CD4+ Cytotoxic T-Cell Clones Specific for bcr-abl b3a2 Fusion Peptide Augment Colony Formation by Chronic Myelogenous Leukemia Cells in a b3a2-Specific and HLA-DR–Restricted Manner

By Masaki Yasukawa, Hideki Ohminami, Shin Kaneko, Yoshihiro Yakushijin, Yasuharu Nishimura, Koiti Inokuchi, Tsuyoshi Miyakuni, Shinni Nakao, Kenji Kishi, Ichiro Kubonishi, Kazuo Dan, and Shigeru Fujita

Although it is well known that CD8+ cytotoxic T lymphocytes (CTLs) play an important role in the suppression of cancer cell growth, the significance of CD4+ CTLs in resistance to cancer is obscure. In an attempt to elucidate the role of CD4+ CTLs in immunosurveillance of chronic myelogenous leukemia (CML), we examined the immunologic functions of bcr-abl b3a2 fusion peptide-specific CD4+ CTL clones. Seven CD4+ T-cell clones that responded to stimulation with b3a2 peptide, but not with b2a2 peptide or physiological counterparts bcr b3b4 and abl 1A-a2 peptides, were established from two healthy individuals. Restriction elements of these clones were HLA-DRB1*0901. These CD4+ T-cell clones exhibited b3a2 peptide-specific and HLA-DRB1*0901-restricted cytotoxicity and produced interleukin-3 (IL-3), IL-4, IL-10, interferon-γ, tumor necrosis factor-α, and granulocyte-macrophage colony-stimulating factor in response to bcr-abl peptide stimulation, indicating they were Th0 clones. The numbers of HLA-DRB1*0901-positive b3a2, but not those of b2a2-positive or HLA-DRB1*0901-negative CML cell colonies increased when CML cells were cultured with b3a2-specific CD4+ CTL clones. These data suggest that bcr-abl-specific CD4+ CTLs recognize CML cells in an antigen-specific and HLA-DR–restricted manner, and that they do not inhibit, but in fact augment, CML cell growth.

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cytes can recognize the bcr-abl fusion peptide in the context of HLA class II molecules expressed on CML cells, resulting in the production of growth factors for CML cells.

**MATERIALS AND METHODS**

**Generation of bcr-abl fusion peptide-specific T-cell clones.** Peptides were synthesized to a minimum of 90% purity using an automated peptide synthesizer (Model 432A Synergy; Applied Biosystems Inc, Foster City, CA) with the Fmoc procedure. The sequences of the 17-mer amino acid peptides synthesized (underlined amino acids indicate the breakpoints with a new amino acid) were as follows: bcr-abl b3a2.

IPLINKKEALQRQEV; bcr-abl b3a2, ATGFKQSSKALQRQEV; bcr b3b4, ATGFKQSSNLYCTLEVD; and abl 1A-a2, 88SSCLEYLARQPV.

Peripheral blood mononuclear cells (PBMCs) from healthy individuals suspended in RPMI 1640 medium supplemented with 10% heat-inactivated human AB type serum (referred to hereafter as the culture medium) and synthetic peptide at a concentration of 10 µg/mL were seeded in round-bottom microtiter plate wells at a concentration of 1 × 10^3 cells/0.2 mL. After 7 days in culture, half of the medium was exchanged for fresh culture medium and a second stimulation was performed by addition of 1 × 10^5 autologous PBMCs treated with mitomycin C (MMC) as APCs and peptide at a concentration of 10 µg/mL. After a further 7 days, a third stimulation was performed for the second stimulation. Four days after the third stimulation with peptide, human recombinant interleukin-2 (IL-2; Boehringer-Mannheim, Mannheim, Germany) was added to each well at a concentration of 10 U/mL. The growing cells were transferred from the microtiter wells to 16-mm diameter wells and their proliferative responses were examined. Bulk cells showing a proliferative response to stimulation with bcr-abl fusion peptide were cloned by the limiting dilution method described previously.

**Generation of bcr-abl fusion peptide-specific T-cell clones.** T-cell clones were cultured continuously in IL-2–containing culture medium and MMC-treated autologous PBMCs and bcr-abl fusion peptide were added to the wells every 2 weeks.

