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Immunology

Characterization of antigen association with accessory cells: Specific removal of processed antigens from the cell surface by phospholipases

(antigen processing/antigen presentation/T-cell activation/plasma membrane)

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ABSTRACT **To characterize the basis for the cell surface association of processed antigen with the antigen-presenting cell (APC) we analyzed its sensitivity to enzymatic digestion. Antigen-exposed APC that are treated with phospholipase and then immediately fixed lose their ability to stimulate antigen-plus-Ia-specific T–T hybridomas. This effect is seen with highly purified phospholipase A_2 and phospholipase C. In addition it is observed with three distinct antigens—ovalbumin, bovine insulin, and poly(LGlu^{56}LLys^{35}LPhe^{9}) [(GluLysPhe)$_n$]. The effect of phospholipases is highly specific. Identically treated APC are equivalent to controls in their ability to stimulate alloreactive hybridomas specific for precisely the same Ia molecule that is corecognized by antigen-plus-Ia-specific hybrids. Furthermore, the antigen-presenting function of enzyme-treated, fixed APC can be reconstituted by the addition of exogenous *in vitro* processed or "processing independent" antigens. In parallel studies ^{125}I-labeled avidin was shown to specifically bind to APC that were previously exposed and allowed to process biotin-insulin. Biotin-insulin-exposed APC that are pretreated with phospholipase bind significantly less ^{125}I-labeled avidin than do untreated, exposed APC. Identical enzyme treatment does not reduce the binding of avidin to a biotinylated antibody already bound to class II major histocompatibility complex molecules of APC. At least some of the biotin-insulin surface sites are immunologically relevant, because the presentation of processed biotin-insulin by fixed APC is blocked by avidin. This effect is specific. Avidin binding to biotin-insulin-exposed APC does not inhibit allospecific stimulation nor the presentation of unconjugated insulin. These studies demonstrate that phospholipase effectively removes processed cell surface antigen.**

T-cell receptors, in contrast to immunoglobulins, do not bind native unprocessed antigen and are often specific for sequential determinants in denatured, unfolded chains (1–9). Immunogenicity implies the transformation, generally described as processing, of a water-soluble native antigen into a membrane-associated protein, or denatured protein fragment, capable of specific interaction with class II major histocompatibility complex (MHC) molecules on the membrane by antigen-presenting cells (APC) (10, 11).

The precise mechanism of this processing step and the nature of the association of processed antigen with the membrane have not been ascertained, although recognition is increasing that the documented affinity (10, 11) of the specific processed antigen–class II MHC molecule interaction (12–14) does not explain the stability of the membrane association. Moreover, identification of processed antigen on the cell surface has been difficult because of the absence of reagents appropriately specific for the cell-associated, processed antigen.

In a previous communication (15), we have characterized the processing and presentation of the soluble polypeptide antigen, bovine insulin, by murine APC. Analyzing the sensitivity of processed and membrane-associated bovine insulin to enzyme digestion, we noted that APC exposed to this antigen and treated with phospholipase A2 lost the ability to stimulate antigen-specific MHC-restricted hybridomas; in contrast, the ability of the same APC to stimulate allospecific hybridomas was unaffected. This effect was not observed following treatment with Pronase under conditions that do not affect the class II MHC molecules. These results suggested that one of the consequences of antigen processing may be an antigen–lipid association that contributes to the anchoring of antigen to the APC membrane.

In the present report we extend our studies to other antigens—ovalbumin (OVA) and poly(LGlu^{56}LLys^{35}LPhe^{9}) [(GluLysPhe)$_n$] in addition to bovine insulin, as well as to treatment with another lipase, phospholipase C. We further demonstrate that the loss of reactivity for antigen-specific T-cell hybridoma following treatment of bovine insulin-exposed APC with phospholipases A2 or C is associated with a loss of detectable cell surface biotin-labeled antigen as detected with ^{125}I-labeled avidin.

MATERIALS AND METHODS

Reagents. (GluLysPhe)$_n$], Poly(LGlu^{55}LLys^{35}LLeu^{10}) [(GluLysLeu)$_n$], and chicken OVA were purchased from Miles. Bovine insulin, biotinylated bovine insulin, avidin, and bovine serum albumin were obtained from Sigma. Antigens were prepared as described (12). Where indicated, OVA was denatured and trypsin-treated by the method of Shimonkevitz *et al.* (5). Avidin was labeled with Na^{125}I using chloramine-T. Phospholipase C (*Clostridium perfringens*), phospholipase A_2 (*Naja naja* venom), and Pronase (*Streptomyces griseus* protease) were purchased from Sigma. Phospholipase preparations were purified by acid and heat (100°C, 10 min) denaturation/renaturation, ion-exchange and gel-exclusion chromatography, and phospholipase C preparations were further purified by substrate-affinity chromatography by the manufacturer.

