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Antigen presentation of lysozyme: T-cell recognition of peptide and intact protein after priming with synthetic overlapping peptides comprising the entire protein chain

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Summary. Recently, using synthetic overlapping peptides which encompass the entire protein chain of hen egg lysozyme, the full submolecular profile of continuous regions on the protein recognized by T cells (T sites) was localized. In the present report, we have examined in two mouse strains the proliferative response to peptides and to native protein of lymph node cells from mice primed with synthetic overlapping peptides, either individually or as a mixture. It was found that the pattern of T-cell recognition observed after priming with peptides differs from that obtained when the native protein is used as the immunogen. Some, but not all, of the T-site containing peptides were effective in priming for an anti-lysozyme T-cell response. Several peptides which were highly immunogenic as free synthetic peptides were not associated with any of the known protein T sites. Further, some peptides were effective in priming for T cells that respond *in vitro* to the priming peptide, but not to the whole protein. If antigen processing proceeds via fragmentation, then only those regions containing T sites would be expected to be effective in priming for a T-cell response to the intact protein. Since this was not found to be the case, it is unlikely that fragmentation of lysozyme is a prerequisite for antigen presentation. Rather, we suggest that the critical aspects in the presentation of a protein antigen predominantly involve recognition of an intact protein, the interaction of which with the cell membrane triggers cellular activating events.

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Abbreviations: Con A, concanavalin A; PPD, purified protein derivative.

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INTRODUCTION

The role of macrophages or accessory cells in H-2 restricted immune recognition is well established (for reviews see Moller, 1978; Unanue, 1981; Rosenthal, 1980). In presentation, an antigen is recognized in association with Ia molecules on the cell surface (Keck, 1975; Melchers, Rajewsry & Shreffler, 1973; Lozner, Sachs & Shearer, 1974; Okuda *et al.*, 1978; Berzofsky, 1978), and each antigenic site of a multideterminant protein is presented in relationship to a particular Ia subregion (Okuda *et al.*, 1979b). It is widely believed that, during presentation, the antigen must be fragmented (Ziegler & Unanue, 1981, 1982; Chesnut, Colon & Grey, 1982; Shimonkevitz *et al.*, 1983; Lee, Wong & Spitzer, 1982; Allen & Unanue, 1984; Allen *et al.*, 1984a, b). This 'processing' event, however, has not been clearly resolved because of the antigenic complexity of the models used. In contrast, antigens whose full profiles of T- and B-cell recognition are known, such as myoglobin (Atassi, 1975; Bixler & Atassi, 1983, 1984a), lysozyme (Atassi, 1978;

Bixler, Yoshida & Atassi, 1984a, b; Bixler & Atassi, 1984b) and haemoglobin (Kazim & Atassi, 1980, 1982; Yoshioka & Atassi, 1983, 1985; Yoshioka, Yoshioka & Atassi, 1985), offer distinct advantages for the unravelling of antigen processing.

Lysozyme contains three 'discontinuous' (for terminology, see Atassi & Smith, 1978) antigenic (i.e. antibody binding) sites which were synthetically confirmed (Lee & Atassi, 1976, 1977a, b; Atassi & Lee, 1978a, b), and account for the majority (> 98%) of the anti-lysozyme antibodies (Atassi, 1978, 1979). Traces of anti-lysozyme antibodies to region 38–54 (Takagaki *et al.*, 1980) and some activity to the loop region, residues 64–80, (Arnon *et al.*, 1971) have also been reported. In initial studies of T-cell recognition, T-cell activity to cyanogen bromide cleavage fragments as large as 93 residues was reported (Maizels *et al.*, 1980) but, because of size, resolution of individual sites was not possible. However, in recent studies with synthetic peptides, four continuous lysozyme T sites were localized (Bixler *et al.*, 1984a, b). The T site residing in region 49–62 (Bixler *et al.*, 1984a, b) corresponds closely to the region 46–61 which was independently demonstrated using tryptic fragments (Allen *et al.*, 1984b). In addition, the discontinuous antigenic sites also stimulate lysozyme-specific T cells (Bixler & Atassi, 1984b). Thus, the full profiles of T- and B-cell recognition of lysozyme are known and are confined to discrete regions of the molecule. In contrast, it should be noted that other workers (Benjamin *et al.*, 1984) have postulated, mostly from studies of protein variants, that the antigenic determinants of a protein are a complex, overlapping array of regions which 'approach a continuum', and that the sites recognized by T cells on a protein are different from and fewer than the sites recognized by B cells. Neither of these postulates is supported by the extensive amount of knowledge on B- and T-cell recognition of proteins derived by chemical and synthetic methods (for recent reviews, see Atassi, 1984; Bixler & Atassi, 1985, and references therein). In this communication, we have examined T-cell proliferative responses after priming with synthetic peptides which encompass the entire protein. We have found that the pattern of recognition obtained after peptide priming differs from that obtained after priming with the native protein.