**Proliferative response to synthetic peptide.** Proliferative response of T cells to stimulation with peptide was examined as described previously with a slight modification. Briefly, 2 × 10^5 T cells and 2 × 10^5 MMC-treated PBMCs or 3 × 10^5 MMC-treated HLA-DR gene-transfected murine L cells as a source of APCs in 0.2 mL culture medium were seeded into flat-bottom microtiter wells, to which synthetic peptide was added. In preliminary experiments to determine the optimal concentration of peptide, bcr-abl b3a2 fusion peptide-specific T-cell clones proliferated maximally in the presence of over 10 µg/mL peptide. Therefore, we used synthetic peptide at a concentration of 0.1 µg/mL for 2 hours. Then, various numbers of effector cells suspended in 0.1 mL assay medium were added to each well. After incubation for 4 hours, 0.1 mL supernatant was collected from each well and its radioactivity was determined using a gamma counter. For the experiment of specific 3^11Cr release was calculated as follows: (cpm experimental release – cpm spontaneous release)/(cpm maximal release – cpm spontaneous release) × 100. Each cytotoxicity assay was performed at least twice and yielded identical data.

**Cytotoxicity production.** T-cell clones were collected from the wells and washed twice with RPMI 1640 medium to remove IL-2 and any other cytokines present in the culture medium. Then 5 × 10^3 effector cells were cultured in 2 mL assay medium and cultured in 16-mm wells in the presence or absence of peptide. After 72 hours, the supernatant was collected from each well and assayed for the production of various cytokines by enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, MN).

**Detection of cytolytic mediator mRNA expression.** Expression of various cytolytic mediator mRNAs in the T-cell clones was investigated by reverse transcriptase-polymerase chain reaction (RT-PCR). The total RNA was extracted from each T-cell clone that had been stimulated with peptide 5 days previously and cDNA was synthesized by reverse transcription with Moloney murine leukemia virus RT, as described previously. Amplification of cDNAs by the PCR was performed using the following primers: perforin, 5′-ACCAAGAATGTGATGTTCTGTTA-3′; granulocyte/macrophage colony-stimulating factor (GM-CSF), 5′-GGGCTCACTGTTTCCT-3′; bcr-3; 5′-TGGAGGAGCGCTCATTGTTCTGCT-3′; Fas ligand, 5′-ATAGAAGCTTGTGCTCTTTGCTCTCCACTCTACAGAAGG-3′ and 5′-ATAGAAGCTTGTGCTCTTTGCTCTCCACTCTACAGAAGG-3′; and lymphotoxin-β, 5′-ATAAGCTTGAAGGGAGGAGCTGTCCAAAGGAGG-3′ and 5′-ATAAGCTTGAAGGGAGGAGCTGTCCAAAGGAGG-3′. The expected lengths of the amplified cytolytic mediator cDNAs were as follows: perforin, 459 bp; granulocyte/macrophage colony-stimulating factor, 180 bp; Fas ligand, 506 bp; TNF-α, 375 bp; and lymphotoxin-β, 655 bp.

** Colony formation assay.** The effects of the T-cell clones on CML cell growth were examined by performing the colony formation assays described previously with a slight modification. Bone marrow cells were isolated from patients with chronic-phase CML when the Ph chromosome was detected in all bone marrow cells analyzed and had been cryopreserved until use. Cloned T cells and CML bone marrow cells were seeded into assay medium at an E:T ratio of 5:1, centrifuged at 1,000 rpm for 3 minutes to ensure close cell contact, and then coincubated in assay medium at 37°C for 4 hours. To examine the role of HLA-DR in the interaction between cloned T cells and CML cells, CML bone marrow cells were preincubated with anti–HLA-DR MoAb before centrifugation with cloned T cells. Control CML bone marrow cells were centrifuged and incubated without T-cell clone in the same manner. After incubation, 5 mL Iscove’s Modified Dulbecco’s medium containing 1% methylcellulose, 5% GCT conditioned medium, 1% bovine serum albumin, 30% FCS, 10^-5 M 2-mercaptoethanol, and 3 U/mL erythropoietin (Stem cell CFU Kit; Baxter, Deerfield, IL) was added to the cell pellet at the final CML cell concentration of 1 × 10^5 cells/mL. Then, each cell suspension was plated in triplicate 24-mm wells and cultured at 37°C for 12 to 14 days, after which the numbers of colony-forming unit granulocyte-macrophage (CFU-GM) and burst-forming unit erythroid (BFU-E) were counted using an inverted microscope. For the colony assay, bone marrow cells were isolated from a HLA-DRB1*0901-positive patient with a nonhematopoietic disorder and cocultured with cloned T cells as described above. To examine the effect of soluble factors produced by T-cell clones on CML cell growth, the culture supernatants of T-cell clones that had been stimulated with peptide for 2 days were obtained, and then added to the CML bone marrow cells. The significance of differences between values.
Table 1. HLA-DRB1*0901-Restricted Proliferative Responses of bcr-abl b3a2-Specific CD4+ T-Cell Clones

