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Antigen Presentation by Liposomes

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A. Introduction

T cells respond to foreign antigen only when the latter is presented on the surface of an antigen-presenting cell (APC) together with a molecule encoded in the major histocompatibility complex (MHC). The nature of this antigen presentation is poorly understood. The difficulty of demonstrating soluble antigen serologically on the surface of APC, the finding that in some cases peptides of a certain protein are more antigenic than the whole molecule [1], the observation that T cells respond to native and denatured antigen equally well irrespective of which form was used for priming [2], and the fact that cells can rapidly degrade the antigen have led to the concept of antigen processing. According to this hypothesis the antigen is internalized and structurally altered (possibly enzymatically degraded) by the APC, and is then redisplayed on the surface of this cell in association with MHC molecules. Only antigens thus converted are recognizable by T cells [3].

To determine whether antigen processing is necessary for T-cell activation, we constructed liposomes carrying a foreign protein antigen and MHC class II molecules, and tested whether these liposomes could activate antigen-specific class II-restricted T cells in the absence of APC. The results presented here demonstrate that T cells can recognize unprocessed, native antigen.

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The protocol used to produce the liposomes is described in detail elsewhere [4]. A summary is given in Fig. 1. The liposomes produced by this procedure contain MHC molecules inserted into the lipid bilayer by their transmembrane portion and a protein antigen covalently bound to DPPE (dipalmitoylphosphatidylethanolamin) lipids via a disulfide bond.

B. Results and Discussion

Table 1 summarizes a series of experiments that were performed with a lactate dehydrogenase B (LDH_B)-specific A^b-restricted mouse T-cell clone. Under the conditions when the T cells did not proliferate to LDH_B without adding APC they could be stimulated by liposomes containing the antigen and the restriction molecule (A^b) but could not be stimulated by either liposomes containing only one of these two components or liposomes with LDH_B together with another class II molecule. A mixture of liposomes carrying the antigen and liposomes carrying the MHC molecule or a mixture of A^b-containing liposomes with soluble LDH_B were also ineffective. These results show that the antigen and the restriction molecule combined in the same membrane provide a sufficient signal for the activation of T cells. Thus an APC-dependent processing step is not required for antigen recognition by T cells. Apparently the antigenic site seen by the T cell is only determined by the molecular properties of the antigen and is not influenced by the APC.

