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Original Article

The Comparison of Cytotoxic T-Lymphocyte Effects of Dendritic Cells Stimulated by the Folate Binding Protein Peptide Cultured with IL-15 and IL-2 in Solid Tumor

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The current modalities for treating cancer employ not only single but multiple approaches involving surgery, radiotherapy and chemotherapy. Unfortunately, the survival outcome is not promising even with these approaches. Alternative approaches for cancer therapy are now emerging. Immunotherapy is aiming at both increasing the power, and in redirecting the specificity of the patients' immune system to attack the tumor cells. Recently, many studies using tumor associated lymphocytes (TAL) isolated from malignant ascites cultured in a media containing interleukin-2 exhibit antitumor responses. IL-2 is a lymphokine produced by T-cells. It facilitates activation, sustained growth and rescue from apoptosis. Lately, newly developed IL-15 has also exhibited antitumor activity similar to IL-2. IL-15 is a newly described cytokine produced from monocytes-marcrophages and T-cells. It has a different molecular structure but it functions like IL-2 by binding to the IL-2R β and γc chain. These antitumor responses are mediated by the cytotoxic T lymphocytes (CTL) that recognize the antigen in the context of the MHC molecules using the T cell receptors. CD8+-CTL recognize the peptide epitopes that are processed from the cellular proteins in the context of the MHC class I molecules. These peptides have a restricted length of 8-11 amino acids. The folate binding protein (FBP) is over-expressed in over 90% of ovarian and 20-50% in breast cancers. The FBP is the source of the antigenic peptides that are recognized by a number of these CTL-TAL, and is antigenic to both ovarian and breast cancer *in vivo*.

To define the antitumor response of IL-15 and its' FBP immunogenicity, a peptide defining epitope E39 and E75 were presented by the PMBC derived dendritic cells (DC) from healthy donors isolated by the CD14 method to ovarian and breast CTL-TAL. Stimulating both ovarian and breast CTL-TAL by E39 or E75 pulsed DC (DC-E39, DC-E75), in the presence of IL-15 and IL-2 can rapidly enhance or induce the E39 or E75 specific CTL activity. The antitumor activities were measured by a chromium release assay for the tumor specific lysis activity using the ovarian and breast cancer cell lines. The tumor specific lysis activity for the ovarian TALs for IL-15 vs IL-2 were 28.6 $\pm$ 3.9% and 30.3 $\pm$ 3.2%, respectively and in the breast TALs, they were 14.8 $\pm$ 3.1% vs 13.5 $\pm$ 2.9%, respectively. Using autologous tumor cells, a slightly higher tumor specific lysis activity was obtained for the ovarian TALs cultured in IL-15 compared to IL-2 (72.0 $\pm$ 8.2% vs 68.5 $\pm$ 3.6%). However, for the breast TALs, they were 39.5 $\pm$ 4.2% vs 41.5 $\pm$ 3.3%, respectively.

IL-15 is a newly developed cytokine that shows promising antitumor activity similar to IL-2. However, it requires lower dosage and is less toxic. Therefore, IL-15 might be a potential anticancer immunotherapeutic agent.

Key Words: Folate binding protein, cytotoxic T-lymphocytes, tumor associated lymphocytes, IL-2, IL-15.

INTRODUCTION

The results of treating advanced cancer patients are often very disappointing. The current modalities for treating cancer employ not only a single but also a combined approach involving surgery, radiotherapy and chemotherapy. Unfortunately, the survival outcome is not so promising even with these multi-modalities. However, an alternative new approach for cancer therapy, immunotheray is now emerging.[1] The main goal of immunotherapy is to reinforce and bolster the immune system by redirecting the specificity of

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the patient's immune system to attack the cancer cells resulting in a rejection of the tumor. Successful immunotherapy is based on the recognition of a tumor antigen by host T-cells. The identification of a tumor antigen that can be recognized by the CTL in melanoma as well as in other cancers such as ovarian cancer has attracted interest for developing novel molecular cancer therapies based on the tumor Ag stimulation of the CTL.[2,3] Since the tumor Ag recognized by the CTL consists of short amino acid sequences (8-11 residues long), which define the epitopes presented by the MHC-I molecules, the central hypothesis of all these studies is that these specific sequences can induce anti-tumor CTL immunity. The definition of the immunogenicity of these epitopes is based on their ability to stimulate the CTL both *in vitro* and *in vivo* to expand and express the specific CTL function.[4] Although T cell stimulation and vaccination with short defined sequences is expected to overcome the concerns of the specificity of recognition and focus the responses to a well defined epitope, tumor specific CTL stimulation/induction by short peptides has encountered some difficulties.[5-8] This is expected given the reported complexities in inducing a CTL capable of recognizing an endogenously presented Ag, after being stimulated with exogenously added monomer peptides.[9,10] In general, exogenous peptides pulsed on various APCs (antigen presenting cells) inadequately stimulate CD8+ cells from the PBMC, and lead to a CTL that-recognizes to a greater extent-the exogenous but not the endogenously presented Ag. Ongoing studies have focused on approaches to overcome the poor immunogenicity of the tumor Ag when delivered in a peptide form. One of these approaches uses the DC as an APC. This aims to enhance the peptide immunogenicity by increasing both the Ag levels and the levels of the co-stimulatory molecules. because the DC has the capacity to uptake higher amounts of peptides than other APCs. While the DC approach appears to require fewer stimulation cycles for CTL induction than the PBMC as an APC, and its use for therapeutic purposes depends on the availability of DC precursors. This is an important issue for cancer patients particularly for those with advanced disease with low blood counts and functionally impaired DCs.[11,12]

The fact that a PBMC derived DC cultured in GM-CSF plus IL-4 exhibit poor proliferation and a limited life span,[13] has raised the possibility of using the DC from healthy donors as an APC for stimulating the CTL. Tumor infiltrating lymphocytes (TIL) and/or TAL show a higher frequency of Ag-specific CTL than the PBMC and consist of activated memory effectors.[14] This has raised the possibility of stimulating the TIL/TAL with a peptide pulsed DC to expand the Ag-specific clonal populations, based on the rationale that lower Ag concentrations and fewer co-stimulatory interactions are needed for memory activation than naive T cells.