Cell Lines and Monoclonal Antibodies. The following T–T hybridomas were used: RF28.4 [BALB/c anti-bovine insulin-plus-I-A^{d}) × BW5147], RF21.8 {BALB/c [anti-(GluLysPhe)$_n$-plus-I-E^{d}] × BW5147} (13), D011.10 [BALB/c (anti-OVA-plus-I-A^{d}) × BW5147], kindly provided by J. Kappler and P. Marrack (National Jewish Hospital, Denver, CO) (5), RF19.52 [B10 (anti-I-A^{d}) × BW5147; ref. 15] and RF26.12

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Abbreviations: APC, antigen-presenting cell(s); mAb, monoclonal antibody; MHC, major histocompatibility complex; OVA, ovalbumin; IL-2, interleukin 2.

[B10 (anti-I-E^d) × BW5147] (9). The A202J cell line, Ia positive BALB/c B-lymphoblastoid cells, was obtained from J. Kappler and P. Marrack. These cell lines are passaged *in vitro* in Dulbecco's modified Eagle's medium (DME medium), supplemented as previously described (16). Monoclonal (anti-I-E^d) antibody was purified from culture supernatant of the cell line 14.4.4.S (ref. 17; made available by D. Sachs, National Institutes of Health, Bethesda, MD) and was conjugated with biotin succinimide ester (Sigma).

Cell Culture. A202J APC were antigen-exposed, enzyme-treated, and fixed as previously described (15). Hybridoma cell cultures and IL-2 assays were done as described (15, 16).

Avidin Binding Assay. Antigen-exposed or unexposed A202J APC were washed three times. In some experiments the unexposed APC were incubated with 100 μl of biotinylated (anti-I-E^d; 100 μg/ml) per 10^7 cells for 45 min at 0°C followed by extensive washing. APC were then treated with enzymes (15). Two × 10^6 Ficoll/Hypaque-purified cells were incubated with 2 × 10^6 cpm of ^{125}I-labeled avidin (≈80–140 × 10^6 cpm/μg of avidin) for 40 min at 0°C in 125 μl of phosphate-buffered saline (0.01 M PO_4^{3-}, pH 7.4) with 2 mg of bovine serum albumin per ml. In some groups 100 μg of unlabeled avidin was added to compete for specific binding. After incubation with labeled avidin, samples were washed extensively and the radioactivity of the cell pellets was counted. Data are expressed as the arithmetic mean cpm of triplicate or quadruplicate samples, unless otherwise indicated.

RESULTS

Phospholipases Selectively Inhibit Antigen Presentation by Exposed APC. OVA, bovine insulin, and $(GluLysPhe)_n$ were chosen as model immunogens to characterize the nature of processed-antigen association with the APC. T-cell responses to these antigens have the following characteristics: (*i*) The native protein requires a processing step that is sensitive to metabolic inactivation (fixation) prior to presentation (5, 9). (*ii*) After processing, each antigen is accessible to T cells on the APC surface (5, 9). (*iii*) Immunogenic fragments of these proteins interact specifically with I-A or I-E molecules on the APC surface (13, 18).

The cell surface association of each antigen was analyzed functionally by assessing the ability of antigen-exposed APC to present antigen after enzymatic digestion. APC were incubated with antigen for 18 hr to allow uptake, processing, and display of immunogen on the cell surface; these cells were then treated with various enzymes and immediately fixed to prevent reexpression of antigen.

As shown in Fig. 1 *A*, *C*, and *E*, treatment of OVA-, bovine insulin-, or $(GluLysPhe)_n$-exposed APC with the enzyme phospholipase A_2 significantly reduces their ability to stimulate IL-2 production from MHC-restricted T-cell hybridomas specific for the corresponding antigen.

