Analysis of B Cell Dysfunction in Patients with Common Variable Immunodeficiency by Using Recombinant Interleukin 2

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ARIGA, T., OKANO, M., TAKAHASHI, Y., SAKIYAMA, Y. and MATSUMOTO, S. Analysis of B Cell Dysfunction in Patients with Common Variable Immunodeficiency by Using Recombinant Interleukin 2. Tohoku J. exp. Med., 1987, 152 (1), 53-61 — Recombinant interleukin 2 (RIL2) induces proliferation and differentiation of the Staphylococcus aureus Cowan I (SAC)-activated normal B cells to immunoglobulin (Ig) producing cells. We applied this finding to an analysis of heterogeneity in the differentiation states of B cells in patients with common variable immunodeficiency (CVI). B cells from 5 of 7 patients with CVI were tested for their ability to proliferate under the stimulation of SAC, or SAC and RIL2, and for their differentiation to Ig producing cells in the presence of SAC plus RIL2. The results suggest that the differentiation status of B cells in CVI could be divided into four states based on their responses to SAC and RIL2. In the first state, no B cell proliferation or differentiation was demonstrated in our assays. B cells in the second state showed normal proliferative responses to SAC, but not to SAC plus RIL2, and no differentiation to Ig-secreting cells. The third state showed normal proliferation in response to SAC and SAC plus RIL2, but no differentiation to Ig-secreting cells. The fourth state had a normal proliferation response to SAC and SAC plus RIL2, and normal secretions of IgG and IgM in response to SAC and RIL2. These results show that some B cells in CVI have defects in response to RIL2, and at least in terms of defect of B cells, CVI is heterogeneous.

It has been shown that one of the ways B cells produce immunoglobulin (Ig) is a process of activation, proliferation and differentiation of resting B cells to Ig secreting cells (Kishimoto et al. 1984). Many cytokines have been reported to transmitting signals for the proliferation and/or differentiation of B cells, and they are named B cell growth factor (BCGF) or B cell differentiation factor (BCDF) and so on. Recently complete purification and synthesis of some of these

Received January 14, 1987; accepted for publication March 19, 1987.
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cytokines have been achieved by the technique of gene rearrangement (Hirano et al. 1986; Kinashi et al. 1986; Noma et al. 1986). On the other hand, some reports have shown that recombinant interleukin 2 (RIL2), a transcription product of a cloned cDNA for IL2, exerts a direct effect on B cell function (Mingani et al. 1984; Ralph et al. 1984; Romagnani et al. 1986).

Common variable immunodeficiency (CVI) is a primary immunodeficiency disease that is characterized by hypogammaglobulinemia involving all immunoglobulin classes with normal or decreased number of circulating surface Ig⁺ (sIg⁺) cells, and which causes recurrent infections of the respiratory tract and gastrointestinal problems. Although intrinsic B cell defects have been postulated to be one of the primary pathogenetic factors in the disease (Siegal et al. 1978; WHO Scientific Group on Immunodeficiency 1986), not many precise analysis of B cell defects in the activation and differentiation process, especially using purified lymphokine, has been reported.

In the present study, we examined the effects of RIL2 on Staphylococcus auerus Cowan I (SAC)-activated normal B cells and attempted to analyze the heterogeneity of B cell dysfunctions in the patients with CVI using this system.

**Materials and Methods**

*Patients*

Seven patients with CVI were studied. The clinical parameters of these patients are listed in Table 1. As they received treatment by intravenous gammaglobulin at 2-3 weeks intervals, the level of serum Ig shown in Table 1 are the minimum values shown before the initial replacement therapy was started. In 2 patients (patient 6, 7) the number of circulating sIg⁺ cells were too small to be used in the study.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (year)</th>
<th>Sex</th>
<th>Serum Ig (mg/100 ml)</th>
<th>Lymphocyte subsets (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgG</td>
<td>IgA</td>
</tr>
<tr>
<td>CVI 1</td>
<td>5</td>
<td>M</td>
<td>350</td>
<td>&lt;5</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>M</td>
<td>327</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>F</td>
<td>180</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>M</td>
<td>228</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>M</td>
<td>330</td>
<td>56</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>M</td>
<td>165</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>M</td>
<td>120</td>
<td>10</td>
</tr>
<tr>
<td>Normal range value</td>
<td>708</td>
<td>65</td>
<td>49</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>1560</td>
<td>377</td>
<td>223</td>
<td>75.0</td>
</tr>
</tbody>
</table>

Lymphocyte subsets were determined by flow cytometry analysis or fluorescent microscopy.
**Analysis of B Cell Dysfunction in CVI**

