Antigen Presentation in an HLA-DR–Restricted Fashion by B-Cell Chronic Lymphocytic Leukemia Cells

By Masaki Yasukawa, Tomoo Shiraguchi, Akira Inatsuki, and Yuzuru Kobayashi

The ability of B-cell chronic lymphocytic leukemia (B-CLL) cells to present antigen to antigen-specific T cells was investigated. B-CLL cells present herpes simplex virus (HSV) antigen and purified protein derivative (PPD) to HSV- and PPD-specific, interleukin-2–dependent T-cell lines in an antigen-specific manner. Treatment of B-CLL cells with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) induced markedly increased levels of HLA-DR expression. TPA-treated B-CLL cells showed substantially more effective presentation, especially at low antigen concentrations, than did untreated B-CLL cells. By culturing different allogeneic combinations of B-CLL cells and T cells and by adding anti–HLA-DR monoclonal antibody to cultures, it was found that antigen presentation by B-CLL cells was restricted by HLA-DR in the same way as for macrophages. We concluded from these experiments that B-CLL cells have a capacity to serve as antigen-presenting cells in an HLA class II–restricted fashion and that increasing the amount of HLA class II antigen and activation of B-CLL cells resulted in effective antigen presentation.

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From the First Department of Internal Medicine, Ehime University School of Medicine, Japan.

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Address reprint requests to Masaki Yasukawa, MD, First Department of Internal Medicine, Ehime University School of Medicine, Shigenobu, Ehime 791-02, Japan.

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Antigen-specific helper T cells recognize antigen in association with the major histocompatibility complex (MHC) class II gene (Ia) products expressed on antigen-presenting cells (APC).1,2 Although the tissue distribution of Ia antigen has been extensively examined, the nature of the cells capable of antigen presentation is not completely clear. The most widely recognized APC is the adherent, nonspecific esterase–positive macrophage, but antigen-presenting ability has been found in a variety of other cells including dendritic cells,3 epidermal Langerhans' cells,4 hepatic Kupffer's cells,5 and vascular endothelial cells.6 Recently, several investigators have turned their attention to B cells because of their ability to present antigen to helper T cells. In a murine system, it was shown that several Ia-negative B cells were capable of presenting antigen to antigen-specific T cells.7-9 Issekutz et al showed that human B cells transformed by Epstein-Barr virus (EBV) present soluble antigen to T cells in association with HLA class II antigen.10 However, the antigen-presenting function of human B-cell neoplasms has remained unknown. In this paper we report that human B-cell chronic lymphocytic leukemia (B-CLL) cells can serve as APC and that increased expression of HLA-DR on the cell surface and activation of B-CLL cells by treatment with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) resulted in a more effective antigen presentation.

MATERIALS AND METHODS

Cell separation. Peripheral blood mononuclear cells (PBMC) were isolated from both patients with an established diagnosis of CLL and normal individuals by Ficoll-Conray centrifugation. T cells accounted for, on average, 5% of PBMC in CLL, as shown by staining with CD3 monoclonal antibody (OKT3) (Ortho Pharmaceutical Corp., Raritan, NJ). PBMC were cryopreserved for future use.

Antigens. The KOS strain of herpes simplex virus (HSV) type 1 was grown in HEp2 cells. UV light–irradiated HSV was used as the HSV antigen at a concentration of 1 x 106 plaque-forming units (PFU)/mL. Purified protein derivative (PPD) was purchased from Nihon BCG Seizo, Tokyo, and was used at a concentration of 1 μg/mL.

Generation of antigen-specific T-cell lines from B-CLL patients. Fifty million PBMC from patients with B-CLL were suspended in 10 mL RPMI 1640 medium supplemented with 10 mmol/L HEPES buffer and 10% heat-inactivated normal human serum (this medium is referred to as the culture medium) with HSV antigen or PPD. Cells were then seeded in a 16-mm–well plate at 10 x 106 cells/well and cultured at 37°C in a 5% CO2 atmosphere. After 6 days, the culture medium was exchanged for RPMI 1640 medium supplemented with 10 mmol/L HEPES buffer, 10% human serum, and 5% interleukin-2 (IL-2) (Boehringer Mannheim, Mannheim FRG). The growing cells were expanded in culture medium containing IL-2 and were used for antigen-specific T-cell lines (TCLs) of B-CLL patients.

