Liposomes with Incorporated MHC Class II/Peptide Complexes as Antigen Presenting Vesicles for Specific T Cell Activation

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Purpose. The purpose of this study was to design a well-characterized liposomal carrier system for multivalent antigen presentation in order to activate T cells.

Methods. MHC class II molecules were loaded with peptide and subsequently reconstituted into liposomes. A FACS assay was developed to monitor peptide loading and MHC class II incorporation in the liposomes. For in vitro testing of the resulting MHC class II/peptide liposomes, a T cell hybridoma assay was employed.

Results. The FACS assay provided a qualitative means to visualize the amount of incorporated MHC class II and peptide molecules that were oriented in the appropriate way for antigen presentation to the T cells. Interestingly, when MHC class II molecules were loaded with the appropriate peptide prior to liposome incorporation, such liposomes were fully capable of inducing IL-2 production of a T cell hybridoma.

Conclusions. This is the first article showing that MHC class II/peptide liposomes can serve as 'artificial antigen presenting cells' for activation of a CD4+ T cell hybridoma. As compared to soluble MHC class II/peptide complexes, the multivalency of liposomal complexes may be an important advantage when studying possible applications in immunotherapy.

KEY WORDS: liposomes; MHC class II; peptides; FACS analysis; T lymphocytes.

INTRODUCTION

Interaction of specific major histocompatibility complex (MHC) class II/peptide complexes with the T cell receptor (TCR) on the cell surface of CD4+ T cells is the key event in T cell activation (1). The way of antigen presentation to the T cell is important for the ultimate effect on T cell function. For full activation of a T cell resulting in the complete cascade of T cell functions, additional signaling is needed apart from the interaction between the MHC class II/peptide complex and T cell receptor. These co-stimulatory signals are provided by the antigen presenting cell (APC) (2). Antigen presentation in the absence of proper co-stimulation has been shown to induce a state of antigen-specific T cell unresponsiveness (3). The physical association between the MHC/peptide complex and the TCR was analyzed by studies employing planar membranes containing MHC/peptide complexes (4), or peptide-MHC tetramers (5). Surface plasmon resonance studies indicated that binding of the MHC/peptide complex to the TCR and the rate of the subsequent dissociation reaction is correlated to T cell responsiveness (6,7). *In vitro* studies employing isolated MHC/peptide complexes demonstrated that such complexes were capable of triggering T cell responses (8,9). Moreover, similar studies using dimers or tetramers of MHC/peptide complexes indicated the importance of multivalency in T cell activation (5,10,11). Indeed, recent reviews on the various models of T cell activation have elaborated on the necessity of multiple triggering (12) and the formation of a (two-dimensional) contact area between T cell and APC (13). This led us to propose a liposomal formulation as a carrier system for MHC class II/peptide complexes, in order to present these complexes in a multivalent way to the immune system. Membrane proteins like MHC molecules can be incorporated into the phospholipid bilayer in a 'cell-like' fashion (14,15). Others have employed MHC class II-containing liposomes (16) and planar membranes prepared from these liposomes to study basic aspects of antigen presentation (16–19). However, antigen-specific interleukin 2 (IL-2) production of a T cell hybridoma in response to these MHC class II-liposomes could not be observed (16). Here we now show that it is possible to prepare functionally active MHC class II/peptide liposomes by complexation of the purified detergent-solubilized MHC class II molecules with the desired peptides under optimal loading conditions prior to their incorporation into liposomes. In order to visualize peptide loading and MHC class II incorporation, a FACS assay for liposomes was developed. Using such a strategy, well-defined MHC class II/peptide liposomes containing different types of MHC class II molecules (mouse, rat and human) could be prepared. To demonstrate the antigen presentation capacities of such vesicles, peptide-loaded rat MHC class II molecules, RT1.B^L, were incorporated into liposomes and used as antigen presenting vesicles in a co-stimulation-independent T cell hybridoma assay for specific T cell activation.

