Rapid induction of primary human CD4+ and CD8+ T cell responses against cancer-associated MUC1 peptide epitopes

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Abstract

Antigen-specific MHC class II- and class I-restricted helper and cytotoxic T cell responses are important anti-cancer immune responses. MUC1 mucin is a potentially important target for immunotherapy because of its high expression on most human adenocarcinomas. MUC1 peptide-specific type 1 T cell responses were generated in vitro using human peripheral blood lymphocytes (PBL), incubated with liposomes containing synthetic MUC1 lipopeptide antigen. Only two weekly stimulations with the liposomal MUC1 formulation led to the generation of potent anti-MUC1-specific T cell proliferation as well as class I-restricted cytotoxic responses. Thus the use of PBL pulsed with liposome-encapsulated antigen provides an effective approach of rapidly generating effective antigen-presenting cell (APC) function as well as antigen specific T cells in vitro. It may be feasible to use this technology for the rapid and effective generation of APC and/or T cells as cellular vaccines for adenocarcinomas.

Introduction

Antigen-specific MHC-restricted T cell responses are important components of immune responses against viral infections and tumors. Effective immunotherapy intervention depends on the understanding of the target antigen and its capability to be efficiently presented by MHC class I and II molecules. While with modern techniques it is quite possible to determine the affinity of antigenic peptide for the antigen binding groove of MHC class I and II molecules, it is generally not an easy task to predict the immune response towards a given peptide in the context of varying T cell repertoires in outbred human populations. In attempts to develop various immunotherapeutic approaches, it is desirable to evaluate primary human T cell responses in vitro against potential peptide epitopes.

Human MUC1 mucin is an interesting tumor-associated molecule because it has been shown to be both immunostimulatory and immunosuppressive (1–3). Most adenocarcinomas abundantly express on the cell surface and secrete abnormal (underglycosylated) MUC1 mucin (4,5) resulting in the exposure of multiple tandem repeat core peptide epitopes. Adenocarcinoma patients who have high levels of serum MUC1 mucin have a poor prognosis and it has recently been shown that MUC1 mucin inhibits human T cell responses (6,7). In contrast, cancer patients can generate MHC-unrestricted T cell precursors which can recognize MUC1 core peptide tandem repeats (8). Recently, it has been reported (9) that following immunization of cancer patients with a 105mer MUC1 polypeptide vaccine, cross-reactive anti-Gal antibodies form immune complexes with MUC1 and increase the presentation of MUC1 by antigen-presenting cells (APC) which favor antibody rather than cellular immune responses. This observation could also provide an explanation why multiple re-stimulations were required to isolate anti-MUC1 cytotoxic T lymphocytes (CTL) from cancer patients (1). In contrast, breast cancer patients immunized with 16mer MUC1 peptide conjugated to keyhole limpet hemocyanin (KLH) generated strong MHC class I-restricted, MUC1-specific CTL responses (10). It is possible that immunization with a shorter peptide (16 amino acids) obviates the immune complexes and does not divert the response to antibody away from MHC class I presentation. It has been demonstrated that the highly ordered structure of MUC1 in solution is dependent on the multiple tandem repeats, whereas MUC1...
peptide of one tandem repeat is a random coil structure (11). It is likely that due to the highly ordered three-dimensional structure of 105mer MUC peptide in MUC1 mannan fusion protein, natural anti-Gal antibodies bind to multiple conformational determinants leading to the observed lack of cellular response, whereas the MUC1 16mer-KLH antigen would not allow natural anti-Gal antibodies to bind to the antigen due to the lack of conformational determinants and hence would allow cellular immune response against it.

The efficient generation of CD4+ and CD8+ T cell responses against a target antigen is usually dependent upon in vivo priming, either through natural infection or deliberate immunization. However, recent studies have demonstrated that CD8+ CTL responses can be generated in vitro against viral antigens following stimulation of T cells from unprimed individuals with peptide-loaded mutant T2 or RMA-S cell lines (12–14). In addition, it was shown that antigen-specific CTL could be generated in vitro using murine spleen cells incubated with high concentrations of exogenous peptide (14–17). These reports suggest that primary CTL responses could be generated due to the presence of a high density of peptide–MHC class I complexes on the surface of APC (12–14,16,17).