**B cell separations**

Heparinized venous blood from patients and normal controls was obtained and incubated with silica suspension at 37°C for 1 hr to deplete monocytes. Then the monocyte-depleted mononuclear cells were centrifuged on Ficoll-Conray gradients and separated into sheep red blood cells (SRBC) rosette-positive (E+) and-negative (E-) cells, by using 2-aminoethylisothiouronium (AET)-treated SRBC. E+ cells were further treated with monoclonal antibody Leu 1 (Becton-Dickinson Monoclonal Center, Inc., Mountain View, CA, USA) and OKM1 (Ortho Diagnostic Systems, Inc., Raritan, NJ, USA) plus rabbit complement (Pel-Freez Biologicals, Rogers, AR, USA) to obtain B cell enriched populations. These B cell-enriched suspensions contained more than 70% surface Ig+ cells, less than 3% of E+ and less than 5% of peroxidase positive cells. Proliferation of these cells in response to phytohemagglutinin (PHA)-P (Difco Laboratories, Detroit, MI, USA) was not detected.

**B cell mitogen and lymphokines**

SAC was obtained from Zyland Laboratories Inc. (CA, USA) and used at 0.005% (v/v). Recombinant γ-interferon (Rγ IFN) and RIL2 were kindly provided by Shionogi Co., Ltd. (Tokyo). Crude T cell replacing factor (cTRF) was prepared according to the method of Kuritani (Kuritani and Cooper 1983) and used at a dose of 25%.

**Assays of B cell function**

*Proliferation in response to SAC.* B cells (5 × 10^4/well) were cultured in triplicate in 0.2 ml of complete medium composed of RPMI 1640 medium (GIBCO, Grand Island, NY, USA) supplemented with 10% fetal calf serum (Flow Laboratories, Inc., McLean, VA, USA), 100 U/ml penicillin and 100 μg/ml kanamycin in a U-shaped microplate (76-013-05 Flow Laboratories). For the proliferation assays, SAC was added to the wells and the cells were incubated for 3 days at 37°C in humidified 5% CO₂/95% air. 1 μ Ci of [³H]-thymidine (New England Nuclear, Boston, MA, USA) was added for the last 16 hr. Then the cells were harvested, and the incorporations of [³H]-thymidine were measured by standard liquid scintillation counting techniques.

*Assay for the SAC-activated B cells in response to lymphokines.* For the proliferation assay, RIL2, Rγ IFN and cTRF were respectively added to SAC-activated B cells in triplicate and cultured for another 3 days. Then [³H]-thymidine was added for the last 16 hr and incorporations of [³H]-thymidine was measured. For differentiation assay to Ig secreting cells, B cells (1 × 10^5/ml) were cultured in complete medium in culture tube (2058, Falcon Labware, Oxnard, CA, USA) with SAC and lymphokines for 7 days. For the determination of Ig production, the culture supernatants were stocked at −20°C and the amount of Ig in them was measured by an enzyme-linked immunosorbent assay (ELISA) (Voller et al. 1976).

**RESULTS**

*Effect of lymphokines on proliferation of SAC-activated control B cells*  

RIL2 and cTRF augmented DNA synthesis of SAC-activated control B cells in 6-day culture (Fig. 1). We used RIL2 at the dose of 10 U/ml in experiment of proliferation, as 1 U/ml had little effect on proliferation of SAC-activated B cells, and more than 10 U/ml of RIL2 revealed to have the effect as much as 10 U/ml. Rγ IFN had no effects on resting or SAC-activated control B cells, even if it was added with RIL2 or cTRF at various doses (1-1000 U/ml).
Effect of lymphokines on Ig secretion in SAC-activated control B cells

RIL2 and cTRF induced SAC-activated B cells to secrete Ig, but had no effects on resting B cells. In this experiment we took the dose of 100 U/ml of
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RIL2, because the maximum response was shown in this dose. Various doses (1-1000 U/ml) of Rγ IFN showed no effects on Ig secretion using a combination of other lymphokines.

**Analysis of B cell function of patients with CVI**

It was suggested that RIL2 and cTRF had an effect on proliferation and differentiation to Ig secreting cells in SAC-activated B cells. Using this assay system, we examined the following B cell functions in the patients: proliferation in response to SAC, proliferation in response to RIL2 of SAC-activated B cells and Ig secretion by SAC plus RIL2. The B cells of patient 1 showed no proliferative response to SAC or SAC plus RIL2 and no Ig secretion by SAC plus RIL2. Patient 2 showed low but significant response to SAC, but not SAC plus RIL2 and no Ig secretion. Patients 3 and 4 showed normal proliferative response, but no
secretion of Ig. Patient 5 showed a normal response of differentiation and proliferation in this assay system.