MATERIALS AND METHODS

Purification of MHC II Molecules

Lewis rat MHC class II molecules, RT1.B^L, were purified from the Z1a T cell clone through affinity chromatography using the OX6 monoclonal antibody (mAb) as described before (20). Balb/C mouse MHC class II molecules I-A^d, were purified from the A20 B cell lymphoma using the MKD6 mAb, human HLA-DR4 molecules from Huly.138 I C2 cells (A.M.H. Boots, N.V. Organon, Oss, the Netherlands) using the L243 mAb,

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ABBREVIATIONS: APC, antigen presenting cell; ECL, enhanced chemiluminescence; FITC, fluorescein isothiocyanate; HSP65, mycobacterial 65 kD heat-shock protein; IL-2, interleukin 2; IMDM, Iscove's modified Dulbecco's medium; mAb, monoclonal antibody; MBP, myelin basic protein; MHC, major histocompatibility complex; OG, n-β-octylglucopyranoside; PBS, phosphate-buffered saline; PL, phospholipid; TCR, T cell receptor.

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according to the same method. Affinity-purified MHC molecules were solubilized in 1% n-β-octyl glucopyranoside (OG) (Sigma).

Peptides

The RT1.B^L binding peptides, peptide 176–190 from mycobacterial 65 kD heat shock protein (HSP65 176–190; EESNTFGLQLELTEG) and peptide 72–85 from guinea pig myelin basic protein (MBP72-85; QKSQRSQDENPV) were synthesized by standard solid phase Fmoc chemistry (21). Peptides were purified by reversed-phase high-performance liquid chromatography, and checked by fast atom bombardment mass spectrometry. When used in the MHC-peptide binding assay, peptides were biotinylated during synthesis. I-A^d binding peptide 126–138 from hemagglutinin (HA126-138; HNTNGVTAACSHE) and biotinylated HA126-138 were kindly provided by Dr. R. Liblau (Paris, France), HLA-DR4 binding peptide HA307-319 (PKYVKQNTLKLAT) by C. J. van Staveren (N.V. Organon, Oss, the Netherlands).

Peptide Loading of MHC Class II Molecules

Optimal peptide loading conditions for MHC class II molecules were determined in a MHC class II-peptide binding assay as described before (20), with minor modifications. Duration of incubation, pH and temperature were varied systematically. Briefly, detergent-solubilized MHC class II molecules (0.5–1 μM) were incubated with a 50–250 fold molar excess of biotinylated peptide for the designated time, pH and temperature without the addition of protease inhibitors. MHC-peptide complexes were analyzed via non-reducing SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Following Western blotting (Hybond-ECL, Amersham), the biotinylated peptides were visualized on preflashed films (Hyperfilm, Amersham) through enhanced chemiluminescence (Western blot ECL kit, Amersham). Optimal binding conditions were derived from the films, by evaluation of the density of the spots at the position of the MHC class II dimer-peptide complex. The presence of the MHC class II dimer under the tested conditions was monitored by means of non-reducing SDS-PAGE followed by silver staining (BioRad).

Liposome Preparation

Liposomes were prepared from egg-yolk phosphatidylcholine (Lipoid), egg-yolk phosphatidylglycerol (Lipoid) and cholesterol (Sigma) (10:1:4, molar ratio) through the method of detergent removal (adapted from (22)). Appropriate amounts of phospholipids (PL) and cholesterol were dissolved in ethanol (absolute) and mixed in a round-bottom flask. A fluorescence-labeled phospholipid, Texas Red phosphatidylethanolamine (Molecular Probes) was added to a final concentration of 0.5 mole% of total PL. A thin lipid film was obtained through evaporation of the ethanol under reduced pressure at 40–50°C, followed by a N_2 flush of at least 15 minutes. The lipid film was dispersed in phosphate-buffered saline (PBS) (pH 7.2), containing 1% OG and MHC class II molecules (initial MHC to PL molar ratio 1/1000 to 1/4800). Liposomes were formed via controlled dilution with detergent-free PBS, followed by two subsequent ultracentrifugation steps (230,000×g, 90 min, 4°C). Final liposome pellets were redispersed in PBS or Iscove's Modified Dulbecco's Medium (IMDM, Gibco) at a phospholipid concentration of 8 mM. For control experiments, peptide liposomes were prepared with comparable amounts of soluble peptide in the absence of MHC.