Methods

Peptides

Various synthetic peptides were prepared by automated solid-phase synthesis with Fmoc amino acids using a Milligen/Bioresearch Model 9050 peptide synthesizer (Millipore, Marlborough, MA). For the synthesis of lipopeptide glycine was attached to the resin followed by coupling palmitoylated lysine residue. This was followed by usual solid-phase synthesis. The following peptides were used for these studies which were 95% pure as determined by HPLC: a human MUC1 24 amino acid peptide (BP24), previously referred to as p-24H (2), TAPPAGTSAPDTRPAAPGSTAPP, human MUC1 25 amino acid peptide (BP-25) STAPPAGTSAPDTRPAAPGSTAPP, a mouse MUC1 24 amino acid peptide (BP-24M) DSTSSPVHSQTSSPATSAPEDSTS; lipopeptides BLP-24 PAHGVTSAPDTRPAAPGSTAPP-Lys(Pal)G and control 24 amino acid peptide from HLA.Aw68.1 (61–84) (p-HLA) DRNTRNVK-PAHGVTSAPDTRPAAPGSTAPP-Lys(Pal)G, BLP25 STAPPAHGVTSAPDTRPAAPGSTAPP-Lys(Pal)G and control 24 amino acid peptide from HB104, OKT4 and OKT8) and control antibody IgG1 were used to block T cell responses. The antibodies (W6/32, HB104, OKT4 and OKT8) and control antibody IgG1 were purified from culture supernatants of hybridomas obtained from ATCC (Rockville, MD). For FACS staining anti-CD4-FITC/anti-CD8–phycoerythrin (PE), anti-CD25–PE and anti-CD3–FITC, CD3–FITC, CD8–FITC, CD3–FITC, CD69–PE and isotype control antibodies were used to block T cell responses. The antibodies (W6/32, HB104, OKT4 and OKT8) and control antibody IgG1 were purified from culture supernatants of hybridomas obtained from ATCC (Rockville, MD). For FACS staining anti-CD4–FITC/anti-CD8–phycoerythrin (PE), anti-CD25–PE and anti-CD3–PE, anti-CD28–PE, anti-TCRαβ–FITC, CD4–FITC, CD8–FITC, CD3–FITC, CD69–PE and isotype control antibodies were obtained from Becton Dickinson (Mountain View, CA).

Preparation of liposomes—ethanol injection process

The bulk lipid composition of liposomes consisted of dipalmitoyl phosphatidyl choline (DPPC), cholesterol (Chol) and dimyristoyl phosphatidyl glycerol (DMPG) (Genzyme, Cambridge, MA) in a molar ratio of 3.1:0.25 and at a final

![Fig. 1.](image-url)
primary in vitro anti-MUC1 T cell responses

Fig. 2. Antigen specificity of T cells stimulated with liposomal BLP25-pulsed autologous APC. The T cells isolated from PBL of normal donors were cultured in the presence of autologous PBL pulsed with liposomes containing BLP25 (10 µg) for 2 weeks with weekly re-stimulations. These T cells were tested for their proliferative response against liposomal lipopeptide antigen or soluble peptide-loaded autologous APC. The results are shown as mean c.p.m. ± SD of four replicate wells. As antigen specificity controls, liposomal mouse BLP25M and soluble 24 amino acid peptide from HLA.Aw68.1 (residue 61–84) were used.

Table 1.

<table>
<thead>
<tr>
<th>Stimulatory liposomal antigens</th>
<th>IFN-γ (pg/ml) (duplicate tests)</th>
<th>IL-4 (pg/ml) (duplicate tests)</th>
</tr>
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<tbody>
<tr>
<td>(a) Cytokines detected in the supernatants of liposomal antigen stimulated bulk T cell cultures</td>
<td>(b) Cytokines detected in the supernatants of T cells cultured in 96-well plates following stimulation with various liposomal antigens</td>
<td></td>
</tr>
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<td>no antigen (empty liposomes)</td>
<td>65, 145</td>
<td>&lt;31, 45</td>
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<td>BLP25, 1 µg</td>
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<td>884, 1082</td>
<td>&lt;31, &lt;31</td>
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</tr>
<tr>
<td>BLP-24M, 10 µg</td>
<td>198, 226</td>
<td>&lt;31, &lt;31</td>
</tr>
</tbody>
</table>

The data shown are representative of three repeated experiments (with three different high-responder donors). The ranges of sensitivity of cytokine ELISA assays were 25–1600 pg/ml (for IFN-γ) and 31–2000 pg/ml (for IL-4). Supernatants obtained from cultures of T cells grown with liposomal antigen-loaded APC for 2 weeks were used in (a); in (b) supernatants were obtained from 96-well plate cultures after the end of two weekly re-stimulations and one 6 day culture.

