<td>12.8</td>
<td>13.0</td>
<td>10.6</td>
<td>10.3</td>
<td>13.6 ± 3.5</td>
<td>14.7 ± 5.8</td>
</tr>
<tr>
<td>IgG (%)</td>
<td>1.0</td>
<td>4.0</td>
<td>ND</td>
<td>5.0</td>
<td>2.0</td>
<td>3.0</td>
<td>3.0 ± 1.6</td>
<td>2–12</td>
</tr>
<tr>
<td>IgM (%)</td>
<td>6.0</td>
<td>15.0</td>
<td>ND</td>
<td>7.0</td>
<td>6.0</td>
<td>12.0</td>
<td>9.2 ± 4.1</td>
<td>6–14</td>
</tr>
<tr>
<td>IgA (%)</td>
<td>2.0</td>
<td>3.0</td>
<td>ND</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.6 ± 0.9</td>
<td>1–5</td>
</tr>
<tr>
<td>IgD (%)</td>
<td>8.0</td>
<td>7.0</td>
<td>ND</td>
<td>2.0</td>
<td>3.0</td>
<td>5.0</td>
<td>5.0 ± 2.5</td>
<td>5–12</td>
</tr>
<tr>
<td>x (%)</td>
<td>3.0</td>
<td>9.0</td>
<td>ND</td>
<td>5.0</td>
<td>2.0</td>
<td>10.0</td>
<td>5.8 ± 3.6</td>
<td>8–18</td>
</tr>
<tr>
<td>λ (%)</td>
<td>6.0</td>
<td>7.0</td>
<td>ND</td>
<td>4.0</td>
<td>4.0</td>
<td>3.0</td>
<td>4.8 ± 1.6</td>
<td>5–9</td>
</tr>
<tr>
<td>CD2 cell (%)</td>
<td>79.1</td>
<td>73.7</td>
<td>80.4</td>
<td>80.2</td>
<td>73.4</td>
<td>79.7</td>
<td>77.8 ± 3.3</td>
<td>74.7 ± 5.8</td>
</tr>
<tr>
<td>CD4 cell (%)</td>
<td>43.9</td>
<td>56.2</td>
<td>57.5</td>
<td>48.4</td>
<td>47.4</td>
<td>53.6</td>
<td>51.2 ± 5.7*</td>
<td>37.6 ± 5.8*</td>
</tr>
<tr>
<td>CD8 cell (%)</td>
<td>19.3</td>
<td>16.0</td>
<td>14.3</td>
<td>21.7</td>
<td>15.2</td>
<td>13.4</td>
<td>16.7 ± 3.2**</td>
<td>28.7 ± 7.0**</td>
</tr>
<tr>
<td>CD4/CD8</td>
<td>2.3</td>
<td>3.5</td>
<td>4.0</td>
<td>2.2</td>
<td>3.1</td>
<td>4.0</td>
<td>3.2 ± 0.8**</td>
<td>1.5–2**</td>
</tr>
</tbody>
</table>

* p < 0.01, ** p < 0.05, ND: not done, CD: cluster of differentiation.

Fig. 1. Proliferative response of peripheral B and T lymphocytes of patients with primary selective IgM deficiency (slgMD). B cells were stimulated with 0.001% SAC (2) and T cells with 0.1% PHA-P (2) or 10 μg/ml of Con A (2). The response is shown as the stimulation index compared with that of the control described in Materials and Methods. Con A: concanavalin A, PHA-P: phytohemagglutinin-P, SAC: Staphylococcus aureus Cowan strain I.

Fig. 2. IgM synthesis in PWM-driven MLC with (2) or without (2) mitomycin-C treated T cells. Control B cells (Be) were cocultured with control T(Tc) or patients’ T(Tp) cells in the presence of PWM (1%) and patients’ B cells (Bp) were cocultured with patients’ T(Tp) or control T(Tc) cells in the presence of PWM (1%). P: Be + Tp, Q: Bp + Tc, R: Bp + Tp, control: Be + Tc, Be: control B cells, Bp: patients’ B cells, Tc: control T cells, Tp: patients’ T cells, MLC: mixed lymphocyte culture, PWM: pokeweed mitogen.
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Fig. 3. In vitro immunoglobulin production by B cells from patients stimulated with SAC (0.0025%) and BCDF (1 U/ml). The results are shown as IgM (●), IgG (▲) and IgA (△) PFC. BCDF: B cell differentiation factors, PFC: plaque-forming cells, SAC: Staphylococcus aureus Cowan strain 1.

of each patient). However, there was no difference in IgG and IgA production between patient and control B cells under the same conditions (data not shown). On the other hand, IgM production by the control B cells cocultured with patient T cells was similar to the control (Fig. 2, open column of P of each patient).