Characterization of MHC Class II/Peptide Liposomes

Mean particle sizes of the MHC-containing liposomes were determined through dynamic light scattering analysis with a Malvern 4700 system, using a 25 mW Ne-He laser and the automeasure version 3.2 software (Malvern Ltd.). For refractive index and viscosity the values for pure water were used. The particle size distribution was reflected in the polydispersity index, which ranges from 0.0 for a monodisperse to 1.0 for a polydisperse dispersion.

Liposomes were visualized through flow cytometric analysis using a FACSCAN (Becton Dickinson). To enable visualization of liposomes by FACS analysis, it was necessary to select the most sensitive settings of the forward and side scattering detectors. Because of the liposome size, it was not possible to set a threshold for forward scattering to gate out irrelevant particles, as is standard procedure when analyzing cells via flow cytometry. Therefore, fluorescence-labeled liposomes were gated according to their Texas Red fluorescence by placing a threshold at the FL3 channel. For qualitative analysis of the MHC class II incorporation efficiency, MHC class II-liposomes were incubated with a fluorescein isothiocyanate (FITC)-labeled anti-MHC class II monoclonal antibody (FITC-OX6 for RT1.B^L, (1:50, (v/v)), Pharmingen) or the FITC-labeled isotype control, for 30 min on ice. Excess reagent was removed through ultracentrifugation (230,000×g, 90 min, 4°C). For qualitative analysis of the peptide loading of the reconstituted MHC class II molecules, I-A^d-liposomes were incubated with biotinylated HA126-138 or a control (not binding to I-A^d) biotinylated peptide at optimal loading conditions and subsequently labeled with streptavidin-FITC (1:100 (v/v); Pharmingen) following the above described labeling protocol before FACS-analysis.

Residual OG concentrations after liposome preparation were determined via gas chromatography and were always <0.1% of the initial concentration (data not shown). Recovery of phospholipid after liposome preparation was determined in some experiments through fluorescence spectrometrical analysis (LS50B, Perkin Elmer) of the Texas Red concentration in the liposome samples after dilution in methanol and was always >75% (data not shown). Protein concentrations in the liposome formulations were determined via the method as described by Minamide and Bamberg (23). Briefly, aliquots of MHC class II-liposomes were spotted onto filter paper (Whatmann). After washing with methanol for 45 min, protein was stained using 0.5% Coomassie Brilliant blue (Pierce) in 7% acetic acid. The dye was extracted with MeOH/dH_2O/NH_4OH (66/33/1) and optical densities were determined at 600 nm in a well-plate reader (Bio-Rad).

A2b T Cell Hybridoma Assay

For use in the T cell hybridoma assay, detergent-solubilized affinity-purified RT1.B^L molecules were preloaded with peptide at the optimal loading conditions. Liposomes were prepared with these complexes according to the detergent removal method (22). Well-characterized RT1.B^L/peptide liposomes

were tested for their ability to trigger T cell responses. The A2b T cell hybridoma was generated and characterized as described before (24). Activation of the T cell hybridoma was measured in flat-bottomed microtiter plates (Costar) in triplicate cultures in IMDM, supplemented with 2 mM L-glutamine, 50 U/ml penicillin, 50 U/ml streptomycin and 10% heat inactivated fetal calf serum (PAA Laboratories). Each well contained 2×10^4 hybridoma cells and various concentrations of the liposomes. Control experiments were performed with a dose range of soluble antigen in the presence or absence of 1×10^6 irradiated (3000 rads) Lewis rat derived thymocytes as APCs. Supernatants were removed after 40 hours of culturing, and assayed for IL-2 activity by adding the IL2-dependent cell line CTLL16 (5×10^3 /well). The CTLL16 cells only proliferate when IL-2 is present in the cell culture supernatants. Following 24 h of culturing, cells were pulsed with [^{3}H]-thymidine (0.4 μCi/well; specific activity 1 Ci/mmol, Amersham) for 16–18 h. Cells were harvested on fibreglass filters and [^{3}H]-thymidine incorporation was measured using a scintillation counter (Wallac Oy).

