PURIFICATION AND PHYSICOCHEMICAL CHARACTERIZATION OF MURINE T CELL REPLACING FACTOR (TRF)¹

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Murine T cell replacing factor (TRF) was purified from a cellfree supernatant of a T cell hybridoma (B151K12) that constitutively produces TRF. Two assay systems for TRF activity were employed: 1) induction of anti-DNP IgG PFC responses in cultures of splenic B cells from DNP-KLH-primed BALB/c mice, and 2) induction of IgM PFC in chronic B cell leukemic cells (BCL₁). The purification scheme consisted of ammonium sulfate precipitation, DEAE-cellulose chromatography, Blue-Sepharose chromatography, hydroxyapatite chromatography, gel permeation with fast protein liquid chromatography (FPLC), and disc polyacrylamide gel electrophoresis. Overall, TRF was purified approximately 34,000-fold with a maximum 3.8% recovery of activity, and the specific activity of the purified TRF was approximately 9.6 x 10⁴ U/mg. The TRF that is active in these systems is distinct from the other lymphokines such as IL 1, IL 2, BCGFI (now known as BSF₂), and γ-interferon. The TRF is extremely hydrophobic, with an apparent m.w. of 50,000 to 60,000 on gel permeation chromatography and 18,000 on SDS-PAGE under reducing conditions. Highly purified B151-TRF abrogated the activity by treatment with trypsin but not with RNase. Moreover, it bound to lima bean agglutinin-Sepharose specific for N-acetylgalactosamine residues, indicating that B151-TRF is a glycosylated glycoprotein containing N-acetylgalactosamine residues. The role of N-acetylgalactosamine residues on TRF activity was additionally substantiated by the fact that the addition of appropriate amounts of N-acetylgalactosamine in the assay systems for TRF preferentially induced a profound suppression in TRF-mediated PFC responses.

It has been generally postulated that B cell proliferation and differentiation into immunoglobulin-secreting cells are regulated by several soluble factors derived from macrophages and T cells (1). Furthermore, there is growing evidence to support the idea that different phases of the B cell response to antigenic stimulation are regulated by functionally and biochemically distinct factors (2). Because complex cellular assay systems have usually been used in many studies, the precise mechanisms regarding the observed result of increased B cell function are not well understood.

T cell replacing factor (TRF)³ has been shown to induce terminal differentiation of late-developing B cells to antibody-producing cells rather than to augment B cell proliferation (3). A number of laboratories are involved in the purification of soluble factors that exert stimulatory effects on B cell differentiation (4–8). Progress has been limited primarily because of the minute quantities of active material produced by T cells. Therefore, there is little information available on the biochemical properties of B cell differentiation factors such as TRF.

Of particular interest to us are the properties of TRF that regulate the terminal differentiation of B cells, which had been activated in vivo by immunization with antigen or in vitro by anti-IgM antibody. We previously demonstrated that extensively T cell-depleted splenic B cells from mice, as well as murine chronic B cell leukemic cells (BCL₁), could differentiate into antibody-secreting cells in response to soluble products (TRF) found in the cellfree supernatants (CFS) of a T cell hybridoma (B151K12) established by means of fusion of T thymoma cells and Mycobacterium-primed T cells (9–11). Furthermore, we also demonstrated that the responsiveness of B cells to B151-TRF was under X chromosome control, as determined by genetic analysis of the TRF low responsiveness of B cells from DBA/2Ha mice (12, 13).

Because B151K12 cells constitutively produce TRF, and because the target (BCL₁) cells are monoclonal in nature, there is little ambiguity concerning the origin of the TRF source and the primary target of TRF. To determine the biochemical characteristics of TRF, we have fractionated samples of B151K12-CFS by using a variety of biochemical techniques. The resulting sets of fractions were then tested for TRF activity that had been previously observed on antigen-activated B cells and BCL₁ cells, and several other lymphokine activities were tested as controls.

