

Leukemia-associated fusion proteins, dek-can and bcr-abl, represent immunogenic HLA-DR-restricted epitopes recognized by fusion peptide-specific CD4⁺ T lymphocytes

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Although CD4⁺ helper T lymphocytes have been demonstrated to play an important role in antitumor immune response, only a few epitopes of tumor-associated antigens recognized by HLA class II-restricted CD4⁺ T lymphocytes have been identified. In the present study, we addressed the question of whether leukemia-associated fusion proteins are recognized by CD4⁺ T lymphocytes. Immature dendritic cells (DCs) were loaded with necrotic or apoptotic leukemia cells with t(6;9) or t(9;22) and then cocultured with the dek-can fusion peptide-specific or the bcr-abl fusion peptide-specific CD4⁺ T lymphocyte clone. The dek-can peptide-specific and bcr-abl peptide-specific CD4⁺ T lymphocyte clones produced interferon- γ (IFN- γ) when they were cocultured with HLA-DR-matched but not with mismatched DCs which had been loaded with apoptotic as well as necrotic leukemia cells with t(6;9) and t(9;22), respectively. IFN- γ production by CD4⁺ T lymphocyte clones in response to stimulation with DCs loaded with leukemia cells was inhibited by the anti-HLA-DR monoclonal antibody. These data indicate that the acute myelogenous leukemia-associated fusion protein, dek-can, and chronic myelogenous leukemia-associated fusion protein, bcr-abl, are both processed and presented by DCs to the fusion peptide-specific CD4⁺ T lymphocytes.

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Introduction

Recent clinical observations and experimental studies have revealed that the immunosurveillance system is very important for achieving the cure of malignancies, including leukemia. Among various kinds of immunocompetent cell, CD8⁺ cytotoxic T lymphocytes (CTLs) are undoubtedly considered to play a crucial role in eradication of malignant cells, since they lyse tumor cells directly via recognition of tumor-associated antigens in the context of major histocompatibility complex (MHC) class I which is expressed on the vast majority of tumor cells. On the basis of their direct cytotoxic effect on tumor cells, attention has been focused mainly on tumor-specific CD8⁺ CTLs, and various tumor-associated antigens recognized by MHC class I-restricted CD8⁺ T lymphocytes have been identified. On the other hand, tumor-specific CD4⁺ T lymphocytes have received far less attention, which is remarkable given their central role in regulating most antigen-specific immune responses.

A critical contribution by tumor-specific CD4⁺ T lymphocytes in the development of an effective antitumor response has been consistently found in several murine experimental

systems.^{1–3} In addition, the recent finding that CD8⁺ T lymphocyte-depleted donor lymphocyte infusion is effective for patients with relapsed chronic myelogenous leukemia (CML)⁴ strongly suggests that CD4⁺ T lymphocytes also play an important role in the antileukemia immune response in humans. Although attention has been recently focused on tumor-specific CD4⁺ T lymphocytes, the number of tumor-associated antigens identified so far which are recognized by CD4⁺ T lymphocytes is still limited. These include CDC27,⁵ MAGE-A3,^{6–9} NY-ESO-1,^{10,11} tyrosinase,^{12,13} Melan-A/MART-1,¹⁴ MUC-1,¹⁵ gp100,¹⁶ and Annexin II.¹⁷ These antigens are expressed mainly in melanoma and some solid tumors but not in leukemia, and therefore identification of leukemia-associated antigens recognized by CD4⁺ helper T lymphocytes has been expected.

Fusion proteins produced by chromosomal translocations are expressed in some types of leukemia. Among them, bcr-abl produced by t(9;22) is expressed in most cases of CML, and dek-can produced by t(6;9) is expressed in a certain type of acute myelogenous leukemia (AML). Since these fusion proteins are expressed only in leukemia cells and not in normal cells, their fusion portions can be considered as leukemia-specific antigens. Indeed, the induction of HLA class I-restricted bcr-abl fusion peptide-specific and ETV6-AML1 fusion peptide-specific CD8⁺ CTLs has been successful, and these CTLs appear to lyse leukemia cells in an antigen-specific manner.^{18–21} In the present study, we verified whether leukemia-associated fusion proteins are also recognized by CD4⁺ T lymphocytes. The data obtained from the present series of experiments revealed that dendritic cells (DCs), which are professional antigen-presenting cells (APCs), can process and present dek-can and bcr-abl fusion proteins to fusion peptide-specific CD4⁺ T lymphocytes in an HLA class II-restricted manner. It was also revealed that apoptotic as well as necrotic leukemia cells are both efficiently presented by DCs to CD4⁺ T lymphocytes. On the basis of the data obtained, we discuss the role of leukemia-associated antigen-specific CD4⁺ T lymphocytes in the antileukemia immune response.