Generation of antigen-specific TCLs from normal individuals. Ten million PBMC from normal individuals were suspended in 20 mL of culture medium. HSV antigen or PPD was then added to the cells, which were seeded in a 96-well round-bottomed microtiter plate at 1 x 105 cells/well and cultured at 37°C in a 5% CO2 atmosphere. After 6 days, the cells were transferred to 16-mm wells and cultured in culture medium containing IL-2. The cells were cultured for seven to ten days, after which they were used in proliferation assays as antigen-specific TCLs from normal individuals.

Preparation of purified B cells and monocytes. For isolation of highly purified B cells, PBMC were depleted of sheep erythrocyte (E) rosette–forming T cells, as previously described.11 The E-cells were cultured in 75-cm2 tissue culture flasks (Falcon 3024) at a concentration of about 4 x 106/mL for one hour. E-cells depleted of plastic-adherent cells were suspended at a concentration of 20 x 106 cells/mL in culture medium containing CD11 monoclonal antibody (OKM1) (Ortho Pharmaceutical Corp., Raritan, NJ) at an optimal concentration. After one hour of incubation on ice, rabbit complement (Cedarlane, Hornby, Ontario, Canada) was added at a final dilution of 1:4, and the cells were incubated for one hour at 37°C. The cells were then washed twice and resuspended in culture medium. This population was contaminated with less than 2% T cells and less than 1% monocytes, as shown by staining with CD3 monoclonal antibody and May-Giemsa staining, respectively. Monocytes were isolated by adherence to the surfaces of plastic flasks. PBMC were suspended at a concentration of 4 x 105 cells/mL in
culture medium. Cell suspensions were incubated for five hours in 75-cm² tissue culture flasks (Falcon 3024) at a volume of 10 mL/flask at 37°C. The adherent cells were then scraped off gently with a rubber policeman. These cells consisted of 70% to 80% monocytes.

**TPA treatment of B-CLL cells.** Ten million B-CLL cells suspended in 10 mL RPMI 1640 medium supplemented with 10 mmol/L HEPES buffer and 10% heat-inactivated fetal calf serum (FCS) (GIBCO, Grand Island, NY), with or without 1.6 x 10⁻⁷ mol/L TPA (Sigma Chemical Co, St Louis), were cultured in a 25-cm² tissue culture flask (Falcon 3013). Cultures were performed at 37°C in an incubator with a 5% CO₂ atmosphere for three days. The cells were then harvested and the purified B cells prepared as described earlier.

**Immunofluorescence flow cytometry.** Five hundred thousand cells were incubated with 100 μL phosphate-buffered saline (PBS) containing 5% human immunoglobulin (Hoechst Japan, Ltd, Tokyo, Japan) for 15 minutes at 4°C to block the Fc receptors. This was followed by the addition of 100 μL anti-HLA-DR monoclonal antibody HU-4 at an optimal concentration, and the cells were incubated for 30 minutes at 4°C. After washing three times with PBS containing 5% FCS, the cells were incubated with fluorescein-conjugated goat antimouse IgG (Cappel Laboratories, Malvern, PA) for an additional 30 minutes at 4°C. Cells to be used as the unstained negative control were incubated with human immunoglobulin but without anti–HLA-DR monoclonal antibody; this was followed by incubation with fluorescein-conjugated goat antimouse IgG. After washing three times with PBS containing 5% FCS, the cells were analyzed with a flow cytometer (Epics C, Coulter Electronics, Mialeah, FL).

**Proliferation assay of TCLs.** Purified B cells were suspended in culture medium at a concentration of 2 x 10⁷/mL. The cells were incubated with mitomycin C (MMC) (Kyowa Hakko, Tokyo, Japan) at a concentration of 15 μg/mL at 37°C for 30 minutes. After washing three times, MMC-treated cells were resuspended in culture medium. In some experiments, PBMC and purified monocytes from normal individuals were treated with MMC as described earlier. HSV- and PPD-specific TCL (HSV-TCL, PPD-TCL) cells were cultured in 0.2 mL of culture medium in 96-well round-bottomed microtiter plates. Culture was performed at 37°C in an incubator with a 5% CO₂ atmosphere for seven days. For the final 12 hours of incubation, 1 μCi of [³H]thymidine ([³H]-TdR; New England Nuclear, Boston) was added, and the cells were then harvested onto glass filter paper by a semiautomatic multiple harvester. The incorporation of [³H]-TdR was then determined as described earlier.