In this report, we describe the purification method used to enrich TRF activity from the CFS of a murine T cell

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³Abbreviations used in this paper: CFS, cellfree supernatant; TRF, T cell replacing factor; B151-TRF, TRF in CFS from B151K12 T cell hybridoma; FS6-CFS, CFS from concanavalin A-stimulated FS6-14.13 cells; LBA, lima bean agglutinin; iC'h, lentil lectin; WGA, wheat germ agglutinin; TEMED, N.N.N',N'-tetramethylethylenediamine; PFC, plaque forming cell; BCGFI, anti-IgM antibody-dependent B cell growth factor; ATS, anti-thymocyte serum.
Purification of TRF

MATERIALS AND METHODS

Animals. BALB/cCrSlc mice were obtained from the Shizuoka Agricultural Cooperative Association for Laboratory Animals. Harare, and Harare. BALB/c mice were housed in the Animal Care Unit with approximately 50% relative humidity and 12 hr light/12 hr dark conditions. All animals were supplied with food and water ad libitum.

Reagents. Keyhole limpet hemocyanin (KLH; Calbiochem Corp., San Diego, CA) and hen egg albumin (OVA; Sigma Chemical Co., St. Louis, MO) were used as carrier proteins. 2.4-Dinitrophenyl (DNP) groups were introduced into those proteins by described methods (12). B151K12 monoclonal antibody (MAB) was a gift from Dr. D. M. Davis, Duke University, Durham, NC (13). Subscript refers to the average number of DNP groups per molecule of protein. Various lectin-conjugated agarose gels (LBA-gel, LcH-gel, and WGA-gel) were obtained from E. Y. Laboratories (San Mateo, CA). Various monocarboxylic acid hydrolases were obtained from Sigma. Anti-Thy-1.2 anti-serum (clone F7D5) was obtained from Oac 1976, Bichereh Oxon, England. Rabbit antibody specific for mouse μ-chain was raised by immunizing purified IgM secreted by myeloma MOPC104E cells. Blue-Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) column (10 × 50 mm). Nonbound fractions were received an ATS injection 2 days before sacrifice, were treated in vivo. The factors were harvested by centrifugation, and FCS was used as a B cell source. Those DNP-KLH-immunized B-cell cultures of B cells from DNP-KLH-immunized BALB/c mice, and 2) induction of anti-DNP IgC PFC responses in BCL1 cells, were recovered in the interphase between the 30 and 60% Percoll gradient centrifugation, and were used as a B cell source. Those DNP-KLH-immunized B cells (6 × 10⁵/culture) were co-cultivated with various concentrations of TRF-containing CFS in the presence of DNP-OVA (12 ng/culture). The cultures were set up in triplicate. The numbers of DNP-specific plaque-forming cells (PFC) were enumerated by a modification of the method of Jerne and Nordén (17). Because mainly the IgC class of the anti-DNP PFC response was detected under the conditions employed, only the number of anti-DNP IgG PFC is listed in the Results.

Enzyme treatment. Partially purified preparations of TRF were treated with either insolubilized trypsin or ribonuclease (RNase). The TRF preparation to be treated was obtained by ammonium sulfate precipitation. DE52 column chromatography. Blue-Sepharose column chromatography, hydroxylapatite column chromatography, and gel permeation on TSK columns. The sample contained over 200 U/ml of TRF and no detectable IL-2 activity. The sample was dialyzed against PBS. The reaction mixture was incubated at 37°C for 1 hr on a slowly rotating wheel to prevent the insolubilized enzymes from settling. The factors were harvested by centrifugation, and FCS was used as a B cell source.
and 1/10 and 1/20, as measured by using BCL1 cells. In each experiment, a dose-response titration of unfractionated B151K12 supernatant was also performed. A unit of TRF activity in each fraction was defined as the reciprocal dilution yielding a PFC response that was 50% of the maximal response to a standard TRF preparation.