Materials and methods

Cell lines

FKH-1 is a human myeloid leukemia cell line with t(6;9)(p23;q24) expressing a dek-can chimeric transcript.²² K562 is a cell line established from a patient with CML in blast crisis.²³ The bcr-abl (b3a2) fusion protein is produced in K562 cells. These cell lines were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS).

Generation of dek-can and bcr-abl fusion peptide-specific CD4⁺ lymphocyte clones

The dek-can and bcr-abl (b3a2) fusion peptide-specific CD4⁺ T lymphocyte clones were generated as described previously.^{24,25} Briefly, peptides were synthesized to a minimum of 90% purity using an automated peptide synthesizer (Model 432A Synergy; Applied Biosystems, Foster City, CA, USA) with the Fmoc procedure. The sequences of the dek-can and bcr-abl amino acid peptides synthesized were as follows, where '-' and '┘' indicate the breakpoint of each fusion protein: dek-can, TMKQICKK-EIRRLHQY; bcr-abl (b3a2), ATGFKQSSKALQRPVAS. Peripheral blood mononuclear cells (PBMCs) from healthy individuals were 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, and seeded in round-bottomed microtiter plate wells at a concentration of 1×10^5 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 as 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. Then, 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 each fusion peptide were cloned by the limiting dilution method described previously.²⁶

Detection of IFN-γ production by fusion peptide-specific CD4⁺ T lymphocyte clones in response to stimulation with the fusion peptides

Response of T lymphocyte clones to stimulation with the fusion peptides was examined by determining IFN-γ production. For the assays of IFN-γ production, 1×10^5 clone cells and 2×10^5 MMC-treated PBMCs or 2×10^4 MMC-treated HLA-DR gene-transfected murine L cells as a source of APCs were suspended in 0.2 ml RPMI 1640 medium supplemented with 10% FCS and cultured in flat-bottomed microtiter wells in the presence or absence of peptide. After 72 h, the supernatants were collected from each well and assayed for IFN-γ production by enzyme-linked immunosorbent assay (ELISA) (Endogen, Rockford, IL, USA). To determine the restriction element governing the interaction between the T lymphocyte clones and APCs, monoclonal antibody (MoAb) L243 (anti-HLA-DR) (ATCC, Rockville, MD, USA) or w6/32 (anti-HLA class I) (ATCC) was added to the wells at an optimal concentration, and the inhibitory effect of the each MoAb on IFN-γ production by the T lymphocyte clones was examined.

Induction of necrosis and apoptosis of leukemia cell lines

Necrotic cells were obtained by freezing and thawing. After centrifugation, the cell pellet was used as the source of necrotic cells. Apoptosis of cells was induced by 6 J/cm² ultraviolet (UV) irradiation, then the cells were washed and kept

for 6 h in culture to allow apoptosis to occur. To confirm that leukemic cells were dying by apoptosis or necrosis, DNA fragmentation analyses were performed.²⁷ The cell pellet was lysed with hypotonic lysis buffer, and the DNA was extracted from each lysate using a DNA extraction kit (Sepa Gene; Sanko Junyaku Co, Tokyo, Japan). The samples were electrophoresed in 2% agarose gel, which was then stained with ethidium bromide and visualized by transillumination with UV light.

Coculture of fusion peptide-specific CD4⁺ T lymphocyte clones with immature DCs loaded with leukemia cells

Peripheral blood monocyte-derived immature DCs were generated as described previously.²⁸ Briefly, monocytes were isolated from PBMCs of healthy individuals. Plastic-adherent cells were cultured in RPMI 1640 medium supplemented with 10% FCS, 500 U/ml recombinant human interleukin-4 (IL-4) (Genzyme, Boston, MA, USA), and 800 U/ml recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) (Kirin Brewery, Tokyo, Japan). On day 3 of incubation, fresh medium supplemented with IL-4 and GM-CSF was added. On day 7, the cells were harvested and used as monocyte-derived immature DCs. Generated cells showed typical surface markers of immature DCs (CD1a⁺, CD14⁻, CD80⁺, CD83⁻, and CD86⁺). To examine whether immature DCs can capture apoptotic and necrotic leukemia cells and present antigens to CD4⁺ T lymphocytes, 2×10^4 immature DCs and various numbers of apoptotic or necrotic leukemic cells were suspended in 0.1 ml RPMI 1640 medium supplemented with 10% FCS and cultured in flat-bottomed microtiter wells. After 6 h, 1×10^5 fusion peptide-specific T lymphocyte clone cells were added to the wells. After 3 days of culture, the supernatants were collected from each well and assayed for IFN-γ production as described above. In some experiments, DCs were fixed with glutaraldehyde, as described previously.²⁹ Briefly, immature DCs were washed and suspended in PBS at 10^7 cells/ml. Glutaraldehyde (Sigma-Aldrich Japan, Tokyo, Japan) was added to the cell suspension at a final concentration of 0.0025%, then incubation was carried out for 15 s at room temperature. An equal volume of 0.2 M L-lysine in PBS was then added, mixed gently, and incubation was continued for 1 min at room temperature. DCs were washed twice with PBS and used as the fixed DCs.