The culturing media for the TILs and/or TALs is very important in inducing the CTL. IL-2 is produced transiently by the T-lymphocytes in response to an antigenic stimulation, and is a central regulator of the acute phase of the immune response. IL-2 acts as a strong growth factor, promoting the expansion of the activated T cells population.[15] It signals through a receptor that is composed of an IL-2 specific α and β-chain and a common γ chain. IL-15 is newly described cytokines clone from the simian kidney epithelial cell line, CV1/EBNA. The IL-15 gene was mapped to chromosome 4q31.[16] Although IL-15 does not show a sequence homology with IL-2, both cytokines share many biological functions. IL-15 induces the proliferation of the CD8+ T cell clone, CTLL-2, and the proliferatin of the phytohemagglutinin- activated CD4+ and CD8+ human peripheral blood T lymphocytes.[15]

In this study, ovarian and breast TALs from ten distinct patients were stimulated with the peptide pulsed allogeneic DC cultured in IL-15 and IL-2 in order. The peptide used for stimulation corresponded to an immunodominant CTL epitope mapping of the amino acids, 191 - 199 of the FBP, which is widely overexpressed in ovarian (90%) and breast (20 - 50%)cancer patients and a newly identified tumor Ag.[13,14,17]

MATERIALS AND METHODS

Cytokines

The following cytokines were used in this study:

GM-CSF (Immunex corp., Seattle, WA, U.S.A.), specific activity 12.5×10^7 CFU/250 mg, IL-4 (Biosource International Chiron Corp., CA., U.S.A.), specific activity 2×10^6 U/mg, IL-2 (Cetus, Emeryville, CA, U.S.A.), specific activity 4×10^6 BRMP U/mg, IL-15 (Genzyme, Cambridge, MA, U.S.A.), specific activity 2×10^6 U/mg.

Synthetic peptides

The peptides were synthesized in Synthetic Antigen Laboratory of the U.T. M. D. Anderson Cancer Center using solid phase techniques on an Applied Biosystems 430 peptide synthesizer (Applied Biosystem, Foster City, CA, U.S.A.). The identity and purity of the final material were established by amino acid analysis and analytical reverse phase HPLC (Rainin). All peptides utilized in this study were 92-95% pure. Two FBP peptides were selected for synthesis based on the presence of leucine, isoleucine or valine in the dominant anchors position. From their previously reported recognition by TAL, the peptides position and sequence are as follows: E39 (FBP, 191-199) EIWTHSTKV; E75 (FBP, 245-253) LLSLALMLL. Both peptides are low to moderate binders to HLA-A2.[17]

Cells

To induce the dendritic cells in the presence of the cytokines, GM-CSF and IL-4, the HLA.2+ PBMC was obtained from healthy donors from the Blood Bank of the M.D. Anderson Cancer Center. In order to generate the DC using the CD14 method, the PBMC were distributed in 24 well plates at 4×10^6 cells/well in RPMI 1640 medium. All non-adherent cells were removed after 2 hr of incubation. Complete RPMI medium containing 1000 U/mL GM-CSF and 500 IU/mL IL-4 was added to the wells and the adherent cells were cultured for 5-7 days, while they developed the characteristic DC morphology.

Tumor associated lymphocyte cultures

The tumor associated lymphocytes (TALs) were isolated from fresh collections of malignant ascites and pleural effusions from 6 ovarian and 4 breast cancer patients from ostetrics and gynecology departments. The specimens were processed as described previously.[18] The lymphocyte and tumor cell suspensions were separated by centrifugation over discontinuous 75% and 100% Ficoll-Histopaque (Sigma, St. Louis, MO, U.S.A.) gradients. The freshly isolated TALs were divided in two groups. In group one, the TALs were cultured in RPMI 1640 containing 100 μg/ml L-glutamine (Gibco, Grand Island, NY, U.S.A.) supplemented with 10% FCS (Sigma), 40 μg/mL gentamicin (complete RPMI medium), and 50 to 100 IU/mL IL-2 (Cetus, Emeryville, CA, U.S.A.). In group two, the TALs were cultured as above except that the cytokine used was 20 ng/ml IL-15. Each group of TALs were cultured at 0.5 to 1.0×10^6 cells/mL, placed in a humidified incubator at 37℃ in 5% CO_2 and maintained at this concentration whilst adding the media, and the IL-2 and IL-15 every 2 to 3 days, depending on the growth kinetics.

T cell stimulation by peptide pulsed DC

The DC were washed three times with serum free medium, plated at 1.2×10^5 cell/well in 24-well culture plates and pulsed with the FBP peptide, E39, E75, at 100 μg/ml in serum free medium for 4 hours prior to addig the responders as described.[19] These DC were designated as DC-E39 or DC-E75. Parallel control DC cultures were established and maintained under similar conditions except for the omission of the FBP peptide (designated DC-NP). The responder TALs in complete RPMI medium were added to the DC at 3×10^6 cells/well (stimulator:responder ratio of 1:25). Sixteen hours later, the IL-2 and IL-15 were added to each corresponding well at a final concentration and the cultures were left undisturbed for 5 days whilst the CTL activity was determined.