Anti-IgM-dependent B cell growth factor (BCGFI) assay. Samples were assayed for BCGFI (now known as BSF-2) in a co-stimulator assay with anti-IgM, as described (18). Purified B cells were prepared from BALB/c mice by modification of the procedures described by Leibson et al. (19) as well as Howard et al. (18). The cells were plated at a final concentration of 2.5 x 10^6 cells/ml (5 x 10^6 cells/0.2 ml culture) and were stimulated with rabbit IgG anti-mouse μ-chain at a final concentration of 2 μg/ml, with or without BCGFI, for 3 days. The cells were pulsed with tritiated thymidine (³H-TdR) for the last 18 hr of the incubation period, and were assayed for ³H-TdR incorporation as described (9). A unit of BCGFI is defined as the reciprocal of the dilution yielding a response, in counts per minute (cpm), that is 50% of the maximal response to a standard BCGFI preparation.

IL 2 assay. IL 2 activity was determined by a modified microassay method with the use of IL 2-dependent murine cloned cytotoxic T cell line (GY 1 cell line) according to the method described by Stadler et al. (20). A unit of IL 2 is defined as the reciprocal of the dilution yielding a cpm response that is 50% of the maximal response to a standard IL 2 preparation.

IFN assay. IFN activity was assayed on mouse L929 cells by the 50% plaque reduction method, as described by Epstein et al. (21). The CFS of PPD-stimulated, mouse Mycobacterium-primed spleen cells was used as the standard IFN preparation (17 U/ml).

Statistical analysis. Individual experiments were repeated at least four times. The numbers of PFC/culture were logarithmically transformed, and the geometric means and standard errors were calculated. Group comparisons were made by employing Student's t test.

RESULTS

The CFS derived from a T cell hybridoma (B151K12) that had been established from means of fusion between Mycobacterium-primed T cells with BW5147 thymoma cells was used as a source of TRF, because B151K12 cells constitutively produce TRF without secreting measurable amounts of IL 2 or BCGFI (9, 22). The biochemical characterization of TRF is based on two assay systems: 1) induction of anti-DNP IgG PFC responses in cell cultures of B cells from DNP-KLH-primed mice, and 2) induction of IgM PFC in BCL1 cell cultures. In each experiment, a dose-response curve titration of unfractionated CFS of B151K12 cells was performed. One of the representative results for the TRF assay is shown in Figure 1. First, spleen B cells from DNP-KLH-primed mice that had been pretreated with ATS 2 days before sacrifice, followed by sequential treatment in vitro with anti-Thy-1 antibody plus complement and anti-Lyt-1 antibody plus complement, were tested for the ability to respond to the CFS of B151K12, resulting in anti-DNP IgG PFC. As it is evident from Figure 1A, the addition of CFS of B151K12 alone to the culture of DNP-primed B cells in the presence of DNP-OVA gave rise to a significant number of PFC in a dose-dependent manner after 5 days of culture. Under these conditions, Con A-stimulated, FS6-14.13-derived CFS alone (which contains at least IL 2 and BCGFI) was not sufficient to reconstitute the PFC response.

Secondly, Percoll-purified BCL1 cells were cultured with CFS from various T cell hybridomas for 3 days, and then the number of IgM PFC was enumerated by a reverse PFC assay. As shown in Figure 1B, the addition of B151K12-CFS induced polyclonal IgM secretion by Percoll-purified neoplastic B cells from the BCL1 tumor without any anti-Ig stimulation. In contrast, the supernatant from FS6-14.13 did not induce any polyclonal IgM secretion under the same conditions. These results are in agreement with our previous observations (9, 11), and also indicate that the addition of unfractionated B151K12 supernatant, but not FS6-14.13 supernatant, could induce differentiation of both DNP-primed B cells and neoplastic B leukemic (BCL1) cells into antibody-secreting cells in a dose-dependent manner. Moreover, assay systems with the use of BCL1 cells for TRF activities are more suitable than those with the use of DNP-primed B cells to obtain positive responses in short periods of time (2 days). An arbitrarily chosen unfractionated B151K12-CFS was defined as containing 20 U/ml, because a 1/20 dilution of a test sample gave approximately 50% of the maximal response when using the BCL1 assay (Figure 1B).