Cytokines

Human recombinant cytokines, IL-12 (R & D Systems, Minneapolis, MN) and IL-7 (Intermedico, Markham, Ontario, total lipid concentration of 30 mM. Monophosphoryl lipid A (MPLA) (Ribi Immunchem, Hamilton, MT) was included in the lipid mixture at a concentration of 1–5% (w/w) of bulk lipid and the lipopeptide concentration was 50–1000 µg/ml. Bulk lipids, MPLA and lipopeptide (192 mg DPPC, 33 mg Chol, 15 mg DMPG, 2.4–12 mg MPLA and 0.6–12 mg of lipopeptide) for 12 ml of final product were dissolved in 5.3 ml of ethanol. The ethanol solution was warmed to 50°C and injected through a 30 gauge needle into 100 ml of PBS that was rapidly stirred at the same temperature. The resulting liposome suspension (largely small unilamellar vesicles) was depleted of ethanol and concentrated by diafiltration in a Sartorius cell with a mol. wt cut-off of 300 kDa. The volume was first reduced to 10–20 ml and then the product was washed by continuous replacement of the diafiltrate with 100 ml of PBS. The volume was reduced to <12 ml and reconstituted to the final volume of 12 ml after removal from the diafiltration cell. The product was then passed through a French pressure cell (SLM Aminco, Rochester, NY) 3 times at 20,000 p.s.i. to ensure that all liposome particles would be reduced to a size that would pass through a 0.22 µm filter for sterilization. Size analysis by Brookhaven Instruments Model BI-90 particle sizer showed that the mean particle size was slightly under 0.1 µm.
Fig. 3. Blocking of antigen peptide-specific T cell proliferative responses by mAb specific against CD4, CD8 and MHC class I molecules. All of the blocking mAb and isotype control antibodies were used at a concentration of 20 µg/ml. T cells were cultured in the presence of autologous APC loaded with liposomal BLP25 (10 µg) for 2 weeks with weekly re-stimulations and their proliferative response was examined against autologous APC loaded with BLP25 (10 µg) with or without antibodies or with control peptide BLP-24M containing liposome-loaded APC. The data are shown as mean c.p.m. ± SD of three replicate wells.

Canada), were diluted in serum-free AIM-V medium (Life Technologies, Gaithersberg, MD) prior to use.

Liposomal peptide incubation with APC
Human PBL were purified from buffy coats or heparinized blood by centrifugation in Ficoll-paque (Pharmacia, Uppsala, Sweden). The Ficoll-blood interface layer obtained by centrifugation was collected and washed twice with RPMI before use.

Briefly, to 2–10x10⁶ PBL in 0.9 ml AIM-V medium, liposomes containing lipopeptide formulation were added and the PBL were incubated overnight at 37°C in a CO2 supplemented incubator. After incubation, the PBL were treated with mitomycin C (60 min incubation with 25 µg/ml mitomycin C at 37°C) or γ-irradiated (3000 rad) and then washed 3-4 times with AIM-V medium.

Bulk T cell culture
For T cell enrichment, 30–50x10⁶ PBL were suspended in 1 ml AIM-V medium and loaded on medium pre-conditioned 5 ml nylon wool columns (Robins Scientific, Sunnyvale, CA). The loaded nylon wool columns were incubated at 37°C for 45 min and non-adhering T cells were eluted by washing with warm (37°C) AIM-V medium. Initially, a bulk culture was started in a sterile 25 cm² tissue culture flask (Corning Glass, Corning, NY). T cells (10⁷) were cultured for 16–24 h in the presence of 10⁷ mitomycin C-treated autologous PBL which had been incubated overnight with antigen in a total volume of 10 ml in AIM-V medium. The culture was fed subsequently with human rIL-7 (10 ng/ml) and human rIL-12 (100 pg/ml), and incubated for 6–8 more days. At the end of the culturing period, surviving T cells were collected and re-stimulated with autologous APC which had been incubated with liposomal antigen. After two consecutive cycles of re-stimulation (14–16 days), the T cells were collected and counted. The supernatant was collected from bulk cultures at 14 days for cytokine screening and kept frozen at –80°C until used for the assay.