RESULTS

Preparation and Characterization of MHC Class II Liposomes

Affinity-purified MHC class II molecules were reconstituted into liposomes at various initial MHC:phospholipid (PL) ratios. By carefully monitoring the particle size and MHC incorporation efficiency of these MHC-containing liposomes, a well-characterized carrier system was developed. Particle sizes were typically between 0.10–0.12 μm, with narrow particle size distributions (polydispersity indices: 0.1–0.2). The absolute incorporation efficiency of MHC class II in liposomes was determined quantitatively for the MHC:PL = 1:1000 ratio through the spot-assay and was found to be 68% on average, and not dependent on the MHC type (data not shown). Assuming the MHC class II-liposomes are uni-lamellar, this would correspond to 200–300 protein molecules in total per liposome. Flow cytometric analysis was used to characterize the Texas Red labeled liposome dispersions with respect to MHC class II incorporation. By setting a threshold at the FL3 channel (red fluorescence), all particles in the same size range as liposomes but displaying no Texas Red fluorescence, were ignored. A typical dot plot representation of a liposome dispersion is shown in Fig. 1A. The incorporation efficiency of MHC class II molecules in the liposomes was evaluated through analysis of the FL1 fluorescence (FITC fluorescence). Liposomes, containing varying amounts of rat MHC class II molecules, RT1.B^L, were labeled with FITC-OX6 mAb or the isotype control and analyzed. The OX6 mAb recognizes part of the extracellular domain of the MHC molecule, thus ensuring that only functionally oriented MHC molecules are visualized.

Liposomes prepared with the highest initial ratio of RT1.B^L:PL (1/1000, M:M), displayed the strongest increase in FITC fluorescence (Fig. 1B). The bell-shaped curve indicated furthermore a homogeneous distribution of the MHC class II molecules over the whole liposome population. Decreasing the initial amount of MHC class II used for liposome preparation (MHC/PL=1/4000) resulted in reduced FITC fluorescence. Liposomes containing no RT1.B^L incubated with the FITC-OX6 mAb (Fig. 1B) or RT1.B^L-liposomes incubated with the

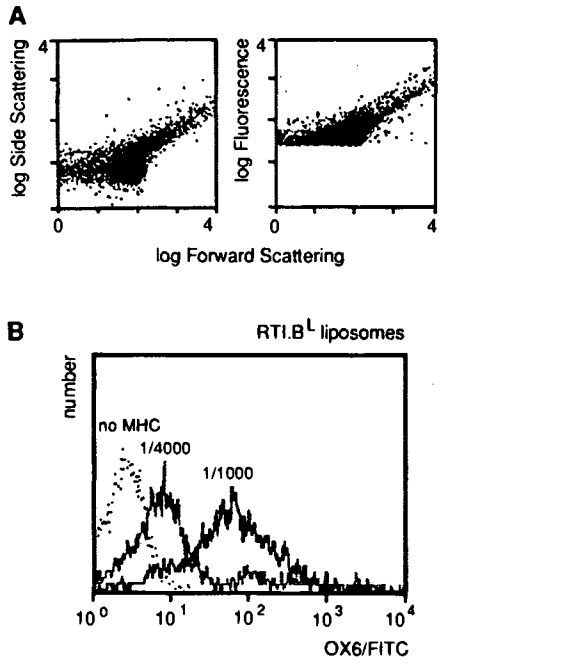

Fig. 1. Visualization of MHC II incorporation efficiency via flow cytometric analysis of MHC class II-containing liposomes. A: Dot-plot representation of a liposome dispersion. Liposomes were gated according to their Texas Red fluorescence, after comparison to non-labeled particles. B: Affinity-purified RT1.B^L molecules were reconstituted into Texas Red-positive liposomes at various MHC:PL molar ratios and subsequently labeled with FITC-OX6, followed by FACS analysis.

FITC-labeled isotype control mAb (data not shown), displayed background green fluorescence, indicating the absence of non-specific labeling.

The FACS assay was applicable for liposomes containing various types of MHC class II molecules in MHC:PL ratios ranging from 1:1000 to 1:5000 (M:M) (data not shown).