<table>
<thead>
<tr>
<th>Clone</th>
<th>APC</th>
<th>MoAb Added</th>
<th>Peptide Stimulation</th>
<th>Proliferative Response (cpm) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MY-1</td>
<td>auto PBMC</td>
<td>None</td>
<td>b3a2</td>
<td>259 ± 37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b2a2</td>
<td>26,771 ± 2,628</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b3b4</td>
<td>324 ± 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1A-α2</td>
<td>332 ± 31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>anti-HLA-DR</td>
<td>b3a2</td>
<td>300 ± 29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>anti-HLA-DQ</td>
<td>b3a2</td>
<td>449 ± 60</td>
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<tr>
<td></td>
<td></td>
<td>anti-HLA-DP</td>
<td>b3a2</td>
<td>26,139 ± 4,559</td>
</tr>
<tr>
<td>TO-1</td>
<td>auto PBMC</td>
<td>None</td>
<td>b3a2</td>
<td>31,309 ± 3,237</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b2a2</td>
<td>279 ± 102</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b3b4</td>
<td>145 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1A-α2</td>
<td>157 ± 11</td>
</tr>
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<td></td>
<td></td>
<td>anti-HLA-DR</td>
<td>b3a2</td>
<td>308 ± 200</td>
</tr>
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<td></td>
<td></td>
<td>anti-HLA-DQ</td>
<td>b3a2</td>
<td>25,664 ± 523</td>
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<td></td>
<td></td>
<td>anti-HLA-DP</td>
<td>b3a2</td>
<td>29,731 ± 2,632</td>
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Table 2. Cytotoxicities of bcr-abl b3a2-Specific CD4+ T-Cell Clones

<table>
<thead>
<tr>
<th>Clone</th>
<th>Target</th>
<th>HLA-DRB1</th>
<th>Peptide</th>
<th>% Cytotoxicity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MY-1</td>
<td>auto-LCL</td>
<td>0901/1406</td>
<td>None</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b3a2</td>
<td>49.9</td>
</tr>
<tr>
<td></td>
<td>allo-LCL</td>
<td>#1 0901/0405</td>
<td>None</td>
<td>−0.4</td>
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<td></td>
<td></td>
<td></td>
<td>b3a2</td>
<td>49.4</td>
</tr>
<tr>
<td></td>
<td>allo-LCL</td>
<td>#2 0901/1406</td>
<td>None</td>
<td>0.8</td>
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<td></td>
<td></td>
<td>b3a2</td>
<td>48.0</td>
</tr>
<tr>
<td></td>
<td>allo-LCL</td>
<td>#3 1406/1502</td>
<td>None</td>
<td>−2.4</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>b3a2</td>
<td>0.2</td>
</tr>
<tr>
<td>TO-1</td>
<td>auto-LCL</td>
<td>0901/0405</td>
<td>None</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b3a2</td>
<td>55.6</td>
</tr>
<tr>
<td></td>
<td>allo-LCL</td>
<td>#1 0901/1406</td>
<td>None</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b3a2</td>
<td>56.9</td>
</tr>
<tr>
<td></td>
<td>allo-LCL</td>
<td>#2 091/0406</td>
<td>None</td>
<td>−0.2</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>b3a2</td>
<td>54.8</td>
</tr>
<tr>
<td></td>
<td>allo-LCL</td>
<td>#3 1406/1502</td>
<td>None</td>
<td>−0.1</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>b3a2</td>
<td>2.0</td>
</tr>
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</table>

Abbreviation: Exp., experiment.
*Incorporation of 3H-TdR into cloned T cells was determined in the presence of autologous APCs with and without various types of synthetic peptide and with and without anti-HLA MoAb.
†Incorporation of 3H-TdR into cloned T cells was determined in the presence of control L cells transfected with the selection marker NeoMouse.