IgM production in the absence of suppressor T cells
To further investigate the failure of IgM production by patient B cells, the suppressor function of CD8+ T cells was inhibited by treatment with MMC as described in detail elsewhere (13, 14). Interestingly, the patient T cells treated with MMC induced much more IgM secretion by control B cells than the untreated patient T cells (Fig. 2, closed column of P of patients 1, 2, 4 and 5). This result suggests that the helper function of T cells in patients with slgMD may possess a higher activity than that of controls, because of the low level of serum IgM.

T cell-independent Ig production
The next examination was designed to investigate B cell function without the influence of T cells. As shown in Fig. 3, patient B cells failed to secrete a comparable amount of IgM after stimulation with SAC and BCDF. In contrast, patient B cells did produce a significant amount of IgG and IgA under the same conditions (Fig. 3). This result suggests that there may be a selective defect in patient B cells during B cell maturation into IgM-producing cells.

Discussion
We report here the in vitro functional analysis of the peripheral blood lymphocytes from six patients with primary slgMD. Surface IgM positive B cells were present at a normal level as previously reported (9, 11). In contrast, there was a significant increase in the percentage of CD4+ positive cells (p < 0.05). Although an increase of the CD4/CD8 ratio is reported in many diseases such as rheumatoid arthritis and autoimmune hemolytic anemia (16), no patients examined in this study had these diseases. There might be a possibility that an increase in CD4+ T cells is the result of hyperfunction of helper T cells to restore a low level of serum IgM, because patient T cells treated with MMC induced an increase in the IgM production by normal B cells (Fig. 2).

Interestingly, two patients had a remarkable elevation of serum IgE levels (patient TN and KT). There are some reports of cases with an elevated serum IgE level (17–19). These patients simultaneously had chronic or atopic dermatitis and recurrent skin abscesses caused by Staphylococcus aureus. The B cells of the present patients showed a significantly low proliferative response to SAC. Thus, a high level of serum IgE may be due to chronic infection or hyper-reactivity to this bacterium (20) due to a lack of antibody production.

We showed that the defect of IgM production of patients with slgMD was the result of the failure of B cell differentiation into IgM secretion as reported previously (9, 21). In contrast to these findings, there are some reports which propose that a lack of helper function of CD4+ T cells (18) or excessive activity of CD8+ T cells (11) is the primary factor in slgMD. Thus, the pathogenesis of this disease may be heterogenous as well as selective IgA deficiency (22). However, there has been no report of detailed analyses of B cell function in slgMD without T cell help. In the present study, we used SAC and BCDF to stimulate B cells to produce Ig without the influence of T cells. Patient B cells did not secrete a significant amount of IgM when treated with SAC and BCDF, although they produced a marked quantity of IgG and IgA under the same conditions as shown in Figure 3. From the present findings, the pathogenesis of slgMD may be an intrinsic B cell abnormality during maturation into IgM-secreting cells. Although the proliferative response of patient B cells to SAC was significantly decreased compared with that of controls (Fig. 1), it seems unlikely that this hypo-responsiveness caused the defect of IgM production because a sufficient amount of IgG and IgA were produced after treatment with SAC and BCDF (Fig. 3).

Patients with slgMD are reported to have a defect in IgM antibody production to some antigens and to have a high titer of IgG isohemagglutinins as substitutes for IgM antibody due to low IgM production (17). We have not examined the serum level of IgM antibodies of patients to E. coli polysaccharide and specific antigens such as cold hemagglutinin and anti-blood group. But these patients had a normal level of antibodies of the IgG class against various types of viruses and bacteria (data not shown).

Recent molecular and genetic investigations suggest
that Ig deficiency with an isolated form may result from an abnormality of B cell function. This abnormality is thought to be a failure of the transcription or RNA splicing at class switch rather than a defect of Ig heavy chain C gene (23). It has been reported that the lymphocytes from 85 patients with selective IgA deficiency had the abnormality of the class switching regulatory gene (23), and there was no defect of the C\textsubscript{\textalpha}_{1} or C\textsubscript{\textalpha}_{2} gene (24). From the present data showing that patient B cells used in this study normally express surface IgM on the cell membrane, it is suggested that B cells with slgMD have an intact Ig heavy chain C\textmu gene. Taken together, the pathogenesis of slgMD may be mainly due to B cell dysfunction during the maturation process into IgM-producing cells. A study of the function of the patient B cells at the molecular level by Northern blot analysis is now underway. Further examinations using lymphocytes of patients with slgMD will be necessary to establish the pathogenesis of this disease.

References