Optimal Peptide Loading of MHC Class II Molecules

MHC class II molecules affinity-purified from cell lysate contain a wide variety of endogenous peptides in their peptide binding groove. In order to obtain MHC class II molecules loaded uniformly with one particular peptide several techniques were described in literature (25,26). In the present study we employed the method of Nag *et al.* who reported that complete loading of HLA-DR2 molecules with peptide derived from myelin basic protein (MBP) was achieved after prolonged incubation with an excess of the peptide at optimal pH and temperature (26).

We determined the optimal peptide loading conditions for rat, human and mouse MHC class II molecules (Fig. 2 and data not shown). In Fig. 2, the optimal peptide loading conditions of rat MHC class II molecule RT1.B^L are compared to those for human MHC class II HLA-DR4. Figure 2A is a reproduction of the film obtained via enhanced chemiluminescence (ECL), showing the dependence of the loading of biotinylated HSP65 176–190 to RT1.B^L on pH, temperature and time. Figure 2B and 2C present the relative peptide loading of RT1.B^L and HLA-DR4 with their respective biotinylated peptides as determined via densitometric analysis of the ECL-films (Fig. 2A

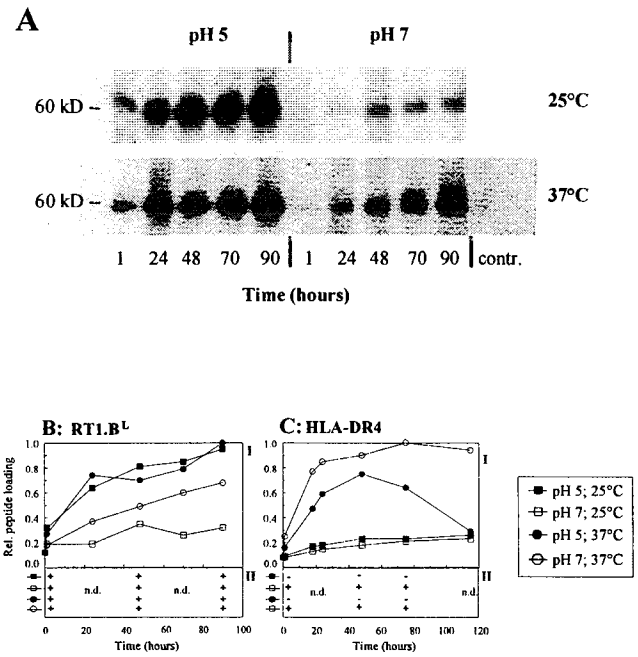

Fig. 2. Optimization of peptide loading of rat and human MHC class II molecules. Detergent-solubilized MHC class II molecules were incubated with biotinylated peptides at the designated temperature and pH for various periods of times. Peptide loading was analyzed through ECL as described in the Materials and Methods section. A: Result of the binding assay for rat RT1.B^L and biotinylated HSP65 176-190. B-C (I): Relative peptide loading of RT1.B^L (B) and HLA-DR4 (C) as determined via densitometry. The condition resulting in the highest peptide loading was set 1.0 for each MHC type. (II): The presence of the αβ-dimer was checked at some timepoints after silver staining of the SDS-PAGE gels and represented as: + only αβ-dimer visible; − mainly separate α/β chains visible. The results were reproduced in at least two independent experiments.

and data not shown). For both MHC types, peptide binding increased at longer incubation times and was higher at elevated temperature. For RT1.B^L, as was also found for mouse MHC class II, I-A^d (data not shown), binding was highest at pH 5. In contrast, for HLA-DR4, peptide binding was at least equally high at neutral pH (Fig. 2B) especially at the highest temperature. At acidic pH however, the HLA-DR4 dimer almost completely dissociated into separated α and β chains (Fig. 2C), whereas in case of RT1.B^L the αβ-dimer remained present under all conditions (Fig. 2B). The dissociation of the αβ-dimer into separate chains in case of HLA-DR4 was probably causing the decrease in peptide loading after 48 hours at acidic pH.