MATERIALS AND METHODS

Materials

DBA/1 (H-2^q) and B10.BR (H-2^d) mice, 6–10 weeks of age, were purchased from Jackson Laboratories, Bar Harbor, ME. Native hen's egg white lysozyme (crystallized three times) and concanavalin A (Con A) were obtained from Sigma Chemical Co., St Louis, MO. Purified protein derivative (PPD) was purchased from Connaught Laboratories Ltd, Swiftwater, PA.

Synthetic peptides

The primary structure, synthesis, purification and characterization of the overlapping peptides encompassing the entire polypeptide chain of lysozyme have been previously described (Bixler *et al.*, 1984a). These synthetic peptides (Fig. 1) correspond to the following residues: 1–19, 16–35, 31–50, 46–65, 61–81, 77–97, 93–113 and 109–129.

Immunization of mice

Mice were immunized subcutaneously at the base of the tail with 25 μg of peptide or 100 μg of a peptide mixture emulsified in Freund's complete adjuvant containing *Mycobacterium tuberculosis*, strain H37Ra (Difco, Detroit, MI) as described by Corradin, Etlinger & Chiller (1977). The peptide mixture was composed of equimolar quantities of all eight overlapping peptides.

Proliferation assay

Seven days after immunization, the inguinal and periaortic lymph nodes were harvested and the proliferative activity assessed as previously described (Yoshioka, Bixler & Atassi, 1983; Bixler *et al.*, 1984a). Lymph node cells ($7{\cdot}5 \times 10^5$/well) suspended in RPMI-1640 containing 1% normal mouse serum were challenged, in triplicate, with various concentrations of antigens or mitogens in 96-well tissue culture plates. After 3 days of incubation at 37° in a humidified 5% CO_2 atmosphere, the cultures were pulsed (18 hr) with 2 μCi [^{3}H]thymidine and then harvested on to glass fibre filters for counting by liquid scintillation. All experiments were performed at least twice.

RESULTS

Proliferative response of peptide-primed DBA/1 lymph node cells to synthetic peptides and to native lysozyme

Lymph node cells from DBA/1 mice immunized with the individual peptides were examined for proliferative activity to the immunizing peptide as well as to the intact protein. A representative experiment is shown in Table 1. When either of the peptides 16–35 or 31–50

Table 1. Proliferative response of peptide-primed DBA/1 lymph node cells to *in vitro* challenge with peptide or the intact lysozyme molecule

	Priming peptide							
Challenge with:	1–20	16–35	31–50	46–65	61–81	77–97	93–113	109–129
Peptide*								
Δ c.p.m.†	765	128,225	108,407	10,929	0	2405	14,795	1090
SI ± SD	1·37 ± 0·07	44·1 ± 1·79	57·6 ± 2·74	5·72 ± 3·06	0·97 ± 0·12	1·93 ± 1·48	6·17 ± 0·27	1·35 ± 0·02
Opt. dose	(1·5)	(25)	(12·5)	(25)	(3·12)	(25)	(50)	(25)
Lysozyme								
Δ c.p.m.	942	15,082	67,667	822	472	731	2022	1719
SI ± SD	1·45 ± 0·55	6·07 ± 0·46	36·3 ± 6·36	1·36 ± 0·09	1·28 ± 0·16	1·28 ± 0·18	1·70 ± 0·38	1·55 ± 0·11
Opt. dose	(50)	(100)	(100)	(50)	(25)	(50)	(100)	(100)
Con A								
Δ c.p.m.	48,206	90,606	55,845	77,412	53,366	94,867	67,637	72,542
SI ± SD	24·1 ± 1·84	31·5 ± 0·71	30·2 ± 0·85	34·4 ± 2·76	32·7 ± 1·64	37·7 ± 3·76	24·6 ± 3·45	24·2 ± 0·69
Dose	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
PPD								
Δ c.p.m.	154,684	153,351	186,298	86,322	124,438	73,704	108,540	139,596
SI ± SD	75·2 ± 1·18	52·6 ± 3·09	98·2 ± 0·25	38·3 ± 6·20	74·8 ± 4·87	29·5 ± 3·00	38·9 ± 1·67	45·5 ± 2·26
Dose	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)

* Peptide refers to the same peptide used to prime the mice initially.