To investigate the specificity of this inhibition, we assessed the activity of the same APC in an allospecific response. In contrast to the inhibition of antigen presentation, these APC are equivalent to controls in their ability to stimulate alloreactive hybridomas (Fig. 1 *B*, *D*, and *F*), even under limiting conditions. Therefore, the enzyme treatment has not disrupted any essential functional component of the APC–T-cell interaction. Furthermore, the allospecific hybrids are stimulated by precisely the same Ia molecule that is corecognized by the antigen-plus-Ia-specific hybrid (I-A^d in Fig. 1 *B* and *D*, I-E^d in Fig. 1*F*). Therefore, treatment has not affected the function of Ia molecules on the APC surface.

As shown in Fig. 2, the ability of APC, exposed to either OVA (Fig. 2*A*), bovine insulin (Fig. 2*C*), or $(GluLysPhe)_n$ (Fig. 2*E*) to present antigen to the appropriate antigen-plus-Ia-specific hybridoma is also significantly reduced by treatment with phospholipase C. As was the case with phospholipase A_2, the treated APC are minimally affected in their ability to stimulate alloreactive hybridomas (Fig. 2 *B*, *D*, and *F*). Taken together, these results demonstrate that the effects of phospholipase treatment are selective. Furthermore, the phenomenon is generalizable to three antigens with two enzymes.

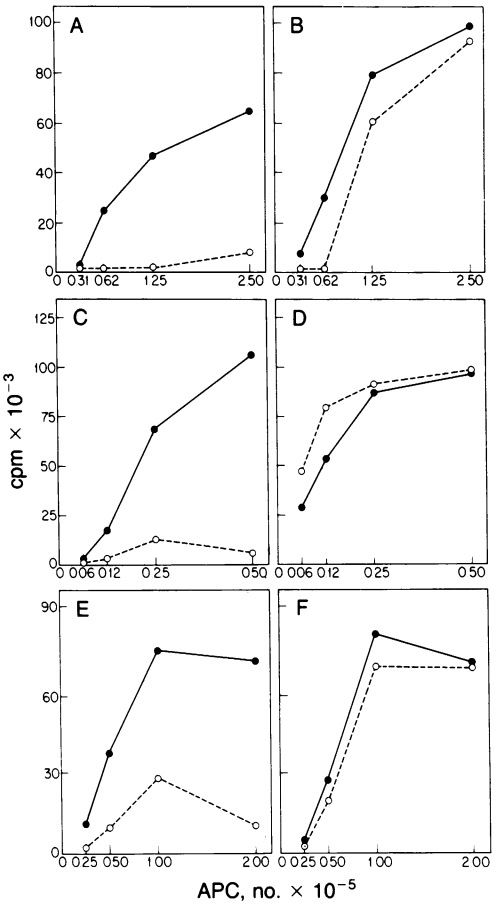

FIG. 1. Phospholipase A_2 treatment of antigen-exposed APC affects antigen-specific but not allospecific presentation. A20 cells were incubated with antigen for 18 hr at 37°C, washed extensively, and then incubated with (○) or without (●) phospholipase A_2 (10 units/ml) in RPMI 1640 medium for 1 hr at 37°C. Then the cells were fixed with 1% (vol/vol) paraformaldehyde and tested for the ability to stimulate the appropriate antigen-specific or allospecific T–T hybridomas in duplicate microcultures. Microcultures (200 μl) were incubated for 18 hr at 37°C, after which 100 μl of supernatant was removed, x-irradiated, and assayed for IL-2 content (incorporation of [^{3}H]thymidine by IL-2-dependent HT-2 cells). Results are recorded as the average cpm of duplicate microcultures. Microcultures containing T–T hybridomas alone or antigen-specific T–T hybrids in the presence of treated or untreated unexposed APC typically averaged less than 1200 cpm; for clarity these points are not displayed. Microcultures contained the following: (*A*) A20 cells exposed to 400 μg of OVA per ml and DO-11.10 (OVA-plus-I-A^d). (*B*) A20 cells identical to those in *A* and RF19.52 cells (I-A^d-allospecific). (*C*) A20 cells exposed to 20 μg of bovine insulin per ml and RF28.4 cells (bovine insulin-plus-I-A^d). (*D*) A20 cells identical with those in *C* and RF19.52 cells (I-A^d-allospecific). (*E*) A20 cells exposed to 200 μg of $(GluLysPhe)_n$ per ml and RF21.8 cells [$(GluLysPhe)_n$-plus-I-E^d]. (*F*) A20 cells identical with those in *E* and RF26.12 cells (I-E^d-allospecific).