Visualization of Peptide Loading on MHC Class II-Liposomes by FACS Analysis

In order to visualize peptide loading on liposomes with reconstituted MHC class II molecules, liposomes were prepared with mouse MHC class II molecules, I-A^d (MHC:PL = 1:2000; anti-MHC/FITC-positive liposomes as determined via FACS analysis: 70% (data not shown)). To exclude the presence of any nonspecifically encapsulated peptide inside the liposomes not bound to MHC, the MHC molecules were not preloaded with peptide prior to liposome preparation for this assay. For I-A^d similar loading efficiencies were achieved upon either preloading MHC with the peptide or upon loading after liposome preparation (data not shown).

Final pellets after liposome preparation were resuspended in PBS and aliquots were incubated with a 2-fold molar excess of biotinylated HA126-138 or control peptide for 16 hours at room temperature. After labeling with streptavidin-FITC, samples were analyzed by FACS. Figure 3 shows the histograms of liposome-associated FITC fluorescence under the different incubation conditions. I-A^d liposomes incubated with biotinylated HA126-138 induced a shift in FITC fluorescence for a large part of the liposome population, indicating the binding of streptavidin-FITC. Since only a 2-fold excess of peptide was used and incubation times were short, peptide binding was not maximal in this assay. I-A^d liposomes incubated with a control biotinylated peptide did not bind streptavidin-FITC (Fig. 3, left panel). The shift in fluorescence as seen in Fig. 3 was paralleled by a shift in the side scattering of the liposomes suggesting an increase in particle/complex size (Fig. 3, right panel). As streptavidin can bind multiple biotin groups it is most likely that upon streptavidin binding aggregates form, which are composed of more than one liposome. This aggregate formation is mediated via the biotin groups associated with the I-A^d/peptide liposomes, since no increase of side scattering was observed in the control sample (Fig. 3, right panel).

These samples were furthermore subjected to SDS-PAGE, followed by the ECL protocol as described for the MHC-peptide binding assay to ascertain that the biotinylated peptide was indeed bound to the MHC molecule (data not shown).

MHC Class II/Peptide Liposomes Induce IL-2 Production of the A2b T Cell Hybridoma

To test the ability of MHC class II/peptide liposomes to specifically activate the A2b T cell hybridoma, affinity-purified, detergent-solubilized RT1.B^L molecules were loaded with the HSP65 176-190 or the MBP72-85 peptide at optimal peptide loading conditions. These MHC class II/peptide complexes were reconstituted into liposomes and added to the hybridoma cells.

As shown in Fig. 4A, soluble HSP65 176-190 in the presence of APCs, activated the A2b T cell hybridoma in a dose-dependent manner, as measured through IL-2 production. In

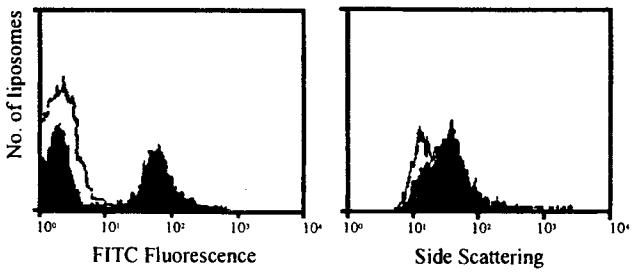

Fig. 3. Visualization of peptide loading on MHC class II containing liposomes. I-A^d liposomes were incubated overnight with biotinylated HA126-138 peptide. The Texas Red-positive liposomes were analyzed via FACS analysis after labeling with streptavidin-FITC. Histogram representation of peptide-loaded MHC class II liposomes (filled histogram), compared to liposomes loaded with a control biotinylated peptide (open histogram), analyzed for FITC fluorescence and side scattering.