RESULTS

Generation of T-cell clones directed against bcr-abl fusion peptide and analysis of HLA restriction. PBMCs from two healthy individuals, MY and TO, whose HLA-DR types were DRB1*0901/0401 and *0901/0405, respectively, were stimulated repeatedly with b2a2 or b3a2 fusion peptide in microtiter wells, as described in Materials and Methods. Of a total of 192 wells per donor seeded with PBMCs, three and four CD4+ T-cell clones that proliferated in response to stimulation with b3a2 peptide in the presence of autologous APCs were generated from MY and TO, respectively. A CD4+ T-cell clone that showed a proliferative response to stimulation with b2a2 peptide in an HLA-DRB1*1406-restricted manner was also generated from PBMCs of MY, but this clone stopped growing during the study. Thus, seven CD4+ T-cell clones, designated MY-1, MY-2, MY-3, TO-1, TO-2, TO-3, and TO-4, that proliferated in response to stimulation with b3a2 fusion peptide were used for further experiments. Southern blot analysis of the T-cell receptor-β genes of these T-cell clones showed distinct rearrangement patterns (data not shown), indicating that these seven T-cell clones originated from different T cells. Because identical results were obtained with each of these seven T-cell clones, only the data for MY-1 and TO-1 are presented. MY-1 and TO-1 proliferated when stimulated with the 17-mer b3a2 peptide, but did not do so in response to stimulation with b2a2 or physiological 17-mer counterpart peptides bcr b3b4 or abl 1A-α2. The proliferative responses of MY-1 and TO-1 to b3a2 peptide were inhibited by adding the anti-DR MoAb to the culture medium, but not by adding the anti-DQ or anti-DP MoAb, suggesting that the proliferative responses of MY-1 and TO-1 were restricted by HLA-DR (Table 1, Exp. 1). The restriction elements of both MY-1 and TO-1 seemed to be HLA-DR9, as both clones proliferated in response to peptide stimulation in the presence of allogeneic APCs bearing HLA-DR9, but not in the presence of HLA-DR9-negative allogeneic APCs (data not shown). To examine this further, we used HLA-DRA and HLA-DRB1*0901 gene-transfected murine L cells (L-DR9) as APCs. As shown in Table 1, Exp. 2, MY-1 and TO-1 proliferated in response to stimulation with the b3a2 peptide in the presence of L-DR9, but not in the presence of HLA-DRA and DRB1*0401 gene-transfected L cells (L-DR4) or control L cells transfected with the selection marker NeoMouse gene alone (L-Neo). These data show that the proliferative responses of MY-1 and TO-1 were restricted by HLA-DRB1*0901. In view of the results of a recent study on peptide motifs for the
HLA-DR9 molecule,23 the fourth amino acid (F) and the seventh amino acid (S) may be binding motifs for HLA-DR9 positions 1 and 4, respectively.

Cytotoxic reactivities of T-cell clones against peptide-loaded and -unloaded target cells. Next, we examined the ability of T-cell clones to lyse b3a2 peptide-loaded target cells. Table 2 shows the cytotoxicities of MY-1 and TO-1 to autologous and various allogeneic B-LCLs in the presence and absence of the peptide. MY-1 and TO-1 were strongly cytotoxic to b3a2 peptide-loaded autologous B-LCL. As with their proliferative responses, the b3a2 fusion peptide-specific cytotoxicities of MY-1 and TO-1 were restricted by HLA-DRB1*0901, as allogeneic targets bearing HLA-DRB1*0901, but HLA-DRB1*0901-negative allogeneic cells were not lysed by MY-1 and TO-1.

Cytokine production by bcr-abl–specific CD4+ T-cell clones. MY-1 and TO-1 were cultured with autologous MMC-treated PBMCs as APCs in the presence or absence of peptide and the supernatants were analyzed for the production of IL-3, IL-4, IL-10, interferon-γ (IFN-γ), TNF-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF). As shown in Table 3, MY-1 and TO-1 secreted all six cytokines after stimulation with b3a2 fusion peptide and, therefore, they were both classified as Th0 type CD4+ T-cell clones.