It is important to establish that the effect of these treatments on antigen presentation is due to phospholipase activity; because the immunogens are peptides, it is particularly important to rule out proteolytic contaminants. Several points make it unlikely that the observed functional effects are due to such a contaminant. First, the same functional

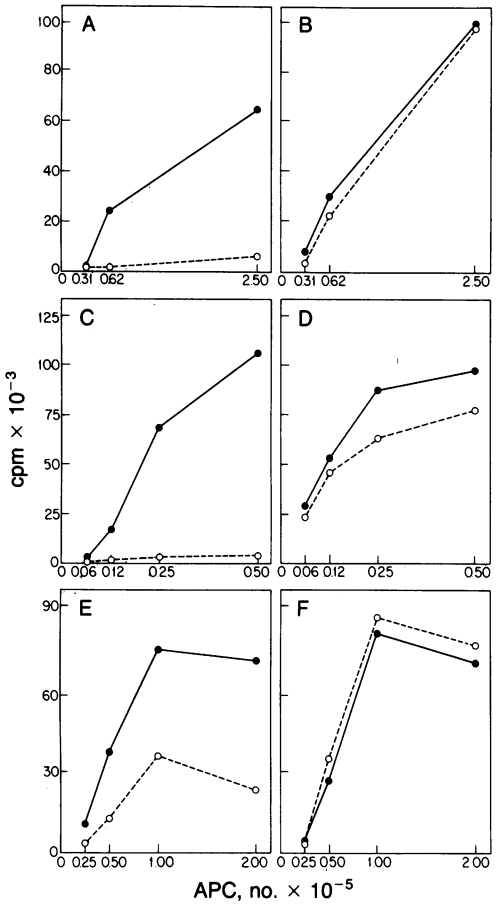

FIG. 2. Phospholipase C treatment of antigen pulsed APC affects antigen-specific but not allospecific presentation. Antigen-exposed A20 cells were treated (○) or untreated (●) as described in Fig. 1, except that phospholipase C (0.5 unit/ml) was substituted for phospholipase A_2. Microcultures were prepared as described in Fig. 1 and contained (*A*) A20 cells exposed to 400 μg of OVA per ml and DO-11.10 (OVA-plus-I-A^d), (*B*) A20 cells identical to those in *A* and RF19.52 cells (I-A^d-allospecific), (*C*) A20 cells exposed to 20 μg of bovine insulin per ml and RF28.4 (bovine insulin-plus-I-A^d), (*D*) A20 cells identical to those in *C* and RF19.52 cells (I-A^d-allospecific), (*E*) A20 cells exposed to 200 g of $(GluLysPhe)_n$ per ml and RF21.8 cells [$(GluLysPhe)_n$-plus-I-E^d], and (*F*) A20 cells identical to those in *E* and RF26.12 (I-E^d-allospecific). Results shown in *C* and *D*, *E*, and *F* are from the same experiment illustrated in Fig. 1 and are shown separately for clarity.

activity is seen with two distinct, highly purified phospholipases isolated from different sources. Second, phospholipases renature after heat denaturation and are enzymatically active (19, 20). Both enzymes have been heat-denatured. Heat-denaturation inactivates Pronase (15) and should inactivate most contaminants. Third, we have shown that the protease-sensitive cell surface protein LFA-1 is not affected on phospholipase-treated APC (ref. 15, and data not shown).

Antigen Reconstitution of Phospholipase-Treated APC. We attempted to reconstitute the presentation by reintroducing antigen. For this purpose, OVA-exposed APC were treated with phospholipase A_2 or C, which inhibited the ability to present this antigen as expected (Fig. 3). As shown in Fig. 3, T-cell responses can be completely restored by the addition of a tryptic digest of OVA (5). Presentation of this antigenic preparation bypasses processing and is presented by fixed APC (5). We next examined whether antigen restoration could be observed in the response to $(GluLysPhe)_n$. As shown in Fig. 4, full reconstitution is also observed when $(GluLysLeu)_n$ is added to cultures with treated and fixed APC. $(GluLysLeu)_n$ is a processing-independent copolymer