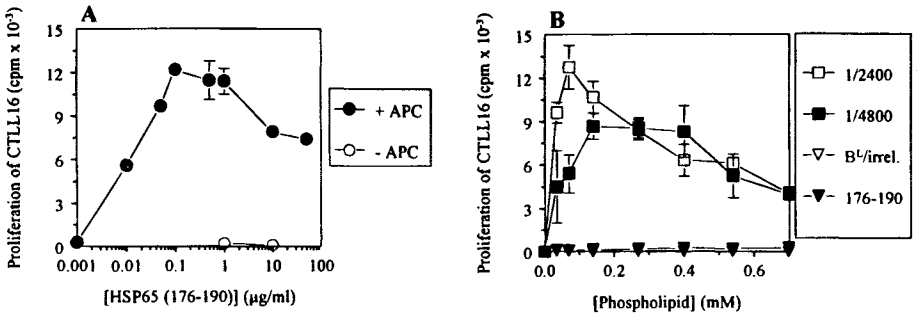

Fig. 4. IL2-production of the A2b T cell hybridoma, in response to A: soluble peptide, in the presence or absence of irradiated thymocytes as APCs or B: RT1.B^L/peptide-liposomes (initial molar ratio MHC:PL = 1/2400 and 1/4800), loaded with the specific or an irrelevant peptide; or control liposomes containing only the specific peptide and no MHC class II. Hybridoma cells were cultured for 40 hours. Supernatants were removed and tested for IL-2 activity via proliferation of the IL2-dependent CTLL16. Data are expressed as mean cpm of triplicate cultures ± SD.

the absence of APCs, no proliferation of the IL2-dependent CTLL16 was observed. Interestingly, (RT1.B^L/HSP65 176-190)-liposomes were fully capable of triggering IL-2 production as measured through proliferation of the CTLL16 (Fig. 4B). No (external) co-stimulation was needed for this activation. Furthermore, activation of the A2b hybridoma by (RT1.B^L/HSP65 176-190)-liposomes was dependent on both the phospholipid dose and the MHC II/peptide complex density on the liposomal surface. The latter could be deduced from the shift of the dose-response curve towards higher phospholipid concentrations when the MHC II/peptide complex density was decreased (1/4800 versus 1/2400 in Fig. 4B). Maximal IL-2 production in response to the (RT1.B^L/HSP65 176-190)-liposomes was comparable to the levels obtained after peptide/APC stimulation (Fig. 4A). MHC class II/peptide liposomes, containing an irrelevant RT1.B^L binding peptide (MBP72-85), and liposomes prepared without MHC class II, but in the presence of the HSP65 176-190 peptide did not elicit IL-2 production (Fig. 4B).

DISCUSSION

In the present report, we show for the first time that MHC class II/peptide liposomes can specifically activate T cells. Following reconstitution of peptide pre-loaded rat MHC class II molecules into liposomes specific activation of a T cell hybridoma was observed. In order to prepare a well-characterized formulation, a FACS assay was developed to visualize both peptide loading and MHC incorporation in the liposomes. Others have used the technique of flow cytometry to study liposomes with respect to size (27) or lateral diffusion of incorporated MHC class I molecules (15). In the present study, small (0.1 μm) liposomes were analyzed through this assay on a single-liposome level by fluorescence labeling of the liposomal bilayer. We were able to show that MHC class II molecules were distributed homogeneously over the entire liposome population. Moreover, since labeling took place at the outside of the vesicles, only the functionally oriented MHC class II molecules were visualized through this method. The loading of peptide molecules onto the liposome-incorporated MHC class II molecules could be visualized following a similar protocol and was confirmed after submitting the liposomes to SDS-PAGE analysis. The FACS-assay however, has a clear advantage as compared to other techniques requiring liposome disruption prior to analysis, since by FACS analysis only liposome-associated and externally oriented protein/peptide molecules are visualized while other methods are based on the determination of total protein concentration. Thus, employing the aforementioned methodology a well-defined MHC class II/peptide liposome formulation was obtained, that could be used for the *in vitro* testing. Provided the availability of a MHC source as well as a binding peptide and anti-MHC mAb, the here described method for the preparation and characterization of MHC class II/peptide liposomes is generally applicable.

In the past other groups have studied the response of a T cell hybridoma to MHC class II-containing liposomes or planar membranes. In these reports, antigen-specific IL-2 production was observed only after transforming the MHC class II-liposomes to planar membranes (16–19). An explanation for the apparent discrepancy between our data and these earlier studies involving MHC class II restricted antigen presentation, may be related to the efficiency of peptide loading of MHC. From the present study, it was clear that for each type of MHC class II molecule, optimal peptide loading conditions need to be determined, as was indicated earlier (26). Since MHC-peptide loading and MHC class II dimer integrity turned out to be temperature- and especially pH-dependent, one can easily diminish MHC/peptide induced TCR activation by selecting improper loading conditions. The use of biotinylated peptide in our experiments to determine optimal loading conditions for the unlabeled peptide can be justified because only absolute peptide binding and neither relative binding nor the integrity of the MHC dimer are affected by the biotin group, since the peptide was biotinylated N-terminally.