† The Δ c.p.m. and SI are calculated relative to the background of unstimulated cells which ranged from 1861 to 2860 c.p.m. Although broad dose ranges for peptide (1·56–50 μg/ml) and lysozyme (12·5–100 μg/ml) were examined, only the maximum observed response and corresponding optimum dose are listed.

was used as the priming antigen, high responses to the priming peptide and intermediate or high responses, respectively, to native lysozyme were observed. Cells from mice primed with peptide 93–113 or 46–65 responded to challenge with their respective peptides but were unresponsive to native lysozyme. The remaining groups of peptide-primed cells responded weakly or not at all to challenge with either peptide or whole protein. As a positive control, cells from protein-primed mice were challenged *in vitro* with each of the overlapping peptides; the responses observed (data not shown) agreed with the profile previously reported (Bixler *et al.*, 1984a, b). All of the groups responded to Con A and PPD, but were unresponsive to myoglobin or synthetic myoglobin peptides (unrelated negative controls). Unstimulated cells had a range of background responses from 1861 to 2860 c.p.m.

Proliferative response of peptide-primed B10.BR lymph node cells to synthetic peptides and native lysozyme

A representative experiment showing the proliferative responses of B10.BR lymph node cells from groups of mice primed separately with each of the overlapping peptides is presented in Table 2. Cells primed with peptide 109–129 had the highest *in vitro* responses to both peptide and the native protein. Cells primed with peptide 93–113 also responded strongly to peptide and, in addition, mounted an intermediate response to whole protein. In contrast, although intermediate peptide responses were observed in cells primed with peptides 46–65, 77–97 or 16–35, the cells had either weak or no responses to whole protein. Cells from mice that had been primed with peptide 61–81 and 31–50 were also weakly responsive only to the peptide, whereas cells primed with peptide 1–20 were unresponsive to challenge with either peptide or whole protein. The responses of protein-primed cells (data not shown), which served as positive controls, agreed with previous reports (Bixler *et al.*, 1984a, b). All of the cells were responsive to Con A and PPD but were unresponsive to myoglobin or to the unrelated synthetic myoglobin peptides. In these experiments, the background of unstimulated cells ranged from 1455 to 4988 c.p.m.

Table 2. Proliferative response of peptide-primed B10.BR lymph node cells to *in vitro* challenge with peptide or the intact lysozyme molecule

	Priming peptide							
Challenge with:	1–20	16–35	31–50	46–65	61–85	77–97	93–113	109–129
Peptide*								
Δ c.p.m.†	1151	27,168	3585	45,077	4298	31,923	33,872	72,747
SI ± SD	1·57 ± 0·51	10·9 ± 0·90	3·46 ± 0·08	25·9 ± 4·57	1·86 ± 0·24	13·9 ± 0·19	12·9 ± 3·61	24·9 ± 1·09
Opt. dose	(50)	(50)	(50)	(50)	(12·5)	(50)	(50)	(25)
Lysozyme								
Δ c.p.m.	1407	2017	1181	759	2467	0	13,224	16,567
SI ± SD	1·70 ± 0·61	1·74 ± 0·20	1·81 ± 0·22	1·42 ± 0·05	1·49 ± 0·14	0·88 ± 0·05	5·66 ± 0·01	6·45 ± 0·03
Opt. dose	(50)	(50)	(100)	(50)	(100)	(50)	(25)	(50)
Con A								
Δ c.p.m.	19,906	85,770	50,982	98,823	91,700	79,429	49,360	58,580
SI ± SD	40·8 ± 3·35	32·5 ± 4·95	36·0 ± 6·25	55·7 ± 2·80	19·4 ± 0·41	33·1 ± 0·51	18·4 ± 0·07	20·2 ± 0·14
Dose	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
PPD								
Δ c.p.m.	46,177	40,532	66,707	36,079	39,598	72,626	34,319	154,494
SI ± SD	93·4 ± 14·6	15·9 ± 0·32	46·9 ± 2·25	20·9 ± 2·31	8·93 ± 2·21	30·4 ± 0·03	13·1 ± 0·53	51·8 ± 3·63
Dose	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)

* Peptide refers to the same peptide used to prime the mice initially.