**RESULTS**

Enhanced expression of HLA-DR on TPA–B-CLL cells. Surface HLA-DR expression on B-CLL cells and TPA–B-CLL cells examined by flow cytometry is shown in Fig 1. There was a significant increase in the amount of HLA-DR on TPA–B-CLL cells. Enhancement of HLA-DR expression by TPA was detected on B-CLL cells from all of the four patients examined.

Antigen presentation to antigen-specific TCLs by B-CLL cells and TPA–B-CLL cells. Table 1 shows the results of four experiments that were used to examine the ability of B-CLL cells and TPA–B-CLL cells to present antigen to HSV- and PPD-reactive TCLs. Among them, antigen-specific TCL was not obtained from patient YO, and therefore, TCLs generated from an HLA class II–matched normal individual were used in the experiment. HSV-TCL and PPD-TCL did not proliferate in response to any antigen in the absence of APC, although they proliferated in culture medium containing IL-2. In the presence of MMC-treated B-CLL cells or MMC-treated TPA–B-CLL cells as sources of APC, a proliferative response was observed when antigen was added to the cultures. HSV-TCL proliferated when HSV antigen was added, but either no response or a very weak one was found with PPD. Similarly, PPD-TCL prolif-
cells were added. Similar results were observed in three other experiments examining the effect of cell numbers of B-CLL cells. These results suggest that the interaction between T cells and B-CLL cells, as APC, is antigen specific.

**Effect of cell numbers of B-CLL cells and TPA-B-CLL cells on antigen presentation.** Figure 2 shows representative results of the experiments examining the effect of cell numbers of B-CLL cells and TPA-B-CLL cells on the proliferation of 2 x 10⁵ TCL cells in the presence of an optimal antigen concentration. As the number of B-CLL cells and TPA-B-CLL cells increased, ³H-TdR incorporation by HSV-TCL also increased (Fig 2A). Similarly, the proliferative response of PPD-TCL increased as the number of B-CLL cells and TPA-B-CLL cells increased (Fig 2B). In both experiments, ³H-TdR incorporation by TCL was higher when TPA-B-CLL cells were added as APC than when the same number of B-CLL cells were added. Similar results were observed in three other experiments in which B cells separated from three different B-CLL patients were used as APC.

**Comparison of antigen presentation by B-CLL cells, TPA-B-CLL cells, and monocytes.** To investigate the relative antigen-presenting capacity of B-CLL cells, their abilities to present antigen were compared. In these experiments, monocytes were purified from two healthy individuals who shared one HLA-DR antigen with the B-CLL patient from whom TCL were established since purification of monocytes from B-CLL patients is difficult. As shown in Fig 3, B-CLL cells and TPA-B-CLL cells were approximately one tenth and one quarter as efficient as allogeneic monocytes, respectively. Since the monocytes used in these experiments shared only one HLA-DR antigen with TCL, the relative antigen-presenting capacity of B-CLL cells and TPA-B-CLL cells seemed likely to be lower.

**Effect of antigen concentration on T-cell proliferation.** Figure 4 shows the T-cell proliferative response in the presence of B-CLL cells or TPA-B-CLL cells at various concentrations of antigen. ³H-TdR incorporation by TCL in the presence of TPA-B-CLL cells was higher than in the

Table 1. Antigen Presentation to TCLs Reactive to HSV and PPD by B-CLL and TPA-B-CLL Cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>APC</th>
<th>Antigen</th>
<th>Proliferative Response: ³H-TdR Incorporated, cpm (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HSV-TCL</td>
</tr>
<tr>
<td>Y.U.</td>
<td>B-CLL</td>
<td>HSV</td>
<td>6,821 (321)</td>
</tr>
<tr>
<td></td>
<td>PPD</td>
<td>HSV</td>
<td>1,535 (110)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>HSV</td>
<td>1,032 (39)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PPD</td>
<td>1,009 (141)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None</td>
<td>851 (53)</td>
</tr>
<tr>
<td>U.W.</td>
<td>B-CLL</td>
<td>HSV</td>
<td>5,521 (766)</td>
</tr>
<tr>
<td></td>
<td>PPD</td>
<td>HSV</td>
<td>921 (251)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>HSV</td>
<td>436 (49)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PPD</td>
<td>536 (97)</td>
</tr>
<tr>
<td>D.O.</td>
<td>B-CLL</td>
<td>HSV</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PPD</td>
<td>HSV</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>HSV</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PPD</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None</td>
<td>ND</td>
</tr>
<tr>
<td>Y.O.*</td>
<td>B-CLL</td>
<td>HSV</td>
<td>6,917 (1,309)</td>
</tr>
<tr>
<td></td>
<td>PPD</td>
<td>HSV</td>
<td>1,101 (395)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>HSV</td>
<td>1,047 (109)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PPD</td>
<td>2,510 (305)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None</td>
<td>1,923 (301)</td>
</tr>
</tbody>
</table>

Incorporation of ³H-TdR into 2 x 10⁵ TCL cells in the presence of 2 x 10⁴ MMC-treated autologous B-CLL or TPA-B-CLL cells with HSV antigen, PPD, or no antigen was determined during the last 12 hours of a 72-hour incubation.