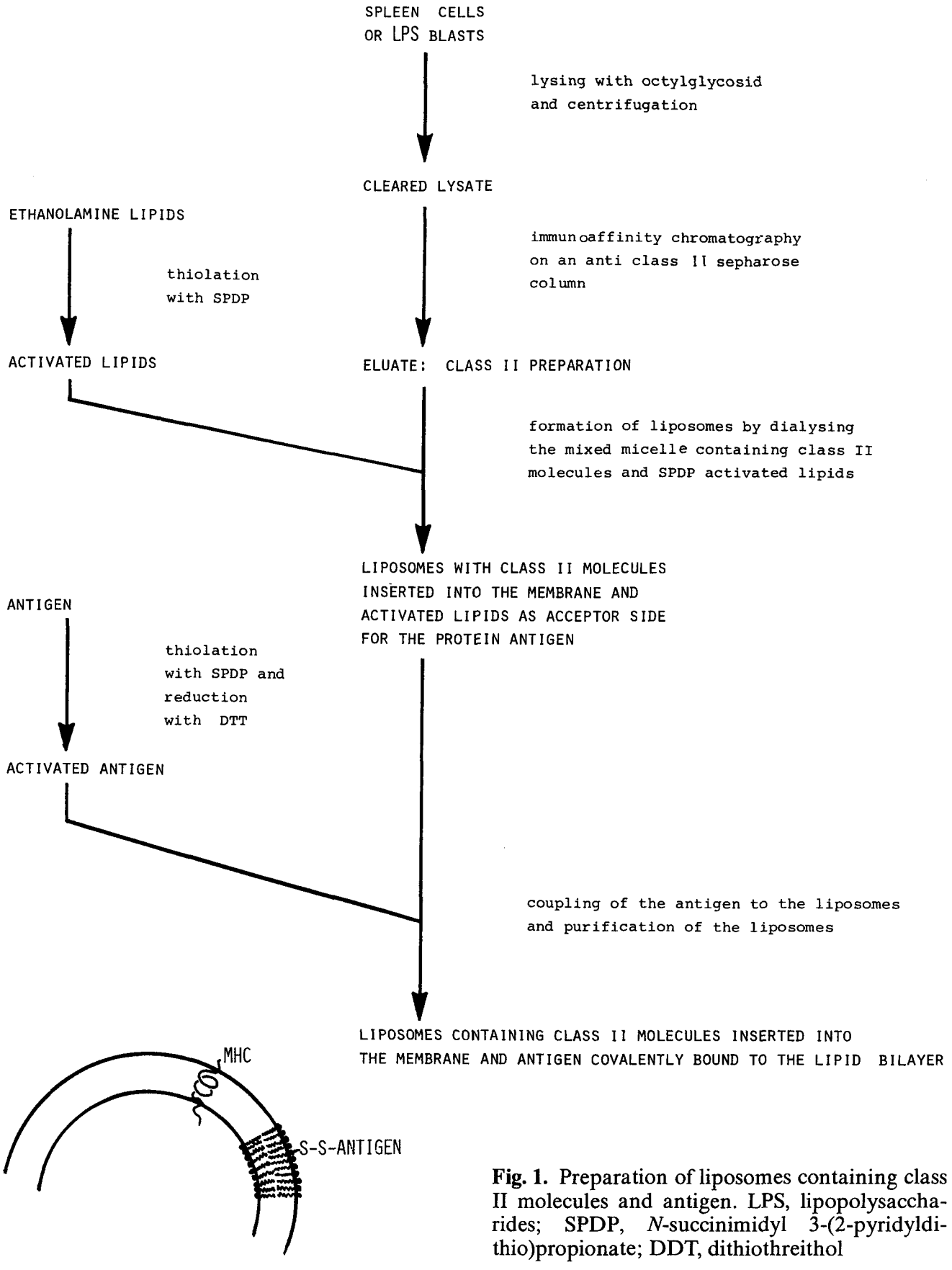

Fig. 1. Preparation of liposomes containing class II molecules and antigen. LPS, lipopolysaccharides; SPDP, *N*-succinimidyl 3-(2-pyridyldithio)propionate; DDT, dithiothreithol

Two further important observations could be made in these experiments. First, the physical properties of the liposome membrane influence the response dramatically. Liposomes composed of lipids that form a liquid crystalline bilayer at the incubation temperature (experiments 1, 2) have a much lower stimulatory capacity than rigid liposomes composed of lipids that result in a bilayer with a high phase-transition temperature (experiments 3, 4). Thus, the mobility of the two essential com-