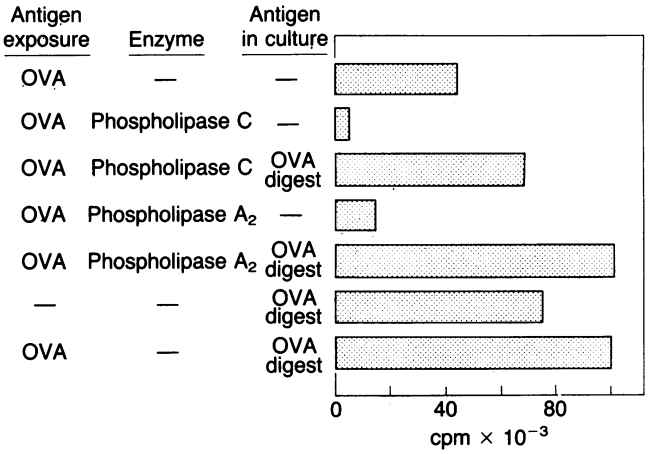

FIG. 3. Ability of OVA-exposed phospholipase-treated APC to present OVA is reconstituted by exogenous *in vitro* processed antigen. A20 cells were incubated with 200 μg of ovalbumin per ml for 18 hr at 37°C, washed extensively, and were then incubated with or without the indicated phospholipase enzyme and immediately fixed as described in the legends for Figs. 1 and 2. Fixed APC (8×10^4) were added to 10^5 DO-11.10 (OVA-plus-I-A^d) cells in duplicate microcultures (200 μl) in the presence or absence of denatured, trypsin-treated OVA (OVA digest) (300 μg/ml). Microcultures were processed and supernatants assayed for IL-2 content as described in the legend for Fig. 1. The fixed APC do not present native OVA added to culture (data not shown).

that is recognized by the $(GluLysPhe)_n$-specific T–T hybrid (9). These results provide functional evidence that phospholipase-treated APC express less processed antigen on their surface, but are otherwise unaffected.

Functional Detection of Membrane-Associated Processed Antigen. The above observation suggests enzymatic removal of cell surface-processed antigen. The ability to directly measure this antigen would be of obvious importance to the present study as well as to the characterization of *in vivo* processed antigen. However, with few exceptions, studies using anti-antigen antibody have failed to detect functionally relevant membrane antigen (21, 22). Part of this failure might reflect the fundamental difference of antibody and T-lym-

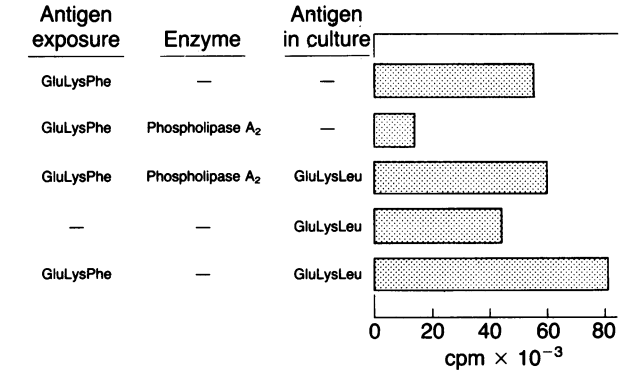

FIG. 4. Ability of $(GluLysPhe)_n$-exposed phospholipase-treated APC to present antigen is reconstituted by the processing independent antigen $(GluLysLeu)_n$. A20 cells were preincubated with 200 μg of $(GluLysPhe)_n$ per ml for 18 hr at 37°C, washed extensively, and were then incubated with or without phospholipase A_2 and immediately fixed as described in the legend for Fig. 4. Fixed APC (6×10^4) were added to 10^5 RF21.8 [$(GluLysPhe)_n$-plus-I-E^d] cells in duplicate microcultures (200 μl) in the presence or absence of $(GluLysLeu)_n$ (2 mg/ml). Microcultures were processed and supernatants assayed for IL-2 content as described in the legend for Fig. 1. The fixed APC do not present $(GluLysPhe)_n$ [which requires processing (9)] that has been added to culture (data not shown).