**DISCUSSION**

We used RIL2 and Rγ IFN, as a single lymphokine, for a functional analysis of B cell in order to make simple and clear the assay system, and we showed that RIL2 had an effect on proliferation and differentiation to Ig secreting cells in the SAC-activated B cells, but that Rγ IFN had no effect. Nakagawa et al. (1985) reported that RIL2 induced proliferation but no production of Ig in the SAC-activated B cells, and that Rγ IFN together with RIL2 was required to obtain optimal Ig production. The difference between our results and theirs might be attributed to the different assay system or to the purification of B cells. We used whole SAC-activated B cells, but in some of their experiments, they used SAC-activated B cell blasts separated by percoll gradient methods, which were not practical to apply because we could not get so much blood sample from the patients. Recently Nakagawa et al. (1986) showed in some Tac-positive EB virus transformed B cell line, combination of RIL2 and Rγ IFN was necessary for induction of the optimal Ig secretion. But on the other hand, there have been reports that RIL2 could augment Ig production in activated B cells as shown by our results (Ralph et al. 1984; Emmrich et al. 1985; Muraguchi et al. 1985; Romagnani et al. 1986), and that RIL2 could augment Ig production in some B cell lines (Tomita et al. 1985). In our system, we must consider about the effects of contaminated T cells and NK cells (Handy et al. 1982). So we used OKM1 monoclonal Ab to deplete NK cells and monocytes. And we thought the effect of contaminated T cells was negligible as purified B cells did not proliferate in response to PHA-P.

Since these findings were applicable to analyze the heterogeneity of B cell dysfunctions of CVI, we examined the function of B cells of 5 patients with CVI using our assay system. Our results showed that the functional status of B cells

<table>
<thead>
<tr>
<th>State</th>
<th>Patient</th>
<th>DNA synthesis by SAC</th>
<th>DNA synthesis by SAC + RIL2</th>
<th>Ig production by SAC + RIL2</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>IV</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Others*</td>
<td>6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* B cell number in peripheral blood was too little to exam. ND, not done.
in the patients with CVI could be classified into four states (Table 2). The first state of B cell (patient 1) did not show any response in our assay system, although he had a normal amount of circulating sIg+B cells in the peripheral blood. It is suggested that the B cells of this state had an intrinsic defects in the mechanisms synthesizing DNA by stimulation of SAC. The B cells of the second state (patient 2) respond low but significant in proliferation to SAC, but not to SAC plus RIL2, or did not differentiate into Ig producing cells in the presence of SAC plus RIL2. It was reported that SAC-activated B cells acquired functional IL2 receptors (Tsudo et al. 1984; Waldmann et al. 1984). This type of defect might be present in the expression of IL2 receptor by SAC or IL2 receptors themselves. The B cells of the third state (patients 3, 4) proliferated well in response to SAC and SAC plus RIL2, but did not differentiate into Ig secreting cells by SAC plus RIL2. The SAC-activated B cells of these patients might be able to express IL2 receptors and proliferate by binding of RIL2 to IL2 receptors. This type of defect may be present in the process of differentiation from proliferation or in the process of Ig secretion. The B cells of the fourth state (patient 5) responded as well as the control B cells in all of our assays. We could not find any difference between the B cells of the fourth state or those of controls. As excessive suppressor T cell function was revealed in this patient by using pokeweed mitogen system (data was not shown), we postulated that the patient 5 had CVI with excessive suppressor T cell and normal B cell function.

Saiki et al. (1984) reported three stages of B cell defects by a functional assay using anti-μ, SAC and conventional T cell factor. One of their groups showed no proliferation in response to anti-μ or SAC, like our patient 1. One of their other groups showed proliferative response to anti-μ and SAC, but no differentiation to Ig secreting cells by SAC plus T cell factor. Our experiment indicated that this type of defects could be classified further into two group by the result of proliferation in response to SAC plus RIL2. Their third group showed proliferation to anti-μ and SAC and differentiation into only IgM secreting cells. Non of our patients fit into this type, and our fourth state (patient 5) was not present in their study.

We also used cTRF and compared it to the effects of RIL2. No difference between cTRF and RIL2 on SAC-activated B cells was detected in either the patients or controls. Mayer et al. (1984) showed different response patterns in B cells of CVI to different factors derived from cloned T cell lines. In our 5 cases, no different response was seen between RIL2 and cTRF. CVI is a heterogenous disorder which is characterized by defects in B cells helper T cells, suppressor T cells, monocytes and presence of autoantibody to lymphocytes (Eibl et al. 1982; WHO Scientific Group on Immunodeficiency 1983). In this study, we analyzed the B cell defects of CVI patients by using SAC and RIL2, and suggested that some B cells in CVI had defects on the responsibility to RIL2. Further experiments are needed to define what affects the responsibility of B cells to IL2 about
IL2 receptor and intracellular events.

References