† The Δ c.p.m. and SI are calculated relative to the background of unstimulated cells which ranged from 1455 to 4988 c.p.m. Although broad dose ranges for peptide (1·56–50 μg/ml) and lysozyme (12·5–100 μg/ml) were examined, only the maximum observed response and corresponding optimum dose are listed.

Proliferative response of DBA/1 and B10.BR lymph node cells primed with a mixture of the synthetic overlapping peptides

It is now known (Atassi *et al.*, 1981; Krco *et al.*, 1981) that recognition of various regions on a multi-determinant complex antigen is subject to inter-site regulatory effects. Therefore, it was necessary to rule out the possibility that differences in the patterns of peptide recognition between protein-primed and peptide-primed mice could be due to some inter-site regulatory effects among various regions of the whole protein which would not play a role when each peptide is used individually for priming. A peptide mixture consisting of equimolar quantities of all of the peptides was used to prime both DBA/1 and B10.BR mice. Representative experiments showing the proliferative responses of these mice to challenge with individual peptides, a peptide mixture or native protein are presented in Table 3.

Lymph node cells from DBA/1 mice responded strongly to challenge with peptides 16–35 and 31–50, as well as to the peptide mixture. The response to the whole protein was intermediate. Several peptides, 46–65, 77–97 and 93–113, evoked only low proliferative responses. No response was seen to challenge with the remaining peptides. Unrelated molecules [myoglobin or synthetic myoglobin peptides of similar size (Bixler & Atassi, 1983)] evoked no response. The cells responded appropriately to both Con A and PPD (Table 3).

In lymph node cells from mixture-primed B10.BR mice (Table 3), the strongest responses to *in vitro* peptide challenge were obtained with peptide 46–65 and, to a lesser extent, peptide 16–35 or with the peptide mixture. Challenge with the whole protein or with peptide 93–113 generated low to intermediate responses. A low response was also observed in cultures challenged with peptide 77–97. The remaining peptides elicited very little or no increases in proliferation. The cells were unresponsive to challenge with myoglobin or synthetic myoglobin peptides, but were responsive to Con A and PPD.

DISCUSSION

The strategy of this work relies on the use of a series of

Table 3. The proliferative response to the overlapping peptides and lysozyme of DBA/1 and B10.BR lymph node cells primed with an equimolar mixture of the overlapping lysozyme peptides*

	DBA/1			B10.BR		
Challenge	Δ c.p.m.	SI ± SD	Opt. dose	Δ c.p.m.	SI ± SD	Opt. dose
Peptides:						
1–20	311	1·09 ± 0·31	50	1965	1·57 ± 0·35	50
16–35	70,486	21·7 ± 0·53	50	10,842	4·16 ± 0·19	50
31–50	33,566	10·8 ± 3·61	50	2989	1·87 ± 0·15	50
46–65	3255	1·95 ± 0·55	50	30,704	9·96 ± 0·39	50
61–81	2489	1·73 ± 0·45	6·3	2084	1·61 ± 0·22	6·3
77–97	3000	1·88 ± 0·01	50	4039	2·18 ± 0·47	50
93–113	3737	2·10 ± 0·20	25	5869	2·71 ± 0·16	50
109–129	209	1·06 ± 0·15	6·3	2221	1·65 ± 0·77	50
Mixture	50,876	15·9 ± 0·74	50	23,245	7·78 ± 0·33	50
Lysozyme	16,603	5·87 ± 1·69	50	6015	2·76 ± 0·08	50
Con A	123,477	37·1 ± 0·16	1	55,141	17·1 ± 0·10	1
PPD	91,148	27·7 ± 4·19	100	24,293	8·09 ± 0·50	100

* Unstimulated DBA/1 cells had a background of 3411 c.p.m. while the background of the B10.BR lymph node cells was 3427 c.p.m.

consecutive synthetic overlapping peptides which encompass the entire protein chain. The size of the peptides, which is uniform, is selected so that it is not smaller than the expected size of the site. The size of the overlap is such that, should an antigenic site fall in the overlap region, it would be expected to possess most (or all) of the binding activity, thereby avoiding inadvertent scission of a site. The peptides designed here (Fig. 1) consisted of 20 or 21 residues and overlapped adjacent peptides at their *N*- and *C*-terminals by five residues. A detailed discussion of this comprehensive strategy has already been reported (Kazim & Atassi, 1980, 1982).