By prior complexation of the MHC class II/peptide complexes at optimal loading conditions, the resulting density of desired MHC class II/peptide complexes on the liposomes will be much higher and better controlled than the density achieved after co-incubation of the MHC class II-liposomes with the peptide in the final lymphocyte stimulation assay, as was done in the study mentioned before (16). Peptide excess or optimal pH and especially optimal incubation times as determined in Fig. 2, will not be reached during the

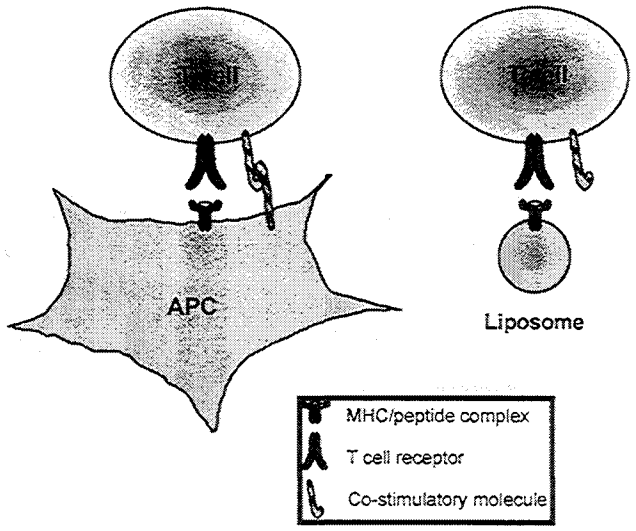

Fig. 5. Concept of induction of T cell anergy by antigen presentation in the absence of co-stimulation, using MHC class II/peptide liposomes (right panel) instead of normal ('professional') APCs (left panel).

course of the actual lymphocyte stimulation assay but can be easily set in the pre-loading step. The dependency of T cell activation on the concentration of MHC II/peptide complexes can be clearly deduced from Fig. 4B, where a decreased initial MHC II/peptide complex density on the liposomes (1/4800 versus 1/2400, initial MHC/PL ratio) resulted in a shift of the dose-response curve towards higher phospholipid concentrations.

The approach of peptide loading under optimal loading conditions, prior to liposome formation and incubation with the target T cells, as was done in the present study, generates a multivalent antigen presentation system. Although the role of multivalency in antigen presentation was not explicitly investigated in some studies employing purified MHC/peptide complexes (8,9), its importance was acknowledged in recent studies involving multimeric MHC/peptide complexes (5,10,11).

A possible point of criticism regarding the study of T cell activation with a T cell hybridoma assay may be that such hybridoma cells are a rather artificial read-out system. As was mentioned earlier, these hybridoma cells do not require co-stimulation for full activation in contrast to normal CD4+ T cells. However, we see this as an important property of the A2b T cell hybridoma allowing us to prove that well-characterized MHC class II/peptide liposomes are indeed capable of efficient delivery of signals through the TCR.

Follow-up studies with these MHC class II/peptide liposomes will focus on the interaction with the corresponding T cell clone. Recently, we have shown that the A2b T clone can be rendered anergic via antigen presentation in the absence of proper co-stimulation. This anergic state was induced by presentation of soluble peptide by MHC class II-positive T cells (28). For the antigen specific immune modulation of certain T cell mediated diseases, induction of T cell anergy may be an interesting approach. We are currently investigating whether T cell activation by MHC class II/peptide liposomes in the absence of co-stimulation can modulate T cell responses by inducing anergy in T cells, as described for isolated complexes (9,10). This concept is schematically presented in Fig. 5. The multivalent presentation of relevant antigens via liposomes and the possibility of preparing a well-characterized and 'tailor-made' carrier system (e.g., by modulating its pharmacokinetic behavior), may give MHC/peptide liposomes an important advantage over isolated complexes.

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