Tumor targets

The FBP+ ovarian SKOV3 line and the breast SKBR3 line were transfected with the HLA-A2 expression vector RSV.5-neo, with resulting high levels of HLA-A2 expression (SKOV3.A2 & SKBR3.A2), as previously described.[18] The cells were maintained in complete RPMI medium and

250 μg/mL G418 (Sigma). Fresh tumors were collected from the malignant ascites after a Ficoll separation and frozen in aliquots in liquid nitrogen until required.

Cytotoxicity assays

Recognition of the peptides used as the immunogens was performed by the standard chromium release CTL assay as described elsewhere.[19,20] The tumor targets were labeled with 200 μCi of the sodium chromate (Amersham, Arlington Heights, IL) for 1.5 hr at 37℃, washed twice and plated at 3000 cells/well in 100 μl in 96 well V-bottom plates (Costar, Cambridge, MA, U.S.A.). The effectors were added at the designated effector: target (E : T) ratios in a 100 μl/well. After 4 hr incubation, 100 μl of the culture supernatant was collected, and the level of ^{51}Cr release was measured using a gamma counter (Gamma 5500B, Beckman, Fullerton, CA, U.S.A.). All determinations were done in quadruplicate. The results are expressed as the percentage specific lysis as determined by the following equation :

(experimental mean cpm - spontaneous mean cpm) /(total mean cpm - spontaneous mean cpm) × 100.

The study was performed to obtain the CTL activities using the ovarian and breast cancer cell line and then applied to the autologous tumor cells to observed the tumor specific CTL activity of the FBP peptides in the presence of different kinds of culturing media, IL-15 and IL-2.

Statistical analysis was performed by a Chi-square and Fisher's exact test.

RESULTS

Patient characteristics

Ten patients were selected for this study. The six ovarian patients TAL (OTAL) and four breast patients TAL (BTAL) were isolated from the malignant ascites and pleural effusion specimens. They were all found to be HLA.A2+, and the concentration of CD8+cells in these ascites ranged from 20 to 40% (data not shown). The patients' ages ranged form 45 to 63 years and the diseases were in the advanced stage. According to the cell types, most patients had a highly differentiated cell grade. All patients received primary cytoreductive surgery followed by adjuvant chemotherapy. The survival period for the study subjects ranged from 22 - 69 months for the ovarian cancer patients and 12 - 38 months for the breast cancer patients from the diagnosis of the disease to initiation of the study. A patients' characteristics are summarized in Table 1.

Freshly cultured ovarian TAL recognize FBP peptide E39 after stimulation with DC-E39

The fresh isolated TALs cultured in media containing IL-15 and IL-2 each expressed either

Table 1. The Clinical Characteristics of the Patients

	Ovarian cancer						Breast Cancer			
	Pt.1	Pt.2	Pt.3	Pt.4	Pt.5	Pt.6	Pt.1	Pt.2	Pt.3	Pt.4
Stage	IIIc	IIIc	IIIc	IIIc	IIIa	IIIb	IIIb	IIIa	IIIa	IIIa
Age	52	50	45	47	62	57	63	56	49	55
History	AC	PSA	AC	PSA	AC	AC	IDC	IDC	IDC	IDC
Grade	III	III	III	III	II	III	III	III	III	III
1st Tx.	TRS	TRS	TRS*	TAH*	TRS	TRS	MRM	RM	MRM	MRM
2nd Tx.	PC	PC	PC	CAP	PC	PC	Taxol	CAP	Taxol	Taxol
Prognosis	Poor	Poor	Fair	Poor	-	-	Poor	Poor	Poor	Poor

AC, adenocarcinoma; PSA, papillary serous adenocarcinoma; IDC, infiltrating ductal carcinoma; TRS, tumor reductive surgery; TAH, total abdominal hysterectomy; MRM, modified radical mastectomy; RM, radical mastectomy; PC, carboplatin + taxol; CAP, cytoxan + adriamycin + cisplatin.
*With bilateral salpingo-oophorectomy.

low levels of Ag specific cytotoxicity or high non-specific lytic activity during the first 7-10 days of culture. Although the non-specific cytolytic activity decreases over time, it is important to identify the approaches that enhance the specific CTL activity early and rapidly. This study focused on the TAL samples that showed low levels of specific recognition of E39. To determine whether the E39 specificity can be induced or enhanced, APC HLA.A2+ matched dendritic cells (DCs) from healthy donors were used as APC. The DC phenotype generated after GM-CSF + IL-4 was as follows: They expressed high levels of MHC-I and CD86 (B7.2) but low levels of B7.1 and CD40. The CD14+ cells were less than 3% of the DCs, while the expanded the CD13+ marker was expressed in more than 97% of cells. This is a characteristic of phenotypically immature DC. The DCs were pulsed with the peptide and DC-E39 was then used to stimulate the OTAL. Since the responders and stimulators were from different individual that shared only HLA.A2, a certain level of allo-specific and/or cross-reactive specificity was expected. Therefore, in all experiments, the OTAL and BTAL were stimulated in parallel with the DC-NP. The parallel stimulations with DC-NP and DC-E39 were done to establish the contribution of the allospecific responses to the overall increase in lysis activity. Furthermore, they would have been detected in the DC-NP stimulated cultures if high affinity E39 specific CTLs were present and were deleted by DC-E39 stimulation.