T cell proliferation assay
T cells (5x10⁴–1x10⁵) obtained at the end of 14–16 days of culture, as described above, were incubated in the presence of autologous mitomycin C-treated APC (5x10⁴–10⁵) loaded with liposomes containing various antigens BP-24, BLP-24 and BLP-24M or with soluble peptides BLP25, BP-24 and p-HLA. The mixtures in a total volume of 200 µl in AIM-V medium, in 96-well flat-bottom tissue culture plates, were incubated for a period of 5 days at 37°C, in a humidified incubator with 7% CO₂. Each group was cultured in five replicate wells. At the end of 5 days, 100 µl of culture supernatant was collected from each well and tested for the presence of IFN-γ or IL-4 by ELISA, as described below. After collecting the supernatant for cytokines, 1 µCi [³H]thymidine (Amersham Canada Limited, Oakville, Ontario, Canada) was added into each well and incubated overnight (~18 h). Incorporation of [³H]thymidine into DNA of proliferating cells was measured after harvesting the plates and counting in a liquid scintillation counter (LS 6001C; Beckman Mississauga, Onatrio, Canada).

CTL assays
For CTL assays, three (HLA.A2⁺) normal donors’ PBL were used. T cells were grown for 2 weeks in bulk cultures as
Fig. 4. The phenotype (CD4/CD8, leukogate CD14/CD45, CD3/TCRαβ, CD4/CD28 and CD8/CD28 expression) of T cells cultured for 2 weeks with weekly re-stimulations with autologous APC pulsed with liposomal BLP25 (10 µg). The phenotype (activation molecules) of T cells cultured in the presence of autologous APC containing empty liposomes (panel I, liposomes with MPLA, no antigen) or BLP25 (10 µg) containing liposomes (panel II). T cells were cultured in the presence of BLP25 liposome-pulsed autologous APC or no antigen containing liposome-pulsed APC for 2 weeks with weekly re-stimulations. The expression of CD25 and CD69 on both CD4+ and CD8+ T cells was examined. The higher percentage of CD25+ and CD69+ T cells in panel II compared to panel I represents antigen-dependent stimulation of these T cells. The markers in all dot-blots are set in a way so as to exclude >98% of isotype control antibody-stained cells, treated in a similar manner (isotype control data not shown). The leukogate (CD14/CD45) and TCR (CD3/αβ) staining was performed to ensure the identity of the cultured cells.

described above. At the end of 2 weeks, live T cells were harvested from flasks and counted. For targets, we used mutant T2 cell lines (14, 15). First, we examined MUC1 peptide-mediated up-regulation of HLA-A2 expression on T2 cells by using two MUC1 9mer peptides STAPPAHGV and SAPDTRPAP according to reported procedures (24). T2 cells were loaded overnight with these two peptides at 200 µM in the presence of exogenous β2-microglobulin (17,18). Peptide loaded T2
target cells were then loaded with $^{51}$Cr (using NaCrO$_4$) for 90 min and washed extensively. We then performed CTL assays (25) using these T2 cells as targets and T cells from bulk cultures as effectors in 5 h $^{51}$Cr-release assays. Percent specific killing was calculated as: experimental release – spontaneous release/maximum release – spontaneous release $\times 100$. The E:T ratio used was 10:1 to 150:1. Each group was set up in quadruplicate and mean percent specific killing was calculated.

Estimation of IFN-$\gamma$ and IL-4 produced in culture supernatant by ELISA

The supernatants collected from the bulk cultures were examined separately for IFN-$\gamma$ and IL-4. From the proliferation assay plate, the supernatants were collected and pooled, and then tested in duplicate for the presence of IFN-$\gamma$ and IL-4. The ELISA assays used for the detection of secreted cytokines (IFN-$\gamma$ and IL-4) were similar to our previous report (25).