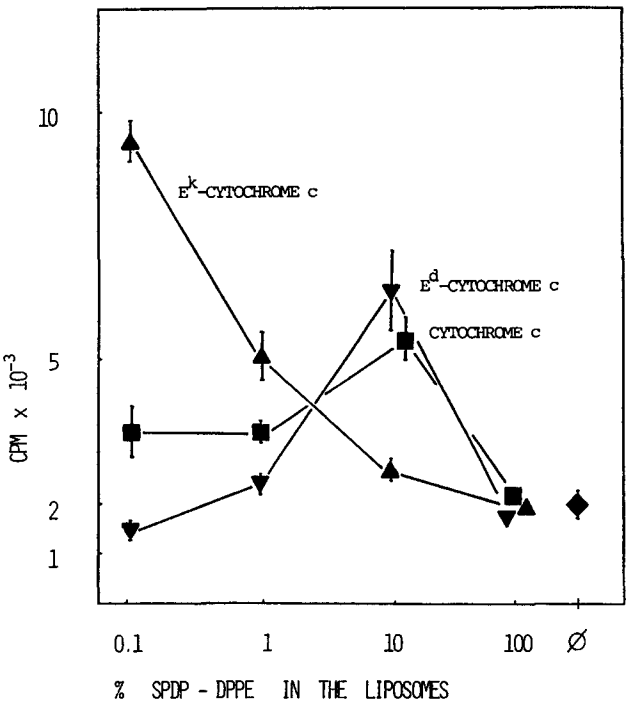

Fig. 3. Effect of antigen density on the induction of IL-2 by the B10.A (5R) anticytochrome c T-cell hybridoma line. The liposomes were produced with DPPE containing varying amounts (0.1%–100%) of SPDP-modified DPPE (efficiency of the modification, 1.7%) and E^k molecules. They were produced as described and tested for their capacity to induce IL-2 production by the T-cell hybridoma 4117 (B10.A[5R]); Heber-Katz et al.). Liposomes containing E^k and cytochrome c (▲), E^d and cytochrome c (▼), and cytochrome c (■) were used. Control values (cpm) for IL-2 production: feeder cells (B10.A), 542 ± 88; antigen (cytochrome c), 2145 ± 348; feeder cells and antigen, 74 680 ± 7384. SPDP-DPPE, SPDP-modified DPPE

Table 2. Secondary response of T cells to liposomes containing MHC class II molecules and antigen (cpm)

Cell line	B10.D2 α KLH	B 6 α OVA	B 6 α Insulin	B10.A α HEL
Responses to				
SC + antigen	179 166 ± 16 012	81 237 ± 9 279	184 657 ± 12 134	29 479 ± 2 936
SC + Con A	299 335 ± 18 599	74 790 ± 4 487	161 640 ± 2 032	158 149 ± 6 271
SC + CM	6 829 ± 557	1 533 ± 632	1 756 ± 377	4 196 ± 513
Antigen	22 721 ± 1 136	881 287	1 536 ± 607	1 198 ± 402
Liposomes containing				
A^d and KLH	172 006 ± 2 916			
A^b and KLH	263 412 ± 21 747	1 413 ± 280		
KLH	247 183 ± 12 841			
A^b and OVA		27 724 ± 4 325	3 163 ± 358	
OVA		6 472 ± 518		
A^b and insulin			81 844 ± 8 074	
Insulin			173 661 ± 10 420	
A^k and HEL				121 273 ± 9 799
A^b and HEL				1 855 ± 312
HEL				4 768 ± 827
A^k and cytochrome c				1 911 ± 392
A^k				4 419 ± 688
A^b		1 178 ± 39	4 063 ± 72	
A^d	5 378 ± 41			
Mixture of liposomes containing				
A^b + Insulin			23 811 ± 1 059	
A^d + KLH	160 828 ± 12 074			
A^b + OVA		6 776 ± 978		
A^k + HEL				3 343 ± 697
A^b + free insulin			3 324 ± 582	
A^d + free KLH	28 715 ± 3 074			
A^b + free OVA		1 095 ± 289		
A^k + free HEL				7 236 ± 973
eeee	8 398 ± 1 696	1 546 ± 344	5 010 ± 512	2 090 ± 711

KLH, keyhole limpet hemocyanin; HEL, hen egg lysozyme; SC, spleen cells; cm, culture medium

Table 1. Proliferative response of the B6 anti LDH_B T-cell clone L_B-E8/G11 to liposomes containing MHC class II molecules and antigen (cpm)

Lipids in the liposomes	DPPC:DOPC:OPPC:SPDP-DPPE 5 : 1 : 3 : 1		DPPE:SPDP-DPPE 9 : 1	
Experiment No.	1	2	3	4
Response to				
SC + antigen LDH_B	31 296 ± 3 090	16 077 ± 1 141	6 496 ± 200	16 786 ± 1 822
SC + Con A	286 983 ± 74 058	ND	56 809 ± 3 413	64 505 ± 3 882
SC + CM	257 ± 125	576 ± 118	572 ± 116	672 ± 247
Antigen LDH_B	424 ± 170	396 ± 269	1 266 ± 40	514 ± 247
CM	315 ± 137	582 ± 127	637 ± 177	ND
Liposomes containing				
A^b and LDH_B	4 812 ± 1 252	3 900 ± 59	153 298 ± 5 600	103 971 ± 5 034
LDH_B	949 ± 63	893 ± 397	ND	5 025 ± 837
A^b	841 ± 152	1 252 ± 236	ND	3 625 ± 649
A^D and LDH_B	1 050 ± 649	1 315 ± 110	ND	ND
Mixture of A^b and LDH_B	ND	750 ± 242	ND	3 874 ± 812
A^b and free LDH_B	ND	1 266 ± 182	ND	ND
No protein	814 ± 387	ND	ND	8 437 ± 978