Abbreviation: ND, not done.

*Incorporation of ³H-TdR into TCLs generated from a normal individual sharing HLA class II antigens with Y.O. was determined in the presence of B-CLL or TPA-B-CLL cells from Y.O. The values indicated are the mean counts per minute (SEM) of triplicate wells.

Fig 2. Effect of numbers of MMC-treated B-CLL cells and TPA-B-CLL cells on the proliferative response of TCLs. Incorporation of ³H-TdR into 2 x 10⁵ TCL cells from patient Y.U. in the presence of various numbers of MMC-treated autologous B-CLL cells (O) or TPA-B-CLL cells (△) with antigen was determined as described in Materials and Methods. Incorporation of ³H-TdR into TCL in the presence of various numbers of MMC-treated B-CLL cells or TPA-B-CLL cells without antigen is shown as solid circles and solid triangles, respectively. The results are shown as means ± SEM of triplicate wells.

Fig 3. Proliferation of TCL in response to antigen in the presence of B-CLL cells, TPA-B-CLL cells, monocytes. Incorporation of ³H-TdR into 2 x 10⁵ TCL cells from B-CLL patient Y.U. in the presence of various numbers of MMC-treated autologous B-CLL cells (O), TPA-B-CLL cells (△), or allogeneic monocytes (□) with antigen was determined as described in Materials and Methods. Incorporation of ³H-TdR into TCL cells in the presence of each APC without antigen was less than 1,500 cpm. The HLA-DR of the donors is as follows: patient Y.U., DR4, w9; donor K.N. (C), DR4, w9; donor M.Y. (C), DR5, w9. The results shown are means ± SEM of triplicate wells.
TCL in the presence of TPA-B-CLL cells and that of or without TPA as described in Materials and Methods. Various cells showed an enhanced stimulatory capacity. The degree of these results indicate that TPA-B-CLL cells are more effective in antigen presentation than are B-CLL cells, especially at low antigen concentrations.

Similar results were observed in three other experiments. As shown in Fig 5, B-CLL cells showed a stimulatory capacity in mixed lymphocyte reaction (MLR). Following the treatment with TPA, B-CLL cells from patients were treated with MMC-treated B-CLL cells (△) or TPA-B-CLL cells. B-CLL cells from healthy individuals, F.U. and S.A., were cultured in 0.2 mL of culture medium for seven days, and then the incorporation of ³H-TdR was determined as described in Materials and Methods. The results are shown as the mean cpm ± SEM of triplicate wells.

Mixed lymphocyte reaction. As shown in Fig 5, B-CLL cells showed a stimulatory capacity in mixed lymphocyte reaction (MLR). Following the treatment with TPA, B-CLL cells showed an enhanced stimulatory capacity. The degree of augmentation in MLR was more prominent at a low concentration of stimulator cells. These results suggest that there is a correlation between the augmented ability of B-CLL cells to produce stimulation in an MLR and the presence of B-CLL cells at every concentration of antigen examined. The difference between ³H-TdR incorporation by TCL in the presence of TPA-B-CLL cells and that of B-CLL cells was marked at low antigen concentrations. Similar results were observed in three other experiments. These results indicate that TPA-B-CLL cells are more effective in antigen presentation than are B-CLL cells, especially at low antigen concentrations.

Antigen presentation by B-CLL cells and TPA-B-CLL cells. It has been established that helper T cells recognize antigen in association with HLA class II antigens on APC. To determine whether antigen presentation by B-CLL cells is also restricted by HLA class II antigens, we examined the proliferative responses of different allogeneic TCLs that shared or did not share HLA-DR antigens with APC. As shown in Table 2, allogeneic TCLs sharing HLA-DR4 or -DRw9 with APC demonstrated proliferative response to antigen stimulation in the presence of APC. On the other hand, TCL that did not share HLA-DR with APC did not show such a proliferative response, although it did respond to antigen stimulation in the presence of autologous APC. These results suggest that B-CLL cells can present antigen to T cells in an HLA-DR-restricted manner that is indistinguishable from the characteristic presentation of conventional APC such as macrophages.