Cell surface immunofluorescence staining

For the detection of cell surface antigens, T cells harvested from bulk cultures were washed once in cold PBS containing 1% BSA, followed by the addition of 10 µl goat Ig (3 mg/ml) to the cell pellet and incubated for 10 min on ice, to which 50 µl of PBS containing 1% BSA and 3% human AB serum was added.

For indirect staining, the cells were incubated with test antibody or isotype control antibody (1 µg/5x10$^5$ T cells) for 30–45 min on ice. PBS containing 10% BSA (100 µl) was added and cells were washed once in cold PBS at 4°C. The second antibody, goat anti-mouse IgG1–PE (70 µl of 1:100 dilution of 0.5 mg/ml concentration) diluted in PBS containing
1% BSA and 3% human AB serum, was added and incubated for 30–45 min on ice at 4°C. PBS containing 10% BSA (200 µl) was added and cells were washed once in PBS at 4°C. For directly labeled test antibody, only one incubation was performed. For single staining, cells were re-suspended in 2% paraformaldehyde. For double staining, 10 µl of mouse Ig (3 mg/ml) was added to the cell pellet and left on ice for 10 min and added with 50 µl of PBS containing 1% BSA and 3% human AB serum. Second directly labeled antibody (10 µl) was added and incubated for 30–45 min on ice, followed by addition of 10 µl of PBS containing 10% BSA, washing once in cold PBS and re-suspending in PBS containing 2% paraformaldehyde. In parallel, appropriate isotype control antibody was always used to stain the cells in a similar way. The samples were generally run and analyzed by flow cytometry using a FACSsort (Becton Dickinson) and Lysys II software. The flow cytometer was calibrated for sensitivity, compensation and photomultiplier tube daily using Becton Dickinson CaliBRITE beads using AutoCOMP software. Viable lymphocytes were shown in the dot-blots after gates were set on forward and side scatter, and confirmed with staining with leukogate (CD45/CD14). The percent positive cells was defined as the fraction of cells exhibiting fluorescence intensities beyond a region set to exclude at least 98% of the control isotype-matched, antibody-stained cells.

Results

Induction of in vitro T cell responses against MUC1 epitopes using autologous APC pulsed with MUC1 lipopeptide-containing liposomes

T cells isolated from PBL of normal donors from a Red Cross Center were stimulated twice with autologous APC which had been pulsed with liposomes containing BLP25. Following this procedure, we observed strong antigen-specific anti-MUC1 T cell responses (Figs 1a and 2). We also determined the in vitro T cell proliferative response against non-liposomal, soluble BLP25 antigen from PBL isolated from buffy coats of three of the high responder donors (from Fig. 1a) and did not observe significant antigen-specific T cell proliferation (Fig. 1b) after three re-stimulations.

In order to examine the peptide antigen specificity of the T cell proliferative response observed in Fig. 1(a), the response of T cells stimulated with liposomal BLP25-loaded autologous APC for 2 weeks was studied against BLP-24 or a 24mer mouse MUC1 lipopeptide (BLP-24M) (Fig. 2). T cells which had been stimulated twice with liposomes containing BLP25 responded strongly to BLP25, weakly to liposomal BLP-24 or soluble BP-24 (Fig. 2) and not significantly against mouse BP-24M MUC1 peptide or liposomal BLP24M. There was no proliferation against a soluble control peptide derived from HLA.AW68.1.

MUC1 peptide BLP25 antigen-specific T cells produce type 1 cytokines

Supernatants from bulk cultures of T cells stimulated with autologous PBL pulsed with BLP25 liposomes at the end of two stimulations (i.e. after 2 weeks in culture) were tested for the presence of a secreted type 1 cytokine, IFN-γ, and a type 2 cytokine IL-4. We detected the presence of IFN-γ, but found no or low quantities of IL-4 (Table 1a). The amount of IFN-γ detected was directly proportional to the BLP25 liposomal lipopeptide antigen. The supernatants from T cells cultured in the presence of autologous PBL incubated with empty liposomes (with MPLA, but no peptide) contained only low levels of IFN-γ. We also determined the cytokines secreted in the supernatants in 96-well microtiter plate in the proliferation assays after three weekly stimulations (Table 1b). Again, we observed that the amount of IFN-γ produced was directly related to the BLP25 liposomal lipopeptide antigen concentra-
Blocking of BLP25 peptide-specific T cell proliferative responses by mAb against CD4 and CD8

In order to examine the type of T cells responding to liposomal BLP25 lipopeptide in the in vitro cultures, we added mAb against CD4, CD8 or HLA class I and II molecules, and polyclonal IgG control antibody in the proliferation assays. As shown in Fig. 3, both anti-CD4 and anti-CD8 mAb partially blocked the antigen-specific T cell proliferative responses (P = 0.0029 and 0.0001 respectively). Anti-HLA class I (W6/32) also partially blocked the T cell proliferative response (P = 0.0001) and the blocking with anti-HLA class II antibody was not statistically significant. As controls, the polyclonal IgG control antibody did not block the T cell proliferative response (P > 0.3).