Purification of B151-TRF. The B151K12 cells were cultured in RPMI 1640 medium containing 10% FCS and 5 x 10^-5 M 2-mercaptoethanol for 2 days, and to 3 liters of CFS were harvested per week. CFS of B151K12 was salted out with (NH₄)₂SO₄ in the range of 50 to 85% saturation. Most of the TRF activity was recovered in this fraction. After extensive dialysis against 10 mM Tris-HCl buffer (pH 8.5), samples were applied to a DE52 column and were eluted with a 0 to 0.25 M NaCl linear gradient in 10 mM Tris-HCl buffer (pH 8.5). The TRF activity at each fraction was determined by using DNP-primed B cells or BCL1 cells. One of the representative results is shown in Figure 2. A single peak of TRF activity was observed that eluted at approximately 75 mM NaCl. Because TRF activity was recovered from fractions before elution of major protein peaks, TRF-active fractions represented a 340-fold increase in specific activity, with a 67% recovery.

Because TRF activities titrated by using DNP-primed B cells were detected at the same fractions as those assayed by using BCL1 cells, the following assay for TRF activity at various purification steps was conducted by using BCL1 cells alone, and the results are expressed as units of TRF activity per milliliter, calculated as described in Materials and Methods.
The TRF-active fraction from the Blue-Sepharose column chromatography was concentrated, dialyzed, and applied to a Blue-Sepharose column. Nonbound materials to the Blue-Sepharose column were recovered, and bound materials were eluted with 2 M KCl in 50 mM Tris-HCl buffer (pH 8.0). The TRF activity of each fraction was titrated by using BCL1 cells.

The TRF-active fractions from the Blue-Sepharose column were dialyzed against 10 mM sodium phosphate buffer (pH 6.7) containing 0.2 M NaCl, and were then applied to a hydroxylapatite column and were eluted with a 10 to 200 mM linear gradient of sodium phosphate buffer at pH 6.7 in 0.2 M NaCl. TRF activity of each fraction was tested by using BCL1 cells. Most of the TRF activity was recovered in the fractions eluted with approximately 70 mM sodium phosphate buffer (pH 6.7) (Fig. 4).

To examine the hydrophobicity of partially purified B151-TRF at this stage, the TRF-active fractions from the hydroxylapatite column were dialyzed against 0.1% trifluoroacetic acid (TFA) and were applied on a ProRPC HR5/10 column (Pharmacia) connected with FPLC and eluted with a 0 to 80% acetonitrile linear gradient in 0.1% TFA. After extensive dialysis against RPMI medium, the TRF activity at each fraction was determined. The TRF activities were recovered at the fraction between 44 to 48% of acetonitrile in 0.1% TFA, suggesting that B151-TRF is extremely hydrophobic in nature (data not shown).

To purify B151-TRF by gel permeation chromatography, TRF-active fractions from the hydroxylapatite column step were concentrated, dialyzed, and applied on a TSK (G3000 SW) column connected with FPLC. The results of TSK-FPLC run in 10 mM HEPES buffer at pH 7.0 in 0.15 M NaCl showed one major and one minor 280 nm absorbing peak. The TRF activity was recovered at the fractions behind the major 280 nm absorbing peak, and it corresponded to an apparent m.w. of 50,000 to 60,000 (at peak 54,000), as shown in Figure 5.

The TRF-active fraction from the gel permeation column step was concentrated and applied to disc polyacryl-
amide gel electrophoresis. After electrophoresis, the gels were sliced at every 2 mm, were eluted in PBS containing 0.5% BSA overnight, and were assayed for TRF activity using BCL1 cells. The results are shown in Figure 6. Two bands, one major and one minor, were observed by staining the gels with Coomassie Brilliant Blue. More than 65% of the applied TRF activity was recovered in association with the minor band. In this step, recovery of the protein was arbitrarily calculated by the method used for characterization of TRF.

Characterization of B151-TRF. To confirm that purified material from B151K12-CFS exerts only TRF activity, other nominal lymphokine activities of highly purified B151-TRF were analyzed. BCGFI activity was measured by co-stimulating assay by using purified B cells with suboptimal doses of soluble anti-IgM antibody. IL 2 activity was determined by using the IL 2-dependent long-term-cultured CTLL line. IFN activity was determined by a standard blocking effect on virus replication in a cultured murine fibroblast cell line (L929) (20).