Results

HLA-DR-restricted IFN-γ production by fusion peptide-specific CD4⁺ T lymphocyte clones in response to stimulation with the fusion peptides

In the present study, we used a dek-can fusion peptide-specific CD4⁺ T lymphocyte clone, HO-1, and bcr-abl (b3a2) fusion peptide-specific CD4⁺ T lymphocyte clone, MY-1. Previous data showed that the proliferative responses of HO-1 and MY-1 were restricted by HLA-DR53 and HLA-DR9, respectively.^{24,25} Since examination of IFN-γ production is a sensitive method for detecting the Th1 response to antigen stimulation, this approach was employed to detect the immune response of CD4⁺ T lymphocyte clones in the present study. We first examined the HLA restriction of IFN-γ production by CD4⁺ T lymphocyte clones. HO-1 secreted IFN-γ

in response to stimulation with the dek-can fusion peptide in the presence of HLA-DR53-positive allogeneic PBMCs or HLA-DR53 gene-transfected murine L cells (L-DR53), but not in the presence of HLA-DR53-negative allogeneic PBMCs or control L cells which were transfected with the selection marker gene (L-Neo) alone (Figure 1a). Similarly, MY-1 secreted IFN- γ in response to stimulation with b3a2 fusion peptide in the presence of HLA-DR9-positive allogeneic PBMCs or HLA-DR9 gene-transfected L cells (L-DR9), but not in the presence of HLA-DR9-negative cells (Figure 1b). These data show that the IFN- γ production as well as proliferative

response of HO-1 and MY-1 to the peptide stimulation are restricted by HLA-DR53 and HLA-DR9, respectively.

Necrosis and apoptosis of leukemia cells

It is well known that degradation of DNA into a ladder pattern of nucleosome-sized fragments is detectable in apoptotic, but not in necrotic, cells by agarose gel electrophoresis. As shown in Figure 2, the ladder pattern was detected in DNAs from FKH-1 and K562 cells which had been irradiated with UV light. In contrast, no fragmentation was detected in DNAs from cell lines which had been treated by freezing and thawing. These data confirmed that FKH-1 and K562 cell lines were undergoing cell death with the classic features of apoptosis and necrosis as a result of UV irradiation and freezing and thawing, respectively.

IFN- γ production by fusion peptide-specific CD4⁺ T lymphocyte clones in response to stimulation with DCs loaded with leukemia cells

We addressed the question of whether fusion peptide-specific CD4⁺ T lymphocyte clones can recognize fusion proteins in apoptotic and necrotic leukemia cells. To verify this possibility, the fusion peptide-specific CD4⁺ T lymphocyte clones were incubated with autologous immature DCs which had been loaded with necrotic or apoptotic leukemia cells. As shown in Figure 3a, HO-1 secreted IFN- γ in response to stimulation with autologous DCs which had been incubated with apoptotic and necrotic FKH-1. Since the degrees of IFN- γ production by HO-1 in the presence of DCs alone and DCs loaded with K562 were scarce, the production of IFN- γ by HO-1 was considered to be dek-can-specific. Similarly, IFN- γ production by MY-1 cells was detected when they were cocultured with autologous DCs which had been loaded with apoptotic and necrotic K562, but not without leukemia cells or with FKH-1 (Figure 3b). These data indicate that dead leu-