Activation markers on T cells responding to the BLP25 lipopeptide liposomal formulation

We further examined the phenotype of T cells stimulated with liposomal BLP25-loaded autologous APC. The CD4:CD8 ratio in these in vitro cultures was 2:1. All of the CD3+ T cells were TCRαβ+ and CD28+ (data not shown). MUC1 antigen induced stimulation of T cells was evident from examining the activation markers on the T cells cultured in the presence of empty (no peptide) liposome pulsed APC (Fig. 4, panel I) as compared to those of BLP25 (10 μg dose) liposome pulsed APC (Fig. 4, panel II). As shown in Fig. 4, in the BLP25 antigen stimulated T cell cultures, ~70% of the CD4+ T cells were CD25+, ~50% of CD4+ T cells were CD69+, and ~50% of the CD8+ T cells were CD25+ and CD69+ (Fig. 4). In contrast, among the empty (no antigen) liposome-stimulated T cells ~15% CD4+ T cells were CD25+, ~<5% of CD4+ T cells were CD69+ and ~<5% CD8+ T cells were CD69+ or CD25+.

In vitro cytotoxic responses of T cells stimulated with autologous APC incubated with MUC1 peptide (BLP25)-containing liposomes

We determined the cytotoxic activity of T cells stimulated with autologous APC pulsed with liposomal BLP25. The source of T cells was PBL from three HLA.A2+ donors. As targets in the CTL assay, we used T2 mutant cell lines (HLA.A2+) loaded with 9mer peptide epitopes (STAPPAHGV and SAPDTRPAP) both of which are contained in the BLP25 peptide sequence. We observed that STAPPAHGV led to a higher up-regulation of HLA.A2 expression on T2 cells than the peptide SAPDTRPAP (Fig. 5a). We found that among the three HLA.A2 donors, T cells stimulated with liposomal BLP25 had the ability to lyse T2 target cells loaded with STAPPAHGV or SAPDTRPAP peptides at various E:T ratios (Fig. 5b). As a negative control, we used an 8mer ovalbumin peptide (SIINFEKL, which has the capability to strongly up-regulate class I HLA.A2 expression on T2 cell surface, Fig. 5a) loaded T2 cells as targets. We further observed that by the addition of pan anti-class I HLA mAb (W6/32) but not the isotype control antibody (IgG1), the specific killing of STAPPAHGV-loaded T2 cells was reduced (data not shown).

Discussion

The synthetic MUC1 lipopeptide (BLP25) used in the present studies was chosen because it contains epitopes with the capacity to bind to several HLA class I molecules including HLA-A11, A3, A2.1 and A1 (26). In addition, we have previously shown that MUC1 peptide BP-24 (which is contained within the sequence of BLP25) appears to be a permissive epitope for HLA class II-restricted CD4+ T cell responses (2). When we used pH-insensitive, liposome-encapsulated BLP25 to stimulate T cells from PBL of normal healthy donors, strong antigen-specific T cell proliferation was found. Using the same culture conditions the non-liposomal, soluble lipopeptide (BLP25) was unable to stimulate T cell responses from the same donors, probably because liposomal delivery of antigen peptide leads to efficient presentation in the context of MHC molecules. Upon examining the response of BLP25 liposome-stimulated T cells against BLP24 liposome-pulsed autologous APC, the T cell proliferative response was reduced as compared to response against BLP25. These results are in agreement with our hypothesis that BLP25 contains additional epitopes for binding to various class I molecules as compared to BLP-24. In addition, the BLP25 liposome formulation-stimulated T cells lysed target cells pulsed with HLA-A2 class I-restricted 9mer peptide epitope peptides contained within the BLP25 peptide.