To characterize the molecular nature of B151-TRF, TRF was treated with protease and nuclease, respectively, and the remaining TRF activity was measured. As can be seen in Table III, TRF activity was abolished by the treatment with trypsin, whereas RNase treatment did not affect the TRF activity, indicating that B151-TRF is a distinct molecule from BCGFI, IL 2, and IFN.

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Functional characterization of B151-TRF. Because it is reported that various lymphokines have binding affinity to plant lectin, the TRF-adsorbing activity of insolubilized lectin was examined. The partially purified B151-TRF was applied to LBA-, LcH-, or WGA-coupled-agarose gel columns. Effluent fractions from each gel were collected, and the adsorbed materials were eluted with the relevant monosaccharide from each column. TRF activity in each fraction from each column was tested. One of the representative results is shown in Table IV. Most of the TRF activity was recovered in effluents from both the LcH-gel and the WGA-gel, whereas little TRF activity was recovered in fractions from the LBA-gel.

![Figure 6. Polyacrylamide gel electrophoresis. The active fractions from the gel permeation chromatography were concentrated and additionally fractionated by polyacrylamide gel electrophoresis, as described in Materials and Methods. After electrophoresis, a portion of the gels were stained with Coomassie Brilliant Blue and were monitored by a densitometer at 550 nm. The gels were sliced at every 2 mm. The TRF activity (-----) in eluted materials from each gel slice was determined by BCL1 cells.](image-url)

**Table 1. Purification of mouse TRF from B151K12 T cell hybrid cell culture**

<table>
<thead>
<tr>
<th>Purification Step</th>
<th>Total Protein* (mg)</th>
<th>TRF Activity (units/mg)</th>
<th>Specific Activity (units/mg)</th>
<th>Purification Increase (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting material</td>
<td>21.000</td>
<td>6.00</td>
<td>2.86</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Ammonium sulfate fraction</td>
<td>10.120</td>
<td>5.67</td>
<td>5.60</td>
<td>1.96</td>
<td>94.5</td>
</tr>
<tr>
<td>DEAE-cellulose chromatography</td>
<td>40.5</td>
<td>4.02</td>
<td>9.93 x 10^4</td>
<td>347</td>
<td>67</td>
</tr>
<tr>
<td>Blue-Sepharose column</td>
<td>16.2</td>
<td>3.68</td>
<td>2.27 x 10^4</td>
<td>794</td>
<td>61</td>
</tr>
<tr>
<td>Hydroxylapatite column</td>
<td>1.51</td>
<td>2.09</td>
<td>1.38 x 10^4</td>
<td>4,859</td>
<td>35</td>
</tr>
<tr>
<td>Gel permeation on</td>
<td>0.24</td>
<td>1.01</td>
<td>4.21 x 10^4</td>
<td>14,714</td>
<td>16</td>
</tr>
<tr>
<td>TSK(G3000 SW)</td>
<td>0.024</td>
<td>0.23</td>
<td>9.58 x 10^4</td>
<td>33,508</td>
<td>3.8</td>
</tr>
</tbody>
</table>

*Supernatant was prepared with 10% FCS and was 3000 ml in vol.

*Protein concentrations were determined relative to BSA standards by using the Bio-Rad protein assay.
Figure 7. SDS-PAGE analysis of partially purified B151-TRF. The TRF active sample from gel permeation column chromatography was analyzed with SDS-PAGE under nonreducing (upper panel) or reducing conditions (lower panel), according to the methods described in Materials and Methods. TRF activity in eluted materials from each gel slice was assayed by using BCL1 cells.

Figure 8. SDS-PAGE followed by silver staining of partially purified B151-TRF. A. The sample of partially purified B151-TRF under the reduced conditions in Figure 7. B. Standard marker proteins.