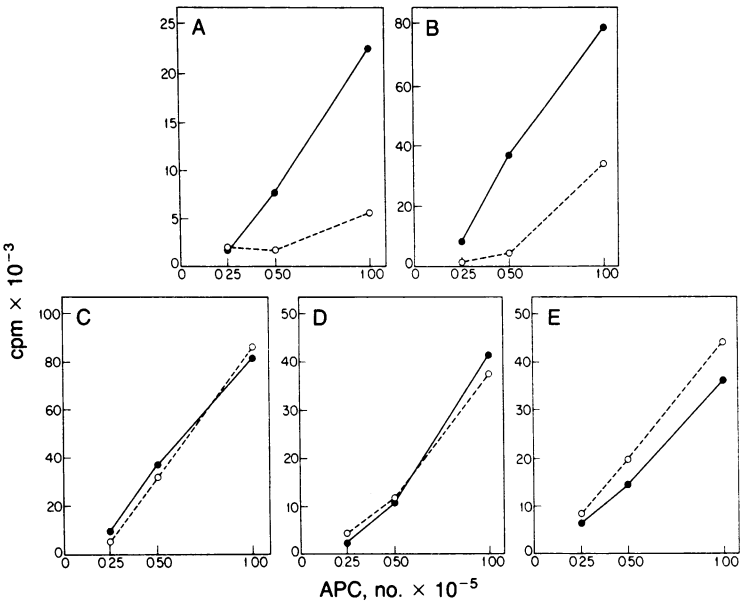

FIG. 5. Avidin specifically blocks presentation of biotin-insulin by exposed and fixed APC. A20 cells were exposed to antigen for 18 hr and subsequently washed and fixed as described in Fig. 1. The indicated number of antigen-exposed, fixed A20 cells were cultured with T–T hybridoma cells in the presence (○) or absence (●) of 100 μg of avidin per ml in triplicate microcultures as follows: (*A*) biotin-bovine insulin (20 μg/ml)-exposed A20 cells plus 10^5 RF28.4 (bovine insulin plus I-A^d specific) cells, (*B*) biotin-bovine insulin (40 μg/ml)-exposed A20 cells plus 10^5 RF28.4 cells, (*C*) bovine insulin (20 μg/ml)-exposed A20 cells plus 10^5 RF28.4 cells, (*D*) biotin-bovine insulin (40 μg/ml)-exposed A20 cells plus 10^5 RF19.52 (I-A^d allospecific) cells, and (*E*) biotin-bovine insulin (40 μg/ml)-exposed A20 cells plus 10^5 RF26.12 (I-E^d allospecific) cells.

phocyte specificity for native and processed antigen, respectively. Given this result, we reasoned that biotin would have several advantages as a potential label to identify processed antigen. Biotin is a small polar molecule that can be conjugated to selected amino acids. Furthermore, it should be resistant to proteolytic/acidic inactivation and could be detected on the basis of its exceedingly high affinity for avidin. For this purpose, we first tested whether bovine insulin could be biotinylated and retain immunologic activity. As shown in Fig. 5, insulin with biotin conjugated to its N terminus is presented to an insulin-plus-I-A^d-specific T–T hybrid, although it is slightly less active compared with the unmodified antigen.

We next tested the effect of avidin on the presentation of biotinylated antigen. As shown in Fig. 5, avidin markedly inhibits the ability of biotin-insulin-exposed APC to stimulate RF28.4 cells; this inhibition is highly specific. The presentation of unmodified insulin to the same hybridoma is not affected by avidin at either the same concentration of antigen (Fig. 5 *A* versus *C*) or at the same level of response (Fig. 5 *B* versus *C*). This indicates that avidin is not inhibitory for either the hybrid or APC *per se*. Additionally, avidin does not inhibit the ability of the same biotin-insulin-exposed APC to present their class II MHC molecules to alloreactive T–T hybrids (Fig. 5 *D* and *E*) or an OVA digest to an OVA-plus-I-A^d-specific hybrid (data not shown) despite equivalent binding to the cell surface. This clearly indicates that avidin directly inhibits the presentation of biotinylated antigen. Finally, in these experiments, the APC have been exposed with antigen and fixed prior to their exposure to either avidin or the T-cell hybrid. Therefore, the avidin inhibition can be directly localized to a specific effect on cell surface-associated, processed antigen. We conclude from these results that avidin can be used to detect immunologically relevant processed biotinylated antigen on the APC membrane.