The synthetic peptides were used either individually or in equimolar mixture to prime mouse strains that are high responders to lysozyme (Okuda *et al.*, 1979a) and in which the profiles of T-cell recognition had been determined (Bixler *et al.*, 1984a, b). Thus, it was possible to compare the patterns of T-cell recognition after priming with peptide or with protein. Priming with intact lysozyme *in vivo* has been shown (Bixler *et al.*, 1984a, b) to generate *in vitro* T-cell proliferative responses to the following overlapping peptides: in DBA/1, 16–35, 31–50, 46–65 and 93–113; in B10.BR, 16–35 and 46–65 (Table 4). These studies were repeated here in order to ensure that the present studies of peptide priming were performed at the same time and with the same mouse batches as those obtained with protein priming. The submolecular profiles obtained here by *in vitro* screening with the overlapping peptides agreed completely with those previously reported (Bixler *et al.*, 1984a, b).

In examining the responses after priming with individual peptides (see Table 4, for summary), it can

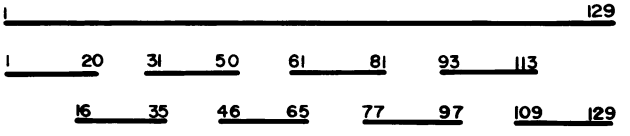

Figure 1. Schematic diagram illustrating the strategy used to localize the continuous sites of T-cell recognition in lysozyme. The identity of the overlapping synthetic peptides is indicated by the numbers which refer to the first and last residues in the covalent structure. (This figure is reproduced from Bixler *et al.*, 1984b, with kind permission.)

Table 4. Summary of the proliferative response of DBA/1 and B10.BR lymph node cells to peptides or whole protein*

Antigen:	DBA/1				B10.BR			
Priming:	Peptide		Protein	Mixture	Peptide		Protein	Mixture
Challenge:	Peptide	Protein	Peptide	Peptide	Peptide	Protein	Peptide	Peptide
Peptide:								
1–20	–	–	–	–	–	–	–	–
16–35	++++	++	+	++++	+++	–	+	++
31–50	++++	++++	++	+++	+	–	–	–
46–65	++	–	+	+	+++	–	++	+++
61–81	–	–	–	–	+	–	–	–
77–97	–	–	–	+	+++	–	–	+
93–113	++	–	+++	+	+++	++	–	+
109–129	–	–	–	–	++++	++	–	–

* The assignment of positive and negative responses for the purpose of this table was based on considerations of both stimulation index and net c.p.m. values. When the response was less than twice that of the background, and when the c.p.m. was less than 3000, the response was denoted (–). For the positive responses, the symbols are used to denote the following net c.p.m. values: (+) 3000–10,000 c.p.m.; (++) 10,000–25,000 c.p.m.; (+++) 25,000–50,000 c.p.m.; (++++) > 50,000 c.p.m. For actual values, see Tables 1, 2 and 3 and the previous reports on the localization of sites of T-cell recognition (Bixler *et al.*, 1984a, b).

be seen that some peptides, which carry T sites, prime cells to respond to the respective peptide as well as to protein (e.g. peptides 16–35 and 31–50 in DBA/1). However, it should be noted that the levels of *in vitro* responses to these peptides were much higher after peptide priming than after protein priming (Table 4). Also, some peptides that are not associated with T sites (i.e. were not recognized after protein priming) did not prime for either anti-peptide or anti-protein *in vitro* responses (in DBA/1, peptides 1–20, 61–81, 77–97 and 109–129; in B10.BR, peptide 1–20). If, as indicated by current dogma, antigen presentation proceeds via antigen fragmentation, then it would be expected that those peptides that are associated with protein T sites should prime for *in vitro* response to both the priming peptide and the protein. Conversely, regions that are not associated with T sites on the protein should not prime for an *in vitro* response to the priming peptide or to the protein. Thus, the results discussed in this paragraph are consistent with the idea (at least qualitatively) that peptide fragments are the species presented after priming with native protein.