It was interesting that, in most OTALs, the E39 specificity was induced at the first stimulation. When the increased E39 specific recognition was not induced at the first DC-E39 stimulation it was induced at an additional stimulation. For example at the first stimulation with DC-E39, the specific lysis activity of DC-NP vs DC-E39 by OTAL was 13.2% vs 15.1% respectively, (Fig. 1A). When the same OTAL were stimulated with DC-E39 again for 1 more week, a significant increase in DC-E39 recognition compared to DC-NP was observed: 25.7% vs 15.3%, ($p<0.0002$) (Fig. 1B).

CTL-mediated cytotoxicity of FBP stimulated TAL cultured in IL-15& IL-2 against cancer cell line

The CTL assays were performed to determine whether or not the DCs stimulated with the FBP peptide increased the recognition levels of the stimulating antigen. The first CTL assays were performed with cancer cell lines against isolated six ovarian and four breast TALs as effectors at an E : T ratio of 20 : 1. The results show that tumor specific lysis activity was noted in SKOV3.A2 cancer cell line as opposed to the SKOV3 cells. The tumor specific lysis activity of SKOV3.A2 for the FBP peptide stimulated TALs cultured in IL-15 and IL-2 was 28.6 ± 3.9% and 30.3 ± 3.2%, respectively, compared to their no peptide counterparts, which was 15.0 ± 2.2% and 21.2 ± 6.5% ($p<0.05$) (Fig. 2). The FBP peptide stimulated TALs exhibited a high specific tumor lysis activity and the

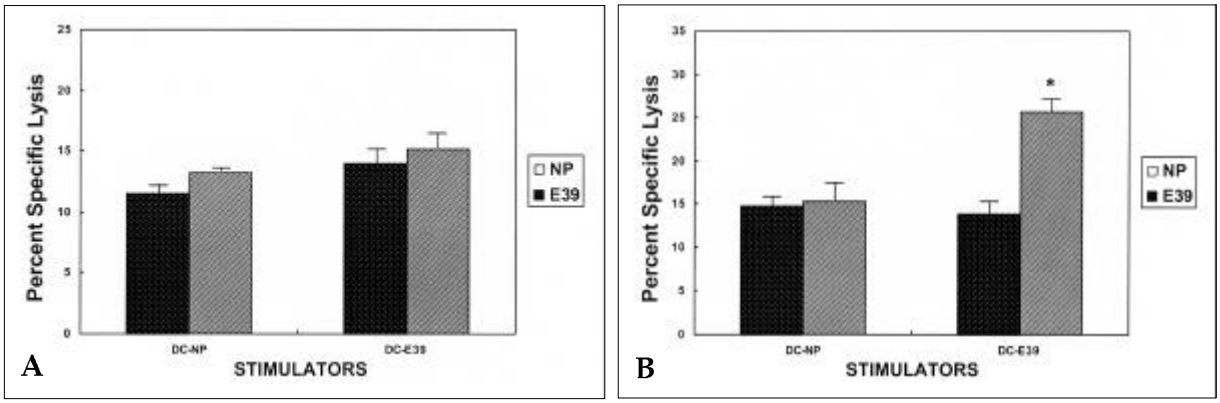

Fig. 1. Induction of E39 specificity in the ovarian TAL requires restimulation with DC-E39. A, in most TALs the E39 specificity was induced after the first stimulation. The OTAL required additional stimulation, as shown here. At the first stimulation, the specific lysis activity of the OTAL stimulated with DC-NP vs DC-E39 was 13.2% vs 15.1%. B, when OTAL again stimulated with DC-E39 after one more week, a significant increase in E39 recognition by the DC-E39 stimulated OTAL was observed compared to DC-NP, 25.7% vs 15.3% ($p<0.0002$).

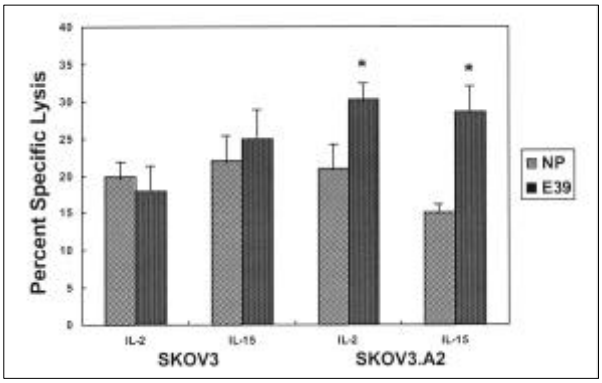

Fig. 2. Increased CTL-mediated recognition of peptide E39 by the DC-E39 stimulated TAL in the ovarian cancer cell line. The CTL assays were performed as effectors at an E:T ratio of 20 : 1. High tumor specific lysis activities were noted in the SKOV3.A2 cancer cell line compared to SKOV3. The tumor specific lysis activity for the FBP peptide stimulated TALs cultured in IL-15 and IL-2 was 28.6 ± 3.9% and 30.3 ± 3.2%, respectively, compared to their no peptide counterparts, which was 15.0 ± 2.2% and 21.2 ± 6.5%, respectively ($p<0.05$). In addition, it is interesting to noted that the SKOV3 cell line also showed a high tumor lysis activity where both the peptide and the peptide stimulated TALs showed similar rates of tumor lysis. However, this was due to a nonspecific activity and not FBP peptide induced lysis.