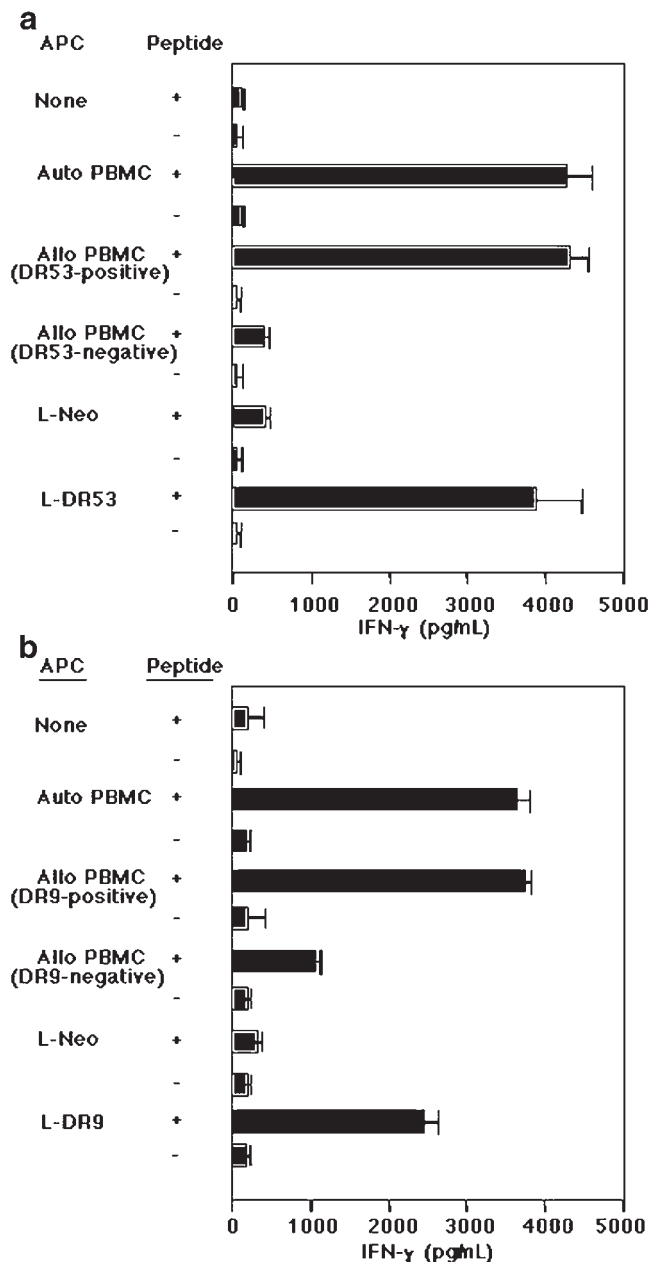


Figure 1 HLA-DR-restricted IFN- γ production of fusion peptide-specific CD4⁺ T lymphocyte clones. The dek-can fusion peptide-specific clone, HO-1 (a) and the bcr-abl (b3a2) fusion peptide-specific clone, MY-1 (b) were cultured in the presence of various kinds of APC with or without the peptide. After 3 days, the culture supernatants were harvested and assayed for IFN- γ production by ELISA. The data are expressed as the mean counts \pm standard deviations of three wells.

M 1 2 3 4 5 6

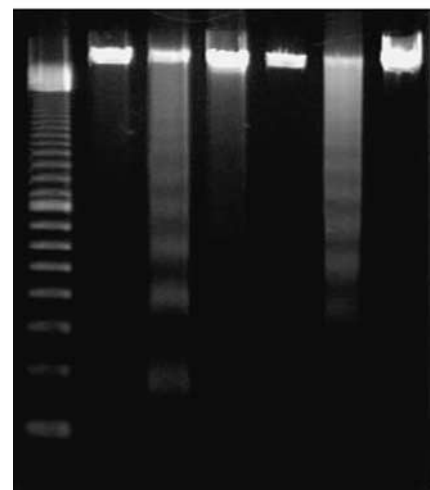


Figure 2 Agarose gel electrophoresis of cellular DNA. DNAs extracted from intact FKH-1 (lane 1), UV-irradiated FKH-1 (lane 2), frozen and thawed FKH-1 (lane 3), intact K562 (lane 4), UV-irradiated K562 (lane 5), frozen and thawed K562 (lane 6) were electrophoresed in 2% agarose gel. Lane M shows 100-bp ladder markers.