Expression of cytolytic mediators by T-cell clones. Because the mechanism underlying CD4+ CTL-mediated cytotoxicity is obscure, we examined the expression of cytolytic mediators reported to be important in cytotoxicity mediated by CD8+ and CD4+ CTLs and natural killer cells, namely perforin, granzyme B, Fas ligand, TNF-α, and lymphotoxin, using the RT-PCR. As shown in Fig 1, mRNAs for all cytolytic mediators examined were expressed in both MY-1 and TO-1. Flow cytometric analysis showed that TNF-α and lymphotoxin-α and -β were expressed on MY-1 and TO-1 as membrane-bound forms (data not shown).

Augmentation of CML cell colony formation by bcr-abl–specific CD4+ T-cell clones. We investigated whether CD4+ CTLs can inhibit CML cell growth by examining the effects of b3a2-specific CD4+ CTL clones on colony formation by CML cells. Unexpectedly, as shown in Fig 2A, the numbers of CFU-GM and BFU-E generated from bone marrow cells of two patients with b3a2 CML who were HLA-DRB1*0901 positive were augmented significantly after coculture with MY-1 and TO-1. The augmentative effects of MY-1 and TO-1 on CML cell colony formation were antigen specific and HLA-DRB1*0901 restricted, as these clones had no effect on colony formation by b2a2 type or HLA-DRB1*0901-negative CML cells and HLA-DRB1*0901-positive normal bone marrow cells. In addition, augmentation of CML cell colony formation by MY-1 and TO-1 was inhibited by anti–HLA-DR MoAb (Fig 2B). The numbers of colonies formed by b2a2 as well as b3a2 CML cells increased when they were cultured in the presence of MY-1 and TO-1 culture supernatants, in comparison with those of CML cells grown in the absence of the culture supernatants (Fig 2C). These data strongly suggest that CD4+ CTLs are not cytotoxic against CML cells, but in fact augment CML cell growth by producing myelostimulatory cytokines, such as IL-3 and GM-CSF, through recognition of leukemia cells in an antigen-specific and HLA-restricted manner.

DISCUSSION

Recently, reports describing the immunogenicity of synthetic bcr-abl fusion peptide to CD4+ as well as CD8+ T lymphocytes of healthy individuals have been accumulating. Binding of b3a2 fusion peptides to HLA class I alleles A3, A11, B8, and B44 has been reported and these peptides have been shown to prime CD8+ CTLs in vitro.4-8 Furthermore, b3a2 peptides have been shown to induce HLA-DR1(1DRB1*0101)-, DR2 (DRB1*1501)-, DR4 (DRB1*0401)-, and DR11 (DRB1*1101)-restricted proliferative responses of CD4+ T lymphocytes.9-12 In this study, we showed for the first time that b3a2 fusion peptide can also elicit a proliferative response of CD4+ T lymphocytes restricted by HLA-DR9 (DRB1*0901), the most frequent HLA-DR allele in the Japanese population. We also believe this study is the first in which b3a2-specific CD4+ CTL clones have been established.