The fragmentation of antigen during presentation, however, is not supported by the rest of the results. Several regions that coincide with protein T sites were effective in priming for an *in vitro* anti-peptide response, but these cells did not respond to native protein (in DBA/1, peptides 46–65 and 93–113; in B10.BR, 16–35 and 46–65). Some peptides which are not associated with protein T sites primed T cells for *in vitro* response only to the immunizing peptide, but not to the protein (in B10.BR, peptides 31–50, 61–81 and 77–97). Further, some peptides, though not associated with the protein T sites, primed T cells for *in vitro* response to the immunizing peptide and to the protein (e.g. in B10.BR, peptides 93–113 and 109–129). These findings do not support, and indeed pose serious problems for, the current dogma.

Since inter-site regulatory influences have been shown (Atassi *et al.*, 1981; Krco *et al.*, 1981) to play an important role in the immune responses to a protein antigen, it was necessary to examine the possibility that the above differences may be due to absence of these regulatory influences when individual peptides are used as the priming antigens. A peptide mixture containing equimolar quantities of all the peptides was therefore employed for priming, and at the same dose as that used in priming with lysozyme. Indeed, differences were found in the pattern of recognition of various peptides after priming with individual peptides as compared to that obtained after priming with the mixture. These differences may well reflect inter-site regulatory effects operating in the case of mixture priming. However, this point needs more study. At

any rate, priming with peptide mixture generated cells that responded *in vitro* to peptides which carry protein T sites (in DBA/1, peptides 16–35, 31–50, 46–65 and 93–113; in B10.BR, peptides 16–35 and 46–65). There were, however, large quantitative differences in the levels of the *in vitro* responses to the peptides that are recognized both after mixture priming or after protein priming (Table 4). In addition, it should be noted that some peptides were recognized *in vitro* after *in vivo* mixture priming but not after protein priming (peptide 93–113 in B10.BR and peptide 77–97 in both DBA/1 and B10.BR). Since all the regions present in the peptide mixture are presumably present in the native protein (if it were processed), then the differences in patterns of recognition cannot entirely be attributed to the absence of inter-site regulatory effects.

This study reveals that the pattern of recognition following protein priming is qualitatively and quantitatively different from that obtained following peptide priming. Furthermore, the response elicited following priming with peptides, either individually or as a mixture, frequently bears little relationship to the T sites of the protein.

Antigen-presenting cells are believed to 'process' and subsequently 'present' an altered antigen to T cells. In presentation, a lag period occurs between the uptake and the capacity to present antigen (Ziegler & Unanue, 1981; Chesnut *et al.*, 1982). During this period, paraformaldhyde fixation (Ziegler & Unanue, 1981; Chesnut *et al.*, 1982) and lysosomotropic agents (Ziegler & Unanue, 1982; Lee *et al.*, 1982; Allen & Unanue, 1984; Chesnut *et al.*, 1982; Allen *et al.*, 1984a, b) interfere with antigen presentation. Prefixed antigen-presenting cells, however, present protein fragments but not the native protein (Shimonkevitz *et al.*, 1983; Allen & Unanue, 1984). From enzymatic degradation studies of membrane-bound antigen, it has been suggested that the antigen may be sequestered in an enzymatically resistant compartment (Chesnut *et al.*, 1982; Allen *et al.*, 1984a). Together, these results have been interpreted as demonstrating that antigen is internalized, degraded in the lysosomes and the fragments subsequently cycled to the cell membrane for presentation.

Fragmentation of a protein obviously destroys its three-dimensional structure. Although native lysozyme and the unfolded derivative with broken disulphide bonds do not cross-react at the antibody level (Gerwing & Thompson, 1968; Young & Leung, 1970; Lee & Atassi, 1973; Scibienski *et al.*, 1978), they do cross-react at the T-cell level (Thompson *et al.*, 1972; Sugimoto *et al.*, 1975; Scibienski *et al.*, 1978; Maizels *et al.*, 1980; Bixler *et al.*, 1984a). These observations have fostered the view that the architecture of the sites of T- and B-cell recognition is inherently different, and they are now being interpreted as demonstrating a requirement for antigen 'processing'. Recent studies, however, conclusively demonstrate that T and B cells can recognize the same sites (Okuda *et al.*, 1979b; Bixler & Atassi, 1983, 1984a, b; Yoshioka *et al.*, 1983; Young & Atassi, 1982, 1983). In this regard, it is important to note that T cells recognize the surface-simulation synthetic sites (Bixler & Atassi, 1984b) which mimic the discontinuous sites of lysozyme that bind to antibody (Atassi, 1978). The integrity of these sites is highly dependent on the retention of the three-dimensional structure of the protein and would be destroyed by its fragmentation. Clearly, the synthetic peptides which simulate surface regions of lysozyme and which, after all, do not exist in nature, could not be generated by a fragmentation process. Further, different populations of T-cell clones are generated following priming with native cytochrome *c* as opposed to the denatured protein (Buchmuller & Corradin, 1982). Thus, the retention of most of the tertiary structure is critical to the generation of an appropriate immune response. The dependence of immune recognition on the conformation of the protein is not easily explained by the current dogma of antigen presentation as being that of 'processed' fragments.