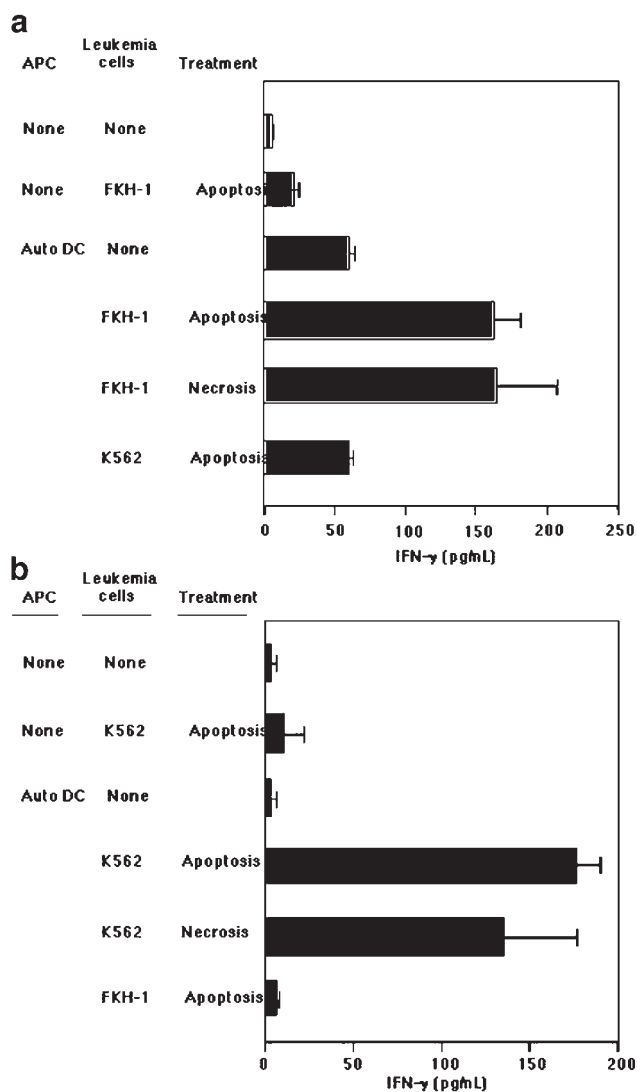


Figure 3 Recognition of apoptotic and necrotic leukemia cells by fusion peptide-specific CD4⁺ T lymphocyte clones. The dek-can fusion peptide-specific clone, HO-1 (a) and the bcr-abl (b3a2) fusion peptide-specific clone, MY-1 (b) were cocultured with autologous immature DCs which had been loaded with various kinds of leukemia cells. After 3 days, the culture supernatants were harvested and assayed for IFN- γ production by ELISA. The data are expressed as the mean counts \pm standard deviations of three wells.

kemia cells were efficiently captured by immature DCs and that dek-can and bcr-abl fusion proteins in apoptotic as well as necrotic leukemia cells can be processed and presented by autologous DCs to the fusion peptide-specific CD4⁺ T lymphocyte clones.

To confirm that fusion peptide-specific CD4⁺ T lymphocytes recognize the peptides processed from fusion proteins by viable DCs, but not peptides contaminating the leukemia cell preparations, fixed DCs were used as APCs. As shown in Figure 4, fixed DCs were able to present fusion peptides to CD4⁺ T lymphocytes, but failed to present fusion proteins in apoptotic or necrotic leukemia cells, indicating that the fusion proteins, dek-can and bcr-abl, were actually processed by immature DCs.

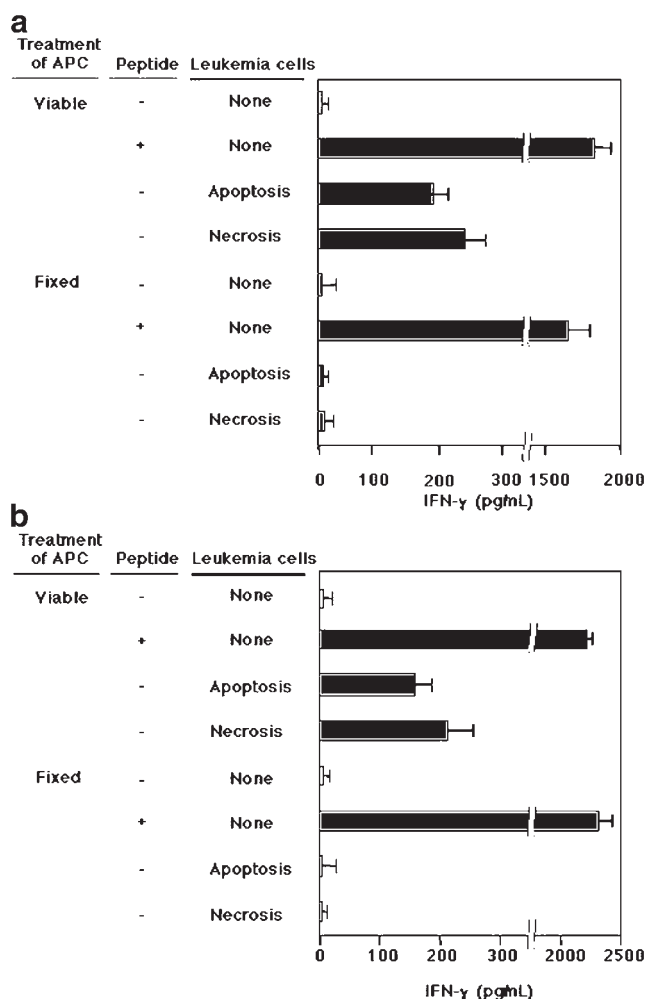


Figure 4 Antigen presentation of viable and fixed DCs to CD4⁺ T lymphocyte clones. The dek-can fusion peptide specific clone, HO-1 (a) and the bcr-abl (b3a2) fusion peptide-specific clone, MY-1 (b) were cocultured with viable or fixed autologous immature DCs which had been loaded with the peptide or leukemia cells. After 3 days, the culture supernatants were harvested and assayed for IFN- γ production by ELISA. The data are expressed as the mean counts \pm standard deviations of three wells.