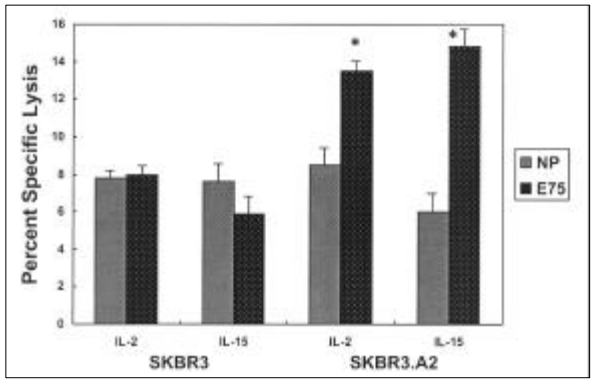

Fig. 3. Increased CTL-mediated recognition of peptide E75 by the DC-E75 stimulated TAL in the breast cancer cell line. The CTL assays were performed as effectors at an E:T ratio of 20:1. High tumor specific lysis activity was noted in the SKBR3.A2 cancer cell line compared to SKBR3. A significant increase in tumor specific lysis activity for the SKBR3.A2 cancer cell line was observed. The FBP peptide stimulated TALs cultured in IL-15 and IL-2 was 14.8 ± 3.1% and 13.5 ± 2.9%, respectively compared to their no peptide counterparts, which was 6.0 ± 1.4% and 8.5 ± 3.0%, respectively ($p<0/05$).

culturing cytokines, IL-15 and IL-2, had similar effect in increasing the antitumor activity. In addition, SKOV3 cell line also exhibited a high tumor lysis activity, both the no peptide and peptide stimulated groups a showed similar tumor lysis activity. However, this was probably due to a nonspecific effect rather than the FBP peptide induced lysis. A similar result was noted with the breast cancer cell line. The 4 hour CTL assay results revealed the higher tumor specific lysis activity of SKBR3.A2 compared to SKBR3. The tumor specific lysis activity of SKBR3.A2 for the FBP peptide stimulated TALs cultured in IL-15 was 14.8 ± 3.1% and that for IL-2 was 13.5 ± 2.9%. Their no peptide counterparts were 6.0 ± 1.4% and 8.5 ± 3.0% ($p<0.05$) (Fig. 3). Again in breast cancer, the antitumor activity induced by the TALs cultured in different types of cytokines, IL-15 and IL-2, were similar.

CTL- cytotoxicity of FBP stimulated TAL cultured in IL-15 & IL-2 against autologous tumor cells

From the results of the CTL assay against the cancer cell lines, specific tumor lysis activity can be obtained from the FBP peptide stimulated DC against isolated the TALs. Based on this, the second part of the study of the CTL assays was performed using an autologous tumor cell. The isolated TAL on each of the different cancer cells were used to determine whether or not the DC stimulated with the FBP peptide increased the recognition levels of the stimulating antigen, which resulted a high specific tumor lysis activity Fig. 4 show the CTL assay against the autologous ovarian cancer cells. The DC-E39 stimulated TAL group cultured in IL-15 and IL-2 both showed a significantly high percentage of tumor specific lysis activity, 72.0 ± 8.2% and 68.5 ± 3.6%, respectively. The DC-NP group showed a very low tumor lysis activity, showing 32.2 ± 6.4% for the IL-15 and 23.8 ± 5.9% for the IL-2, ($+p<0.05$, $*p<0.01$). For the breast cancer cell CTL assay, the IL-15 cultured TAL stimulated with DC-E75 was 39.5 ± 4.2% and the IL-2 cultured TAL stimulated with DC-E75 was 41.5 ± 3.3%. The TALs that were not stimulated with the peptide showed that the IL-15 and IL-2 cultured groups was 23.9 ± 6.0% and 19.8 ± 5.2% ($+p$: NS, $*p<0.05$). The control stimulation with DC-NP, which represented allo-stimulation alone, did not either induce or

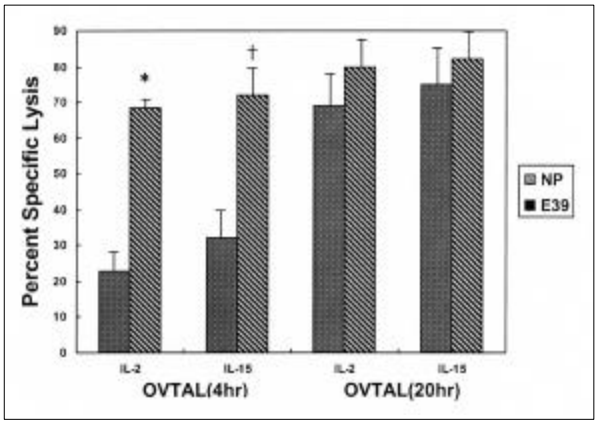

Fig. 4. CTL assay against autologous ovarian cancer cell. The four hour CTL assay for DC-E39 stimulated TAL group cultured in IL-15 and IL-2 both showed a significantly high percentage of tumor specific lysis activity, 72.0 ± 8.2% and 68.5 ± 3.6%, respectively. Whereas the DC-N group showed a very low tumor lysis activity, giving 32.2 ± 6.4% for the IL-15 and 23.8 ± 5.9% for the IL-2 cultured TALs. (+$p<0.05$, *$p<0.01$). However, in the 20 hours CTL assay, all the non-specific tumor lysis activity was observed.