It is not surprising that aldehyde fixation interferes with presentation. It is reasonable to assume that a fluid membrane is necessary to accommodate the insertion of the antigen into the membrane. It has been suggested that the specificity of the insulin receptor, for example, is determined by the receptor and possibly by receptor-ligand movement in the plane of the membrane as well (Fan *et al.*, 1982). Therefore, a large protein could not easily be intercalated into a prefixed, rigid, immobile matrix, whereas fixation after antigen encounter traps antigen on the cell surface and permits its presentation. In contrast, a small peptide may more easily be inserted into a fixed membrane because, with its smaller size, a minimum readjustment of the cell surface components would be necessary. However, as seen here, the T-cell response to a free peptide often has little or no relationship to the recognition by T cells of the same region on the protein. Thus, although protein fragments can be presented and induce an immune response (Shimonkevitz *et al.*, 1983; Allen & Unanue, 1984) and can be

sequestered in the interior of the cell (Chesnut *et al.*, 1982; Allen & Unanue, 1984), their significance to immune recognition of the native protein can not be assessed unless the full profile of T- and B-cell recognition is known. The dependence of presentation on the integrity of the membrane and the protein antigen should also serve as a cautionary note. Any process which alters the characteristics of the membrane(s) (for example, the generation of a hybridoma) or alters the characteristics of the protein antigen (such as chemical modifications associated with labelling proteins) must be carefully evaluated because the results generated may not accurately reflect the events occurring *in vivo*.

Inhibition of antigen presentation by the lysosomotropic agents and by the monovalent carboxylic ionophores (Ziegler & Unanue, 1982; Lee *et al.*, 1982; Chesnut *et al.*, 1982; Allen & Unanue, 1984; Allen *et al.*, 1984a) has been interpreted as showing blockage of acid hydrolyase activity in the lysosomes, resulting in inhibition of protein degradation and presentation. During receptor-mediated endocytosis, however, the activity of the endocytic vesicles which appear very early after internalization and precede lysosomal events (Merion & Sly, 1983; March, Bolzau & Helenius, 1983; Straubinger *et al.*, 1983; Pilch *et al.*, 1983) is dependent on pH and is also inhibited by these agents (Maxfield, 1982; Merion & Sly, 1983; Straubinger *et al.*, 1983). Thus, the precise action of these agents during antigen presentation is unclear.

In view of the uncertainties associated with approaches on which the current dogma is based, and the present findings, we suggest that the events of antigen presentation may not be too different in principle from other biological membrane-mediated cellular activities. Such activities are triggered by the binding event to receptor and lead to elaboration by the cell of chemical signals or some other yet unknown mechanisms. Subsequent internalization of receptor–protein complexes would rescue some receptor molecules, which are recycled to the membrane, and antigen and remaining complexes are degraded. Thus, antigen presentation may closely parallel the events of receptor-mediated endocytosis, shown to involve the recycling of a membrane receptor in several receptor-ligand models (Anderson, Goldstein & Brown, 1977; Basu *et al.*, 1981; Tolleshaug & Berg, 1979; Steer & Ashwell, 1980; Gonzalez-Noriega *et al.*, 1980; Kaplan, 1980; Van Leuven, Cassiman & Van Den Berghe, 1980; Fan *et al.*, 1982). Whether antigen presentation leads to the recycling of a membrane receptor has not yet been determined.

In conclusion, the antigen presentation of lysozyme has been investigated with the advantage of knowing the full profile of sites of T- and B-cell recognition. A comparison of the patterns of T-cell recognition following protein priming, as opposed to peptide priming, revealed significant differences. The findings argue strongly against a mechanism of antigen presentation which is dependent on the generation of peptide fragments, with the latter being the 'presented' species. Rather, the protein molecule must be presented in its intact form.

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