HLA-DR-restricted recognition of DCs loaded with leukemia cells by fusion peptide-specific CD4⁺ T lymphocyte clones

We next examined the HLA restriction of IFN- γ production by fusion peptide-specific CD4⁺ T lymphocyte clones in response to DCs loaded with leukemia cells. As shown in Figure 5a, IFN- γ production by HO-1 was inhibited by adding anti-HLA-DR, but not anti-HLA class I MoAb, to the culture medium. In addition, IFN- γ production was not detected when HO-1 was cocultured with HLA-DR53-negative DCs loaded with apoptotic FKH-1. Similarly, IFN- γ production by MY-1 was inhibited by adding anti-HLA-DR MoAb to the culture medium and was not detected in coculture with HLA-DR9-negative DCs (Figure 5b). These data strongly suggest that dek-can and bcr-abl fusion proteins in leukemia cells are processed and presented by DCs to fusion peptide-specific CD4⁺ T lymphocytes in the context of HLA-DR molecules.

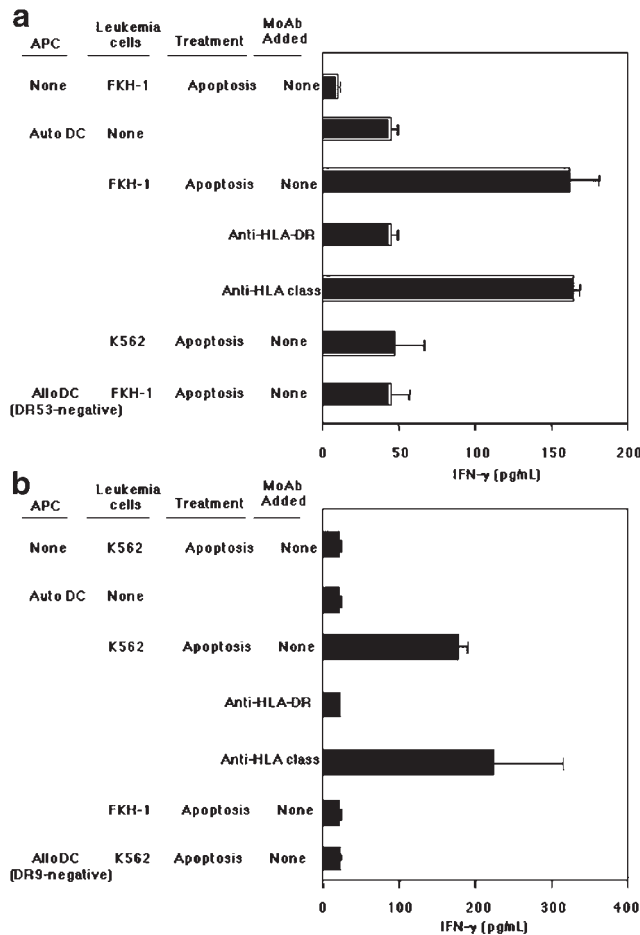


Figure 5 HLA-DR-restricted recognition of apoptotic leukemia cells by fusion peptide-specific CD4⁺ T lymphocyte clones. The dek-can fusion peptide specific clone, HO-1 (a) and the bcr-abl (b3a2) fusion peptide-specific clone, MY-1 (b) were cocultured with autologous and allogeneic immature DCs which had been loaded with apoptotic leukemia cells in the presence or absence of MoAb. After 3 days, the culture supernatants were harvested and assayed for IFN-γ production by ELISA. The data are expressed as the mean counts ± standard deviations of three wells.

Recognition of DCs loaded with various numbers of necrotic or apoptotic leukemia cells by fusion peptide-specific CD4⁺ T lymphocyte clones

We finally compared the efficiencies of processing fusion proteins in necrotic and apoptotic leukemia cells by DCs. IFN-γ production by fusion peptide-specific CD4⁺ T lymphocyte clones in response to stimulation with DCs loaded with various numbers of necrotic or apoptotic leukemia cells was examined. As shown in Figure 6, it appeared that IFN-γ production by CD4⁺ T lymphocytes in response to necrotic and apoptotic leukemia cells was dependent on the number of leukemia cells and that there was no significant difference between the levels of IFN-γ produced by CD4⁺ T lymphocytes in response to stimulation with DCs loaded with various numbers of necrotic and apoptotic leukemia cells. These data suggest that processing efficiencies of fusion proteins in necrotic and apoptotic leukemia cells by DCs are almost the same.