Inhibition of antigen presentation by anti–HLA-DR monoclonal antibodies. To further examine the restriction element of the interaction between T cells and B-CLL cells, we next performed a blocking experiment using monoclonal antibody against an HLA-DR framework. In Table 3, it is apparent that proliferative responses to HSV antigen and to PPD of TCLs were inhibited by monoclonal antibodies directed against an HLA-DR framework. Similar inhibitions of antigen presentation of B-CLL and TPA-B-CLL cells by anti–HLA-DR monoclonal antibody were observed in two other experiments using cells from different donors. These findings confirmed that HLA-DR molecules act as restriction elements in the antigen presentation of B-CLL and TPA-B-CLL cells.

DISCUSSION

Previous studies have shown that normal murine B cells as well as Ia-bearing mouse B-cell tumor cells can serve as APC for antigen-specific T-cell activation. Moreover, it has been reported that EBV-transformed human B-lymphoblastoid cell lines can present the soluble antigen to HLA-DR-compatible T cells. Although these reports suggest the possibility that human Ia-bearing B-cell tumor cells have the capacity to serve as APC, there has been no report concerning the antigen-presenting function of human lymphoid neoplasms. In this paper, we first report that human leukemia cells characteristic of mature B cells can present antigen to T cells in both an antigen-specific and an HLA class II-restricted manner.

Antigen-specific helper T cells recognize antigen in association with Ia molecules expressed on the surface of APC. Matis et al reported that activation of Ia-restricted antigen-specific T-cell clones was dependent on both the number of Ia molecules and the concentration of antigen and that the magnitude of the proliferative response of such clones was a function of the product of antigen concentration and the number of Ia molecules expressed on B-CLL.

increased expression of HLA-DR following treatment with TPA.

Genetic restriction of antigen presentation induced by B-CLL cells and TPA-B-CLL cells. It has been established that helper T cells recognize antigen in association with HLA class II antigens on APC. To determine whether antigen presentation by B-CLL cells is also restricted by HLA class II antigens, we examined the proliferative responses of different allogeneic TCLs that shared or did not share HLA-DR antigens with APC. As shown in Table 2, allogeneic TCLs sharing HLA-DR4 or -DRw9 with APC demonstrated proliferative response to antigen stimulation in the presence of APC. On the other hand, TCL that did not share HLA-DR with APC did not show such a proliferative response, although it did respond to antigen stimulation in the presence of autologous APC. These results suggest that B-CLL cells can present antigen to T cells in an HLA-DR-restricted manner that is indistinguishable from the characteristic presentation of conventional APC such as macrophages.

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Table 2. HLA-DR-Restricted Antigen Presentation Induced by B-CLL Cells and TPA-B-CLL Cells

<table>
<thead>
<tr>
<th>APC</th>
<th>HLA-A11,24</th>
<th>B51,w62</th>
<th>DR4,w9</th>
<th>HLA-Aw19,26</th>
<th>B35,39</th>
<th>DR4, -</th>
<th>HLA-A26,31</th>
<th>B40,w62</th>
<th>DR5,w9</th>
<th>HLA-A26, -</th>
<th>B35,w54</th>
<th>DRw6,w8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative Response</td>
<td>3H-TdR Incorporated, cpm (SEM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autologous TCL</td>
<td>7,614 (1,075)</td>
<td>6,709 (1,240)</td>
<td>9,014 (1,125)</td>
<td>8,683 (713)</td>
<td>6,183 (174)</td>
<td>8,342 (665)</td>
<td>1,607 (296)</td>
<td>1,357 (371)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPA-B-CLL</td>
<td>10,258 (800)</td>
<td>9,988 (1,372)</td>
<td>12,388 (1,746)</td>
<td>11,673 (963)</td>
<td>12,653 (1,234)</td>
<td>11,550 (553)</td>
<td>2,253 (359)</td>
<td>1,911 (70)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autologous APC</td>
<td>13,287 (725)</td>
<td>13,408 (1,346)</td>
<td>13,408 (1,683)</td>
<td>11,187 (2,539)</td>
<td>13,145 (1,050)</td>
<td>8,863 (1,396)</td>
<td>9,407 (977)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Incorporation of 3H-TdR into TCLs generated from B-CLL patient Y.U. or TCLs from donors differing in HLA antigen expression in the presence of MMC-treated B-CLL cells, TPA-B-CLL cells from patient Y.U., or MMC-treated autologous PBMC was determined as described in Materials and Methods. The results shown are the mean counts per minute incorporated (SEM) of triplicate wells.