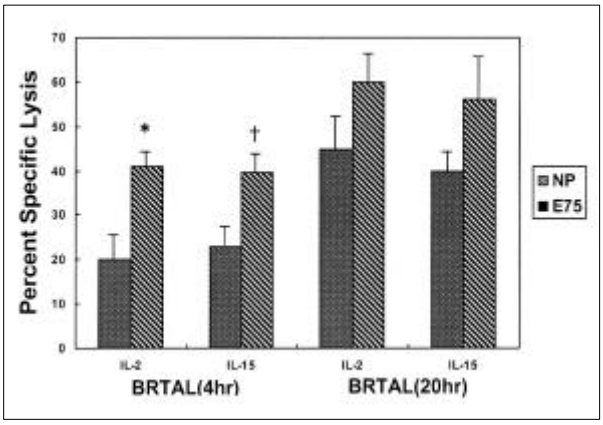

Fig. 5. For the breast cancer cell CTL assay, the IL-15 cultured TAL stimulated with DC-E75 was 39.5 ± 4.2% and IL-2 cultured TAL stimulated with DC-E75 was 41.5 ± 3.3%. In the breast cancer cells, in contrast to ovarian cancer cells, that, a slightly lower tumor lysis activity was noted in the TALs cultured in IL-15 compared to IL-2. The TALs not stimulated with the peptide showed little difference between the IL-15 cultured TALs compared to the IL-2 cultured TALs. IL-15 was 23.9 ± 6.0% and IL-2 was 19.8 ± 5.2% (+p: NS, *$p<0.05$). Control stimulation with DC-NP, representative of allo-stimulation alone did not induce or enhance the specific recognition of the FBP peptide.

enhance the specific recognition of the FBP peptide, E39 and/or E75, (Fig. 5). These results together with the results shown in Figure 4 show that by using the culturing cytokine media, IL-15, E39 and/or E75 specific CTL-TAL recognition could be induced by Ag stimulation as with IL-2. These results demonstrate that the FBP peptide can induce the specific CTL response in the ovarian and breast TALs cultured with either IL-15 or IL-2.

DISCUSSION

In this study, a newly developed cytokine, IL-15 was found to have a similar effect on the FBP peptide stimulated DC activated TALs of the ovarian and breast CTL-TAL as the well known antitumor activity cytokine, IL-2. The important function of the TAL is to lyse the tumor cells. A previous study showed that the E39 specific CTL is present in the ovarian TAL stimulated CTL-TAL can specifically recognize E39 as lyse experimental tumors when cultured in IL-2.[21] IL-15 is clone from the simian kidney epithelial cell line and does not have a sequence homology with IL-2. However, both cytokines share many biological functions. IL-2 and IL-15 exhibit a low sequence similarity. There, it was not surprising that both proteins exert similar biological functions.[22,23] IL-15 induces the proliferation of the CD8+ T cells clone, CTLL-2, and the proliferation of the phytohemagglutinin-activated CD4+ and CD8+ human peripheral blood T lymphocytes.[15] IL-2 and IL-15 utilize the same receptor molecules (β-and γ-chain) for cellular binding and signaling with the noted exception of a cytokine specific α-chain.[24] IL-15 is able to induce FBP peptide specific tumor lysis activity as well as IL-2, which shows that there is a close functional similarity of IL-2 and IL-15. The results showed that the IL-15 cultured TAL stimulated with DC-E39 or DC-E75 showed almost same or a lightly higher tumor specific lysis ability for both ovarian and breast cancer cells compared to the IL-2 cultured TALs (Fig. 4 and 5). The control stimulation with DC-NP, which is representive of allo-stimulation alone, did not either induce or enhance the specific recognition of the FBP peptide, E39 and/or E75. Stimulation and/or restimulation with the peptide pulsed DC may also induce apoptosis or silencing of the CTL if appropriate cytokines are absent. For

this reason each TAL was stimulated in parallel with the DC pulsed with and without the peptide in order to determine whether or not the FBP peptide specificity decreases or increases. It was found from this study that all patients stimulated with E39 in ovarian cancer and E75 in breast cancer resulted in an increased E39 or E75 specific CTL reactivity. It is interesting to note that the levels of the increase in the E39 specificity were higher in the CTL assays performed by the autologous ovarian tumor cell line than the ovarian cancer cell line (Fig. 2 and 4). However, the level of E75 specificity for breast cancer was different from that for ovarian cancer. The E75 specificity was higher in the breast cancer cell line than the autologous breast cancer cell (Fig. 3 and 5). From these results, it can be concluded that the FBP derived peptide can specifically activate the ovarian and breast cancer associated CTL, which suggests it can be applied to immunotherapeutic strategies. Previous studies have shown that adoptive immunotherapy can reduce the tumor size in some of solid tumors such as melanoma and renal cell carcinoma, and it can prolong the survival in advanced ovarian carcinoma when combined with conventional chemotherapy.[25,26] These results were obtained with TIL/TAL using the specific CTL-TAL directed toward the known tumor epitopes such as the FBP derived peptide, which shows that the role of the culturing cytokine is very important. The cytokine plays an important role in immunotherapy. It has many characteristics 1) it can regulate the immune response, inflammation, and hematopoesis, 2) it may have different activities on various cell types, differentiation, proliferation, activation, and suppression, and 3) it have some therapeutic benefit on the systemic application to cancer patients (melanoma or renal cell carcinoma).[27] Most experiments were done with the cytokines, IL-2, IL-4, IL-6 IL-7, IL-10, the tumor necrosis factor, IFN-γ, and GM-CSF.[28-31] However, few studies with the newly developed IL-15 have been reported. From our results, IL-15 is much more beneficial to patients. It requires a lower dosage, and it is less toxic. Therefore, it has a potential for use as an immunotherapeutic agent along with IL-2.