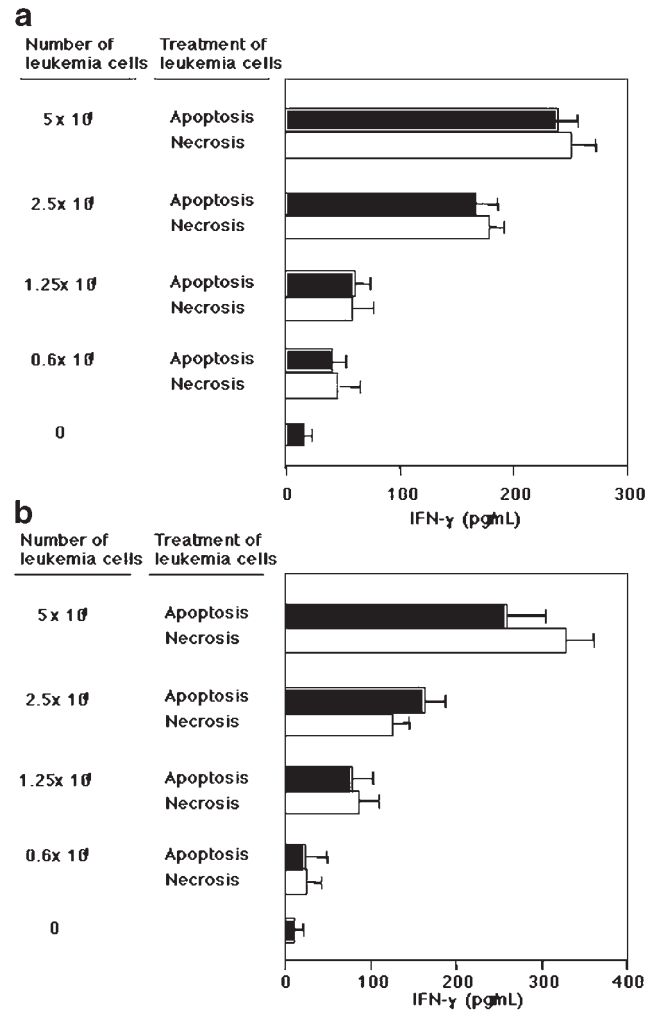


Figure 6 Recognition of various numbers of apoptotic and necrotic leukemia cells by fusion peptide-specific CD4⁺ T lymphocyte clones. The dek-can fusion peptide-specific clone, HO-1 (a) and the bcr-abl (b3a2) fusion peptide-specific clone, MY-1 (b) were cocultured with autologous immature DCs which had been loaded with or without various numbers of leukemia cells. After 3 days, the culture supernatants were harvested and assayed for IFN-γ production by ELISA. The data are expressed as the mean counts ± standard deviations of three wells.

Discussion

Since activation of CD4⁺ helper T lymphocytes initiates the immune response against tumor cells as well as microorganisms, identification of CD4⁺ T lymphocyte epitopes on tumor-associated antigens is essential in order to develop effective cancer immunotherapy.^{30,31} In the present study, we have demonstrated that DCs can process and present dek-can and bcr-abl fusion proteins, which are specifically produced in leukemia cells, to fusion peptide-specific CD4⁺ T lymphocytes in the context of HLA-DR53 and DR9, respectively. These data indicate that dek-can and bcr-abl fusion proteins are leukemia-associated antigens recognized by HLA class II-restricted CD4⁺ T lymphocytes. The present study also revealed that apoptotic and necrotic leukemia cells are both efficiently captured by DCs and that intracellular antigens in both types of dead cells are processed and presented to CD4⁺ T lymphocytes with equal efficiency.

The dek-can fusion protein is produced by t(6;9)(p23;q34)

and is associated with a specific subtype of AML.³² It is well known that the prognosis of AML with t(6;9) is very poor, and accordingly novel therapeutic strategies, including immunotherapy, for this AML subtype have been expected. In the present study, we have demonstrated that dek-can fusion protein represents the immunogenic epitope recognized by HLA-DR53-restricted CD4⁺ T lymphocytes. CD4⁺ T lymphocytes exert several distinct antitumor effector functions. First, they produce soluble factors priming and activating CD8⁺ CTLs in response to stimulation with antigen in the context of MHC class II molecules.³³ Furthermore, it has been reported that CD4⁺ T lymphocytes can eliminate MHC class II-negative tumor cells independently of CD8⁺ CTLs. IFN- γ has been proposed as one of the mediators for this CD8⁺ T lymphocyte-independent antitumor effect.³⁴ In addition to these functions, a subpopulation of CD4⁺ T lymphocytes can recognize viral and tumor-associated antigens in the context of MHC class II molecules and directly lyse MHC class II-positive cells.^{35,36} We reported previously that a dek-can-specific CD4⁺ T lymphocyte clone, HO-1, exerted cytotoxicity against dek-can fusion peptide-loaded target cells in an HLA-DR53-restricted manner.²⁴ Since the t(6;9)-positive leukemia cell line, FKH-1, expresses HLA-DR53 on its cell surface, we addressed the question of whether HO-1 can recognize the dek-can fusion peptide in the context of HLA-DR53 and directly lyse FKH-1 cells. We found that HO-1 failed to produce IFN- γ or lyse FKH-1 cells without addition of the exogenous peptide (data not shown). These findings suggest that the dek-can fusion protein may not be naturally processed via the HLA class II-presenting pathway in leukemia cells and that the conventional exogenous antigen-processing pathway is essential for presenting dek-can fusion protein to CD4⁺ T lymphocytes.