<table>
<thead>
<tr>
<th>Lectin Agarose</th>
<th>TRF Activities (units/ml)</th>
<th>Effluent Eluate</th>
<th>Percent Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBA</td>
<td>26</td>
<td>128</td>
<td>59</td>
</tr>
<tr>
<td>LcH</td>
<td>196</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>WGA</td>
<td>182</td>
<td>21</td>
<td>16</td>
</tr>
</tbody>
</table>

*TRF-active fractions (250 U/ml) from gel permeation chromatography were applied to various lectin-conjugated agarose gels (4 ml gel) and the effluent fractions were collected. The materials adsorbed on LBA-gel, LcH-gel, and WGA-gel were eluted with 0.2 M N-acetylgalactosamine, 0.2 M α-mannose or 0.1 M N-acetylglucosamine, respectively. After extensive dialysis of each sample, the TRF activity was measured by using BCL1 cells, and total TRF activities recovered at each fraction were calculated.

detected in effluent from the LBA-gel. Moreover, more than 60% of the original TRF activity was recovered from the LBA-gel by eluting with 0.2 M N-acetylgalactosamine. By contrast, eluates from the LcH gel with 0.2 M α-methylmannoside or from the WGA-gel with N-acetylgalactosamine did not show significant TRF activity. Taking all of the results together, it is most likely that B151-TRF contains N-acetylgalactosamine residues in the molecule.

Because B151-TRF contains N-acetylgalactosamine residues, it is worthwhile to test whether the N-acetylgalactosamine residue plays an important role in the expression of TRF activity. One of the approaches employed here was to investigate the blocking effect of various monosaccharides on B151-TRF-induced PFC responses. As a control, the effect of monosaccharides on the anti-2,4,6-trinitrophenyl (TNP) IgM PFC response induced by TNP-LPS was also examined. One of the representative results is shown in Table V. The addition of 10 mM N-acetylgalactosamine to the cultures for TRF assay induced profound suppression of anti-DNP IgG PFC responses mediated by B151-TRF, whereas neither N-acetylgalactosamine nor fucose inhibited TRF-induced PFC responses. However, the same concentrations of N-acetylgalactosamine did not inhibit anti-TNP PFC responses induced by TNP-LPS, suggesting that N-acetylgalactosamine residues of B151-TRF may play an important role in the expression of TRF activity.

**TABLE II**

<table>
<thead>
<tr>
<th>Factors</th>
<th>TRF Activity (units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B151-TRF</td>
<td>200.0 &lt;0.5 &lt;0.5 &lt;1.0</td>
</tr>
<tr>
<td>FS6-CFS</td>
<td>&lt;0.5 18.0 8.0 ND*</td>
</tr>
<tr>
<td>PPD-CFS</td>
<td>16.0 &lt;0.5 12.0 17.0</td>
</tr>
</tbody>
</table>

* CFS of purified protein derivative-stimulated Mycobacterium-primed spleen cells.
* ND: Not determined.

**DISCUSSION**

The present experiments defined a molecule (TRF) that induces differentiation and/or maturation of B cells from DNP-KLH-primed mice, as well as chronic B leukemic cells, to antibody-secreting cells. Because the B151K12 cells, when grown in a RPMI 1640 medium containing 2 to 10% FCS and 5 × 10⁻⁶ M 2-mercaptoethanol, produce constitutively TRF (10), we chose CFS of B151K12 cells...
as a starting material for TRF purification. The TRF has been sequentially purified from CFS by DEAE-cellulose chromatography, a Blue-Sepharose column, hydroxylapatite chromatography, gel permeation column chromatography, as a starting material for TRF purification. The TRF has an apparent m.w. of 50,000 to 60,000 (peak at 54,000) on gel filtration, and dissociates to an 18,000 dalton species by SDS under reducing conditions. There is no evidence that B151-TRF could bind to the LBA-conjugated acceptor site on the target cells. Moreover, the addition of N-acetylgalactosamine into the assay system for TRF by using DNP-primed B cells induced a profound suppression of TRF-mediated anti-DNP IgG PFC responses (Table IV), indicating that N-acetylgalactosamine residues in the B151-TRF molecule may play an important role for the expression of TRF activity and/or binding of TRF to the acceptor site(s) on target cells.