Many immunological studies that are focused on ovarian cancer are aimed at identifying the Ag recognized by TAL cultured with IL-2 because it provides a unique model for investigating the immune response to epithelial cancer. Ovarian cancer has distinct tumor growth patterns. It grows either as a single cell in the malignant ascites or as a bulky solid mass. In either case, the tumor specifically induces a T cell response. Tumor associated antigen (TAA) identification in ovarian cancer is significant. These epithelial tumors share a common CTL recognized TAA, and this feature leads to the development of TAA specific vaccines. Previous studies have demonstrated that the endogenous cellular immune response does exist in a variety of epithelial cancers, and that this response involves the specific recognition of antigenic peptides presented by MHC-I. Currently, the established known tumor Ags are MUC-1 and HER2.[32,33] MUC-1 expression is 10 - 40 times higher in breast cancer compared to normal cells. HER-2 has been shown to provide endogenously recognized antigenic peptides but it is overexpressed in approximately 30% of ovarian and breast cancers.[34-36] Therefore it is important to find widely applicable CTL recognized Ags for the development of potential epithelial cancer vaccines, possibly the folate binding protein peptide, as a newly developed TAA for ovarian and breast cancer.

The FBP appears to be the next candidate for use as a target for the cellular immunity. However, further studies on the FBP peptide for possible use as a new tumor antigen are recommended

Current strategies using genetically modified cancer vaccines transfected with genes expressing the cytokines and costimulatory molecules aim to alleviate the inadequacy of tumor specific T cells. To increase the strength of the cytotoxic T cells, the newly developed cytokine, IL-15, may play an important role. IL-15 exhibits similar antitumor activity to IL-2. However, it requires a lower dosage and is less toxic. Therefore, the utilization of IL-15 for potential use as an anticancer immunotherapeutic agent requires further investigation.