It is now well known that endogenous and exogenous antigens are processed and expressed on MHC class I and class II, respectively. However, recent studies have shown that endogenous proteins can also be expressed on MHC class II molecules and recognized by CD4+ T lymphocytes.24-29 These reports suggest that bcr-abl–specific CD4+ T lymphocytes can
distinguish CML and normal cells directly via recognition of the bcr-abl fusion peptide in the context of HLA class II expressed on leukemic cells. The recent study of ten Bosch et al. showed that a b3a2-specific CD4\(^+\) T-cell line proliferated in response to stimulation with b3a2-positive CML blasts in an HLA-DR-restricted manner, which strongly suggests that CD4\(^+\) T lymphocytes can directly recognize bcr-abl fusion peptide that is naturally processed and expressed on CML cells. Although they observed a proliferative response of CD4\(^+\) T lymphocytes to CML cells, they did not show cytotoxic activity against CML blasts. In our study, although b3a2-specific CD4\(^+\) CTL clones exerted strong cytotoxicity against b3a2 peptide-loaded B-LCL, these clones did not inhibit, but actually augmented, colony formation by HLA-DR-matched CML cells. There are several possible explanations for the failure of CD4\(^+\) CTLs to inhibit CML cell growth. The first hypothesis is that the complexes of b3a2 peptide and HLA-DR molecules that CD4\(^+\) CTLs recognize are expressed only on some populations of chronic phase CML cells that have reached a certain differentiation level and that the CML progenitor cells that proliferate in in vitro assays do not express the bcr-abl fusion protein and/or HLA-DR. The second hypothesis is that CD4\(^+\) CTLs recognize bcr-abl fusion peptide in the context of the HLA class II expressed on CML cells, but the CML cells are resistant to CD4\(^+\) CTL-mediated cytotoxicity. MY-1 and TO-1 both seemed to express cytolytic mediators reported to be important for CTL- and natural killer cell-mediated cytotoxicity, namely perforin, granzyme B, Fas ligand, TNF-\(\alpha\), and lymphotoxin, but the main cytotoxic pathway of these CD4\(^+\) CTL clones is unknown. The Fas/Fas ligand system has been reported to account for the main pathway of CD4\(^+\) CTL-mediated cytotoxicity in a murine system and CML cells have been reported to be sensitive to Fas-mediated apoptosis. We found that colony formation by CML cells was inhibited markedly by adding the agonistic anti-Fas MoAb to the assay medium (data not shown). In the light of these findings, the second hypothesis seems unlikely, but it cannot be excluded, because recently, we found that peptide-specific human CD4\(^+\) CTL clones lysed peptide-loaded Fas-deficient mutant target cells (manuscript in preparation). Therefore, the details of the cytotoxic mechanism mediated by bcr-abl fusion peptide-specific CD4\(^+\) CTL clones and the sensitivities of CML cells to various cytolytic mediators need to be elucidated to resolve this issue. The third hypothesis, which we think is the most important, is that bcr-abl peptide cannot be expressed on CML cells as suggested by Pawelec et al., who showed that bcr-abl peptide-specific CD4\(^+\) T lymphocytes did not recognize CML cells. At present, the definitive reason for the lack of inhibition of CML cell growth by CD4\(^+\) CTLs is unknown and further studies to explore the above hypotheses are necessary.

Coculture of CML cells and bcr-abl peptide-specific CD4\(^+\) T-cell clones resulted in increased numbers of CML cell colonies in comparison with those formed by CML cells.
cultured alone. These augmentative effects of CD4+ T-cell clones on CML cell growth were b3a2 specific and HLA-DRB1*0901 restricted, suggesting that bcr-abl–specific CD4+ T lymphocytes can recognize bcr-abl fusion protein in the context of HLA-DR molecules. There are two possible mechanisms whereby bcr-abl protein is recognized by CD4+ T lymphocytes. One is that CD4+ T lymphocytes may directly recognize the bcr-abl peptide and HLA-DR molecule complexes expressed on CML cells. The report by ten Bosch et al10 supports this hypothesis, as described above, but further studies are necessary to confirm this issue, as conflicting data have been also reported.11 The other mechanism is that bcr-abl protein released from CML cells as a result of cell death was processed and expressed on live CML cells or residual normal APCs. Evidence that bcr-abl peptide–specific CD4+ T lymphocytes can respond to peptides derived from purified whole bcr-abl fusion protein and cell lysates containing bcr-abl protein is accumulating. Murine CD4+ T lymphocytes specific for b3a2 peptide have been shown to proliferate in response to whole- andfusion protein purified from a cell extract,35 and recently, human b3a2 peptide–specific CD4+ T lymphocytes were reported to respond to APCs exposed to b3a2-containing cell lysates.12 Furthermore, the findings of Jiang et al36 that CML cells can process and present exogenous antigens suggests that CML cells themselves can present antigens to CD4+ T lymphocytes. These recent reports lend strong support to our observation that bcr-abl–specific CD4+ T lymphocytes responded to bcr-abl fusion peptide derived from CML cells.

In summary, we have reported the establishment and functional characterization of bcr-abl–specific CD4+ CTL clones. It should be noted that the present data do not mean that bcr-abl–specific CD4+ T lymphocytes are ineffective for immunotherapy of CML. In animal models, the adoptive transfer of immune tumor antigen–specific CD8+ T lymphocytes alone had an antitumor effect, but the combination of antigen-specific CD4+ and CD8+ T lymphocytes was generally more effective.37 Therefore, the development of combination adoptive therapy involving various types of immunocompetent cells, including bcr-abl–specific CD4+ and CD8+ T lymphocytes, and effective APCs, such as dendritic cells, is expected to lead to the development of new effective immunotherapy for CML.

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CD4+ Cytotoxic T-Cell Clones Specific for bcr-abl b3a2 Fusion Peptide Augment Colony Formation by Chronic Myelogenous Leukemia Cells in a b3a2-Specific and HLA-DR–Restricted Manner

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