It is clear that we are far from a complete understanding of the molecular mechanism of T cell-derived factors that govern B cell proliferation and differentiation into antibody-forming cells. Many soluble factors have been reported to affect the later stages of B cell differentiation, as reviewed by Howard and Paul (2). Isakson et al. (5) described the existence of two kinds of B cell differentiation factors, termed BCDFγ and BCDFγ. These lymphokines appear to act directly on activated normal B cells (or in the case of BCDFγ, on BCL1 cells) to induce the synthesis and secretion of IgM or IgG, respectively. According to their results, B151-TRF could only induce secretion of IgM from normal B cells, as well as BCL1 cells. Therefore, B151-TRF belongs to BCDFγ but not to BCDFγ (5). However, as we reported in preceding papers and also in the present study, B151-TRF induces remarkable IgG PFC responses. The reasons for the discordant results between ourselves and the other investigators may be due to different assay systems, and additional analysis of mechanisms for class-switching from IgM to IgG by using highly purified B151-TRF may contribute to understanding the functional differences between B151-TRF and BCDFγ.

Leibson et al. (19, 23) reported that IFNγ could induce terminal differentiation of B cells in combination with IL-2. Our results distinguish B151-TRF from a number of previously described lymphokines (IL-1, IL-2, BCGFI, and IFN), because the highly purified B151-TRF material did not show any significant BCGFI, IL-2, or IFN activity (Table II). Furthermore, the addition of CFS containing IL-2 and BCGFI to the assay systems for TRF employed in the present study did not induce PFC responses (Fig. 1). Moreover, the addition of IFNγ produced by recombinant DNA technology does not trigger BCL1 cells for IgM-secreting cells (unpublished observation). These results strongly indicate that the TRF distinct from IL-2 and IFN can induce terminal differentiation of B cells into antibody-forming cells. However, this does not exclude the possibility that IFNγ exerts its activity to B cells in combination with IL-2, giving rise to antibody-secreting cells. It is possible that B cell population responsive to B151-TRF is distinct from that responsive to IFNγ, because B cells that were activated by anti-IgM antibody and BCGFI responded to B151-TRF and EL-TRF with synergism (22).

Leibson and his associates (4) described antigen-non-specific helper factors from helper T cell lines inducing the replication and maturation of B cells. They provisionally named those factors as B cell replication and maturation factors (BRMF). According to their results, BRMF act on B blastoid cells that have been activated with helper T cells and antigen in a major histocompatibility complex-restricted fashion. It is too early to compare our B151-TRF with their factors on a molecular basis.

Recently, Sidman and his collaborators (7) reported the initial biochemical characterization and purification of B cell maturation factors (BMF), which is a lymphokine or family of lymphokines promoting the maturation of some
tumor lines of B cell lineage, as well as normal B cells. According to their results, BMF molecules are distinct lymphokine from IL 1, IL 2, granulocyte-macrophage-CSF, IFN and BCGFI, and are probably heterogeneously glycosylated glycoproteins with an apparent m.w. of 50,000 to 55,000 by gel permeation chromatography and 16,000 by SDS-PAGE. As judged from the apparent m.w. of B151-TRF, BMF has very similar features to B151-

50.000 to 55,000 by gel permeation chromatography and TRF.

question arose in our mind as to whether partially purified B151-TRF exerts BCGFI activity on BCL, cells. Therefore, the growth and differentiation of B blastoid cells, as well as resting B cells, is under investigation.

Besides the possible existence of various T cell factors responsible for B cell differentiation, we have to locate the functional site of various T cell factors at the sequential steps of B cell differentiation. Only this may enable us to define the real functions of the T cell factors. When a cloned B cell line is available in which the differentiation stage is functionally defined, we will be in a position to determine what kinds of helper T cell factors are required in the process of B cell differentiation. As judged from our results described here, we speculate that B151-TRF acts on at least mature B cells at their terminal differentiation stage to antibody-forming cells.

The studies reported herein provide first step purification of sufficient quantities of TRF to distinguish the active molecule responsible for B cell differentiation. At present, studies are in progress to additionally characterize the molecule with respect to its primary structure, as well as its role for B cell differentiation and/or maturation.

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