The bcr-abl fusion protein is produced by t(9;22)(q34;q11) and detected in most cases of CML. It has been reported that the bcr-abl (b3a2) fusion peptide induces a CD4⁺ T lymphocyte response which is restricted by HLA class II molecules, including DR1, DR2, DR3, DR4, DR9 and DR11.^{25,37–41} Mannerling *et al*⁴⁰ reported that bcr-abl (b3a2) fusion peptide-specific CD4⁺ T lymphocytes responded to DCs which were loaded with b3a2-positive, but not b2a2-positive, CML cell lysates. ten Bosch *et al*⁴¹ reported that bcr-abl (b3a2) peptide-specific CD4⁺ T lymphocytes were able to recognize b3a2 fusion protein-expressing blasts from a patient with CML blast crisis in an HLA-DRB1*0401-restricted manner. They have also reported that CD4⁺ T lymphocyte clones specific for the bcr-abl (b2a2) fusion peptide responded to an autologous B lymphoblastoid cell line transfected with invariant chain cDNA in which the HLA class II-associated invariant chain peptide was replaced by a b2a2 fusion oligonucleotide sequence.⁴² We previously demonstrated that colony formation by CML cells was augmented by b3a2 peptide-specific CD4⁺ T lymphocyte clones in a b3a2-specific and HLA-DR-restricted manner,²⁵ and that CML-derived mature DCs presented endogenous bcr-abl (b3a2) fusion protein to b3a2 peptide-specific CD4⁺ T lymphocytes.⁴³ In the present study, we have demonstrated that dead CML cells are captured and bcr-abl fusion proteins produced by CML cells are efficiently processed and presented by normal monocyte-derived DCs to bcr-abl fusion peptide-specific CD4⁺ T lymphocytes in an HLA class II-restricted manner. These data indicate that bcr-abl fusion protein is capable of being presented to CD4⁺ T lymphocytes via exogenous, as well as endogenous, antigen-processing pathways.

Accumulating evidence suggests that combined application of MHC class I- and class II-binding peptides derived from

the same tumor antigen has potential for efficient antitumor immunity. That is, simultaneous vaccination with the tumor-specific helper T lymphocyte and CTL epitopes results in a synergistic antitumor effect.⁴⁴ It has been reported that in addition to bcr-abl-specific CD4⁺ helper T lymphocytes, CD8⁺ CTLs are also generated by stimulating CD8⁺ T lymphocytes with bcr-abl fusion peptide-loaded DCs,^{18–20} indicating that the fusion portion of bcr-abl protein contains both helper T lymphocyte and CTL epitopes. Therefore, bcr-abl fusion peptide is considered as the optimal peptide vaccine for induction of an anti-CML immune response.

There are two major types of cell death: necrosis and apoptosis. Previous reports have shown that necrotic as well as apoptotic cells can function as a source of antigens for CD8⁺ T lymphocyte priming.^{45,46} It has been reported that immature DCs efficiently phagocytose both apoptotic and necrotic cells and present antigens from the internalized dying cells to MHC class II-restricted T lymphocytes.⁴⁷ Although recent papers have described that necrotic as well as apoptotic tumor cells are both efficiently captured by DCs and that the antigens they contain are equally processed and presented by DCs to CD4⁺ and CD8⁺ T lymphocytes as described above, conflicting data have also been obtained. It has been reported that only DCs pulsed with apoptotic cells are able to induce the CD8⁺ T lymphocyte response,⁴⁸ and that DCs can distinguish these two types of tumor cell death. That is, the optimal cross-presentation of antigens from tumor cells requires two steps: phagocytosis of apoptotic cells by immature DCs, which provides antigenic peptides for MHC class I and class II presentation, and a maturation signal that is delivered by exposure to necrotic tumor cells.⁴⁹ In the present study, dek-can and bcr-abl fusion proteins in both necrotic and apoptotic leukemia cells appeared to be presented efficiently by immature DCs to CD4⁺ T lymphocytes, suggesting that leukemia cells showing both types of cell death are effective for priming the CD4⁺ T lymphocyte response by DCs.

In summary, we have demonstrated that the leukemia-associated fusion proteins, dek-can and bcr-abl, in necrotic as well as apoptotic cells represent CD4⁺ helper T lymphocyte epitopes. The recent identification of HLA class II-restricted tumor epitopes should facilitate the development of improved vaccines that have potential for the induction and maintenance of CTL responses via stimulation of tumor-reactive helper T lymphocytes. When considering the importance of CD4⁺ helper T lymphocytes for efficient antitumor immune responses, vaccination with dek-can and bcr-abl fusion peptides will very likely provide an effective form of anti-leukemia immunotherapy.

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