Exogenously added soluble peptides either directly bind to MHC molecules on the cell surface or go through the endo-lyosomal presentation pathway for MHC class II presentation (27,28). For cytoplasmic delivery, liposomes have long been used to deliver drugs, based on the assumption that liposome membranes fuse with cell membranes or the liposomes are endocytosed by the cells and release their contents into the cytoplasm. Liposomes that are pH sensitive have been reported (29) to deliver soluble antigens to peritoneal macrophages for both cytoplasmic class I and endosomal class II presentation pathways. In contrast, pH-insensitive liposomes were shown to sensitize the APC only for class II-restricted presentation. It was also reported that at high concentrations of encapsulated antigen peptide, the pH insensitive liposomes can deliver antigen to both endocytic and cytoplasmic location for presentation by both MHC class I and II molecules (30 and References therein).

Human T cell responses against MUC1 mucin peptide epitopes are relevant for the development of MUC1 as targets for immunotherapy of certain adenocarcinomas (31). The presence of class I non-restricted anti-MUC1 CTL precursors in cancer patients (8,32) and class I-restricted anti-MUC1 CTL have been demonstrated (25,26). Furthermore, in active specific immunotherapy models in mice, we have shown that vaccination with liposomes containing MUC1 peptide could induce potent type 1 anti-MUC1 immune responses and protected animals against development of tumors expressing human MUC1 (33). In addition, MUC1 peptide-specific T cells, obtained from multiparous donors and stimulated with MUC1-derived synthetic peptide, have the capability to kill HLA-matched MUC1+ tumor cells (25). Further, in a phase I clinical study in breast cancer patients using a MUC1 peptide–KLH conjugate formulation for immunotherapy, T1/2 and class I-restricted anti-MUC1 CTL responses were found (10). Our
present in vitro study further strengthens our hypothesis that liposomal formulations containing MUC1 peptides can be used to stimulate potent anti-MUC1 T cell responses. Besides using liposomal peptide/protein antigens as vaccines for certain tumors, dendritic cells pulsed with tumor-derived peptides have been used as cellular vaccines for treatment of certain tumors (34,35). Our studies suggest that autologous PBL pulsed with tumor-associated peptide could be used as cellular vaccines to generate antitumor T cell responses and thereby obviate the need to purify and stimulate the production of dendritic cells. Another approach has been adoptive cellular immunotherapy where tumor-infiltrating lymphocytes (TIL) have been isolated, expanded in vitro and infused back into tumor-bearing hosts (36). However, this process requires surgical harvest of the tumor followed by isolation and expansion of TIL in vitro. Our studies suggest that it might be possible to simply use PBL to obtain T cells which can be primed in vitro using liposomal tumor antigens, followed by their expansion in vitro prior to use in adoptive immunotherapy.

In conclusion, in this report we have shown that strong anti-MUC1 T cell responses (both CD4 and CD8) can be generated from T cells obtained from PBL of normal Red Cross donors. MUC1 and other tumor antigens, e.g. carcinoembryonic antigen (CEA) and prostate-specific antigens (PSA), are class a tumor antigens which are also expressed on normal epithelium and, therefore, might have induced immunological tolerance. However, T cells have been isolated from patients with circulating MUC-1 or CEA antigens that can recognize MUC1 or CEA antigen peptides on target cells (32,37). Furthermore, it has been shown recently (38) that peptides derived from PSA (a self antigen) can induce peptide-specific CTL from PBL isolated from normal control subjects and prostate cancer patients. However, these experiments required six or seven in vitro stimulation cycles and a total of 96–112 days in vitro culturing period to obtain PSA-specific CTL. In agreement with these observations, we have shown previously that using MUC1 synthetic soluble peptides, it took 8–10 weeks of in vitro culture to generate HLA-restricted, MUC1-specific CTL responses from T cells isolated from PBL of multiparous women (25).

In this report, we have shown that it is possible to rapidly generate human Tc cells and CTL responses directed against MUC1-derived peptides in vitro using liposome-encapsulated, antigen-loaded autologous PBL as APC. Based on this demonstration a phase I immunotherapy study, whereby lung cancer patients are immunized with liposomes containing BLP25, has just begun.

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Abbreviations

AIM-V trade name of serum-free medium
APC antigen-presenting cells

References