Table 3. Inhibition of Proliferative Response of TCLs by Anti-HLA-DR Monoclonal Antibody

<table>
<thead>
<tr>
<th>TCL</th>
<th>APC</th>
<th>No Antibody</th>
<th>HU-4</th>
<th>HU-20</th>
</tr>
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<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV-TCL</td>
<td>B-CLL</td>
<td>3,797 (407)</td>
<td>541 (126)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>TPA-B-CLL</td>
<td>7,955 (429)</td>
<td>1,327 (463)</td>
<td>ND</td>
</tr>
<tr>
<td>PPD-TCL</td>
<td>B-CLL</td>
<td>7,171 (774)</td>
<td>2,454 (280)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>TPA-B-CLL</td>
<td>13,448 (1,343)</td>
<td>3,053 (244)</td>
<td>ND</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV-TCL</td>
<td>B-CLL</td>
<td>3,901 (312)</td>
<td>479 (82)</td>
<td>501 (130)</td>
</tr>
<tr>
<td></td>
<td>TPA-B-CLL</td>
<td>6,753 (284)</td>
<td>1,925 (401)</td>
<td>1,096 (371)</td>
</tr>
<tr>
<td>PPD-TCL</td>
<td>B-CLL</td>
<td>5,728 (634)</td>
<td>1,439 (309)</td>
<td>1,018 (194)</td>
</tr>
<tr>
<td></td>
<td>TPA-B-CLL</td>
<td>10,333 (798)</td>
<td>3,835 (518)</td>
<td>2,857 (362)</td>
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</tbody>
</table>

Incorporation of 3H-TdR into 2 x 10^6 TCL cells from patient Y.U. in the presence of 2 x 10^6 MMC-treated autologous B-CLL or TPA-B-CLL cells with and without anti-HLA-DR monoclonal antibodies HU-4 or HU-20 was determined during the last 12 hours of a 72-hour incubation. The results shown are the mean counts per minute incorporated (SEM) of triplicate wells.
provide a costimulator such as IL-1 have been postulated. However, the reason why activated B cells are much more effective in antigen presentation than are resting B cells remains uncertain. Thus, it seems likely that an increase in HLA-DR expression may be one of the mechanisms underlying the enhanced capacity of TPA-B-CLL cells to present antigen in comparison with B-CLL cells, although other mechanisms induced by activation of B-CLL cells may exist. We are further investigating the different properties of antigen presentation between B-CLL cells and TPA-B-CLL cells.

Previous studies have demonstrated that the proliferative responses of human helper T cells are restricted by the HLA class II antigens DR, DQ, and DP. Among these, HLA-DR antigen is the most important restricted element in T cell–macrophage interaction. Therefore, we subsequently examined whether HLA-DR restriction exists between TCL and B-CLL cells. The data obtained from the experiments using an allogeneic TCL panel and a blocking experiment using anti–HLA-DR framework monoclonal antibody revealed that antigen presentation of B-CLL cells was also restricted by HLA-DR in the same fashion as in macrophages.

The significance of the antigen-presenting function of B-CLL cells in vivo is unknown. It has been reported that antимouse IgM antibody–treated mice from which B cells have been eliminated are incapable of generating antigenspecific T-cell proliferative responses. This finding suggests that B cells play a role as APC, certainly in vivo, and that leukemic cells present antigens to helper T cells in patients with B-CLL. From this viewpoint, it can be considered that regulation of the immune response is disordered in B-CLL patients due to the increased number of APC. A variety of immunodeficiencies develop in patients with B-CLL. One of the mechanisms of immunodeficiency found in B-CLL patients may be the results of an impaired immune response induced by the disordered antigen-presenting function of B-CLL cells.

In conclusion, we have clarified that B-CLL cells can present antigen to antigen-specific T cells in a manner similar to that of macrophages. B-CLL is characterized by a clonal expansion of mature B cells. Thus, the present experimental system using B-CLL cells as a source of APC should be useful for the investigation of T-cell–B-cell interaction at a clonal level.

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Antigen presentation in an HLA-DR-restricted fashion by B-cell chronic lymphocytic leukemia cells

M Yasukawa, T Shiroguchi, A Inatsuki and Y Kobayashi