REFERENCES

1. Ressing ME, Offringa R, Toes REM, Ossendorp F, Jong JH, Brandt RMP, et al. Immunotherapy of cancer by

peptide-based vaccines for the induction of tumor specific T cell immunity. Immunotechnology 1996;2: 241-51.
2. Rosenberg SA. Cancer vaccines based on the identification of genes encoding cancer regression antigens. Immunol Today 1997;18:175-82.
3. Ioannides CG, Fisk B, Fan D, Biddision WA, Wharton JT, O'Brian CA. Cytotoxic T cells isolated from ovarian malignant ascites recognize a peptide derived from the HER2/neu proto-oncogene. Cell Immunol 1993;151: 225-34.
4. Dyall R, Bowne WB, Weber LW, Lemaoult J, Szabo P, Moroi Y, et al. Heteroclitic immunization induces tumor immunity. J Exp Med 1998;188:1553-61.
5. Grohmann U, Fioretti MC, Bianchi R, Belladonna ML, Ayroldi E, Surace D, et al. Dendritic cells, interleukin 12 and CD4+ lymphocytes in the initiation of class I restricted reactivity to a tumor/self peptide. Crit Rev Immunol 1998;18:87-98.
6. Murphy G, Tjoa B, Ragde H, Kenny G, Boynton A. Phase I clinical trial: T cell therapy for prostate cancer using autologous dendritic cells pulsed with HLA-A0201 specific peptides from prostate specific membrane. Prostate 1996;29:371-80.
7. Mortarini RA, Anichini A, DeNicola M, Siena S, Bregni M, Belli F, et al. Autologous dendritic cells derived from CD34+ progenitors and from monocytes are not functionally equivalent antigen-presenting cells in the induction of Melan-a/Mart-127-35-specific CTLs from peripheral blood lymphocytes of melanoma patients with low frequency of CTL precursor. Cancer Res 1997; 57:5534-41.
8. Van Elsas A, Van der Burg SH, Van der Minne CE, Borghi M, Mourer JS, Melief CJ, et al. Peptide pulsed dendritic cells induce tumoricidal cytotoxic T lymphocytes from healthy donors against stably HLA-A0201 binding peptides from the melan-A/MART-1 self antigen. Eur J Immunol 1996;26:1683-9.
9. Carbone FR, Moore MW, Sheil JM, Bevan MJ. Induction of cytotoxic T lymphocytes by primary *in vitro* stimulation with peptides. J Exp Med 1988;167:1767-79.
10. Kelly JM, Sterry SJ, Cose S, Turner SJ, Fecondo J, Rodda S, et al. Identification of conserved T cell receptor CDR3 residues contacting known exposed peptide side chains from a major higtocompatibility complex class I bound determinat. Eur J Immunol 1993;23:3318-26.
11. Troy AJ, Summers KL, Davidson PJ, Atkinson CH, Hart DN. Minimal recruitment and activation of dendritic cells within renal cell carcinoma. Clin Cancer Res 1998; 4:585-93.
12. Caux P, Favre N, Bonnotte B, Moutet M, Martin M, Martin F. Tumor infiltrating dendritic cells are defective in their antigen presenting function and inducible B7 expression. A role in the immune tolerance to antigenic tumors. Adv Exp Med Biol 1997;417:525-8.
13. Pickl WF, Majdic O, Kohl P, Stockl J, Tiedl E, Scheinecker C, et al. Molecular and functional characteristics of dendritic cells generated from highly purified CD14+ peripheral blood monocytes. J Immunol 1996;157:3850-9.
14. Ioannides CG, Platsoucas CD, Rashed S, Wharton JT, Edwards CL, Freedman RS. Tumor cytolysis by lymphocytes infiltrating ovarian malignant ascites. Cancer Res 1991;51:4257-65.
15. Dooms H, Desmedt M, Vancaeneghem S, Rottier P, Goossen V. Quiescence-Inducing and Antiapoptotic activities of IL-15 Enhance secondary CD4+ T Cell Responsiveness to Antigen. J Immunol 1998;161:2141-50.
16. Anderson DM, Johnson L, Glaccum MB, Copeland NG, Gilbert DJ, Jenkins NA, et al. Chromosomal assignment and genomics structure of IL-15. Genomics 1995;25:701-6.
17. Peoples GE, Anderson BW, Fisk B, Kudelka AP, Wharton JT, Ioannides CG. Ovarian cancer associated lymphocyte recognition of folate binding protein peptides. Ann Surg Oncol 1998;5:743-50.
18. Fisk B, Blevins TL, Wharton JT, Ioannides CG. Identification of an immunodominant peptide of the HER2/neu proto-oncogene recognized by ovarian tumor specific CTL lines. J Exp Med 1995;181:2709-17.
19. Anderson BW, Swearingen BJ, Wharton JT, Ioannides CG. Primary generation of human antitumor CTL by a HLA-A2 restricted HER-2 peptide. Proc of AACR 1997; 38:A1606.
20. People GE, Coedegebuure PS, Smith R, Lindhan DC, Yoshino I, Eberlein TJ. Breast and ovarian caner specific cytotoxic T lymphocytes recognize the same HER2/neu derived peptide. Proc Natl Acad Sci USA 1995;92:432-6.
21. Kim DK, Lee TV, Castilleja A, Anderson BW, People GW, Kudelka JM, et al. Folate binding protein peptide 191-199 presented on dendritic cell can stimulate CTL form ovarian and breast cancer patients. Anticancer Res 1999;19:2907-16.
22. Doherty TM, Sedar RA, Sher A. Induction and regulation of IL-15 expression in murine macrophage. J Immunol 1996;156:735-41.
23. Giri JG, Anderson DM, Kumaki S, Park LS, Grabstein KH, Cosman D. A novel T cell growth factor that share activities and receptor components with IL-2. J Leukoc Biol 1995;57:763-6.
24. Kumaki S, Armitage R, Ahdieh M, Park L, Cosman D. Interleukin-15 up-regulates interleukin-2 receptor α-chain but down-regulates its own high-affinity binding sites on human T and B cells. Eur J Immunol 1996;26: 1235-9.
25. Repmann R, Wagner S, Richter A. Adjuvant therapy of renal cell carcinoma with active specific immunotherapy using autologous tumor vaccine. Anticancer Res 1997;17:2879-82.
26. Fujita K, Ikarashi H, Tanaka K, Kodama S, Tokunaga A, Takahashi T, et al. Prolonged disease free period in patients with advanced epithelial ovarian cancer after adoptive transfer of tumor infiltrating lymphocytes. Clin Cancer Res 1995;1:501-7.
27. Blankenstein T, Cayeux S, Qin Z. Genetic approaches to cancer immunotherapy. Rev Physiol Biochem

Pharmacol 1996;129:1-49.
28. Blankenstein T, Qin Z, Uberla K. Tumor suppression after tumor cell targeted tumor necrosis factor alpha gene transfer. J Exp Med 1991;173:1047-52.
29. Bubenik J, Simova J, Jandlova T. Immunotherapy of cancer using local administration of lymphoid cells transformed by IL-2 cDNA and constitutively producing IL-2. Immunol Lett 1990;23:287-92.
30. Aoki T, Tashiro K, Miyatake SIY, Kasid A. Expression of murine IL-7 in a murine glioma cell line results in reduce tumorigenicity *in vivo*. Proc Natl Acad Sci USA 1992;89:3850-4.
31. Li W, Diamantstein T, Blankensten T. Lack of tumorigenicity of interleukin 4 autocrine growing cells seems related to the anti-tumor function of interleukin 4. Mol Immunol 1990;27:1331-7.
32. Henderson RA, Nimgaonkar MT, Watkins SC, Robbind PD, Ball ED, Finn OJ. Human dendritic cells genetically engineered to express high level of the human epithelian tumor antigen Mucin (MUC-1). Cancer Res 1996;56: 3763-70.
33. Karanikas V, Hwang LA, Pearson J, Ong CS, Apostolopoulos V, Vaughan H, et al. Antibody and T cell responses of patients with adenocarcinoma immunized with Mannan-MUC-1 fusion protein. J Clin Invest 1997; 100:2783-92.
34. Zaks TZ, Rosenberg SA. Immunization with peptide epitope (p369-377) from HER-2/neu lead to peptide-specific cytotoxic T lymphocytes that fail to recognize HER-2/neu+ tumor. Cancer Res 1998;58:4902-8.
35. Fisk B, Chesak B, Pollack M, Wharton JT, Ioannides CG. Oligopeptide induction of a cytotoxic T lymphocyte response to HER-2/neu proto-oncogene *in vitro*. Cell Immunol 1994;157:412-27.
36. Disis ML, Smith JW, Murphy AE, Chen W, Cheever MA. *In vitro* generation of human cytolytic T cells specific for peptides derived from the HER-2/neu proto-oncogene protein. Cancer Res 1994;54:1071-6.