DPPC, dipalmitoylphosphotidylcholine; DOPC, dioleylphosphatidylcholine; OPPC, oleylpalmitoylphosphatidylcholine; SC, spleen cells; con A, concanavalin A; cm, culture medium; ND, not done

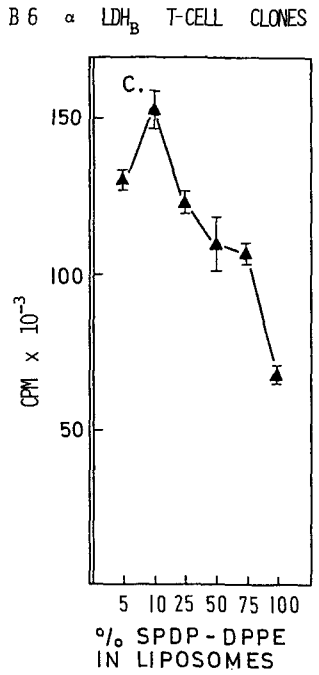

Fig. 2. Effect of antigen density on proliferative T-cell response. The liposomes were produced with DPPE containing different amounts (5%–100%) of SPDP-modified DPPE and A^B molecules. They were allowed to react with a large excess of SPDP-coupled LDH and were purified by sucrose-gradient centrifugation. Control values (cpm) for the response of the clone E8/G11 to: medium, 637 ± 177; LDH_B 1266 ± 40; syngeneic spleen cells 572 ± 116; spleen cells + LDH_B, 6496 ± 200. SPDP-DPPE, SPDP-modified DPPE

ponents appears to correlate negatively with the ability of the vesicles to trigger T cells. This result suggests that cross-linking of T-cell receptors may be a signal for T-cell activation. Second, the antigen density in the membrane with a constant amount of MHC molecule exhibits a sharp optimum (Fig. 2). The finding that high antigen densities, although increasing the probability of MHC-antigen interaction, result in decreased T-cell response, argues against the hypothesis that the formation of MHC-antigen complexes is a prerequisite for T-cell stimulation. Thus T cells may recognize antigen and MHC as separate entities.

That the MHC-antigen ratio in liposomes was critical for T-cell activation was observed also in other experiments: Fig. 3 shows a titration experiment with a pigeon cytochrome C specific B10.A(5R) T-cell hybridoma as an indicator system. In this case, liposomes that contain the antigen at a too high density to activate the T cells in the presence of the appropriate restriction element (here E^k) could trigger T cells in the absence of MHC molecules or in the presence of an irrelevant MHC molecule. This finding can be explained by the cross-linking model, namely, by assuming that

weak interactions can sum up to reach the threshold affinity for the initiation of the response.

The observations reported here can be generalized as shown by the experiments in Table 2. Short-term T-cell lines from different mouse strains that were specific for different antigens were used to test the liposomes. The findings are basically the same as those discussed above. With these polyclonal T-cell populations, we observed in several instances that liposomes containing only the antigen induced a T-cell response.

In conclusion, our data suggest that the most important function of APC is to provide a cell surface with the appropriate density of foreign antigen and MHC molecules for triggering of T cells. Thus the presence of antigen and MHC on the same membrane appears to be the only requirement to activate primed T cells. The results rule out the possibility that extensive processing is necessary to render foreign proteins antigenic for T cells. The question of possible additional functions of APC, such as the secretion of nonspecific mediators required for T-cell differentiation, is not addressed by this study.

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