Phospholipase Selectively Removes Cell Surface-Associated Exogenous Antigen. Given the results of the functional inhibition observed above, we tested whether avidin binding to the cell surface could be directly measured. As shown in Table 1, ^{125}I-labeled avidin binds to biotin-insulin-exposed APC. This binding is specific, as it is significantly higher than background binding to APC exposed to unconjugated insulin, and further, it can be completely inhibited by an excess of unlabeled avidin (Table 1). The appearance of cell surface antigen is markedly inhibited by monensin, a drug that blocks antigen processing. Therefore, this assay system is not

Table 1. Direct detection of cell surface antigen and its removal by phospholipases

Experiment	APC exposure	APC treatment	Specific binding of ^{125}I-avidin,† cpm ± SEM	Inhibition, %
1	Biotin-insulin	None	28,931 ± 950	
	Biotin-insulin	Avidin (unlabeled)‡	496 ± 132	99
	Biotin-insulin	Phospholipase C	7,940 ± *	73
2	Biotin-insulin	None	35,836 ± 2,535	
	Biotin-insulin	Phospholipase A_2	7,099 ± 1,665	80
	Biotin-anti-I-E	None	166,402 ± 17,093	
	Biotin-anti-I-E	Phospholipase A_2	169,826 ± 1,172	0
3	Biotin-insulin	None	6,483 ± 465	
	Biotin-insulin plus monensin	None	1,302 ± 229	80

A20 APC were exposed to biotin-insulin or insulin (both 100 μg/ml) for 18 hr with or without 10 μM monensin at 37° or with biotin-anti-I-E for 45 min at 0°C and were washed extensively. The exposed APC were treated with phospholipase C (0.6 unit/ml) or phospholipase A_2 (8 units/ml) and were subsequently tested for ^{125}I-avidin binding as described. Samples were tested in triplicate or quadruplicate except where noted by * (individual sample points 8180 and 7699). SEM, standard error of the mean.

†Calculated as cpm bound to biotin-exposed APC minus cpm bound to unconjugated insulin-exposed APC. Background binding to unconjugated insulin-exposed APC was 1546 cpm (exp. 1) and 10,019 (exp. 2).

‡100 μg of unlabeled avidin was added to the binding assay to compete for specific binding.

simply detecting adsorbed antigen. These results suggest that it should be feasible to directly test the effect of phospholipases on membrane-associated antigen. For this purpose, APC were exposed to biotin-insulin and subsequently treated in the presence or absence of phospholipase. After treatment, the binding of ^{125}I-labeled avidin was measured. As shown in Table 1, phospholipase A_2-exposed APC bind significantly less avidin. A similar result is observed with phospholipase C. The enzyme treatment fails to reduce the binding of avidin to a biotinylated antibody already bound to the APC class II MHC molecules. Therefore, the enzyme is not affecting the biotin group, an integral membrane protein, nor a noncovalently bound protein (antibody). We conclude from these results that lipase treatment is selectively and efficiently removing cell surface-associated exogenous antigen.

DISCUSSION

The present studies were initiated to investigate the basis for antigen association with the APC surface. Previously we have detected a specific interaction of processed antigen with class II MHC molecules before their recognition by the T-cell receptor (12–14). Recently this interaction has been confirmed and quantitated by equilibrium dialysis (10, 18). It appears to be of relatively low affinity, as expected. However, antigen pulsed onto APC persists for considerable time even when the cells are fixed to prevent reexpression (9). These observations suggest that there must be an additional basis for the stable association of antigen with the APC, apart from antigen–Ia interaction.

Evidence for this association has previously come from functional studies. The present studies have detected immunologically relevant antigen on the cell surface by two independent methods. First, processed antigen is accessible to selective enzymatic digestion as discussed below. Second, the presentation of processed, biotin-conjugated bovine insulin by fixed APC can be specifically blocked by avidin. Taken together, these results demonstrate that processed antigen is on the APC surface.

Highly purified phospholipases inhibit the ability of antigen-exposed APC to stimulate specific T–T hybridomas. This effect is selective, because the treated cells are equivalent to controls in their ability to stimulate alloreactive hybrids. Therefore, class II MHC molecules are functionally intact as are any putative cell-interaction structures. This has also been confirmed by antibody binding to I-E (Table 1) as well as to I-A, LFA-1, and other integral membrane proteins (data not shown). Several observations argue against carry-over of enzyme as a basis for the observed inhibition. Thus, alloresponses are intact, antigen responses can be reconstituted, and finally the T-cell hybrids can be directly treated without effect (data not shown).

Phospholipase A_2 and C have similar effects on cell surface antigen. These enzymes hydrolyze the fatty acid ester and phosphoric acid-glycerol bond of phospholipids, respectively. Although they have a distinct profile of activity on purified phospholipid substrates, this is not apparent in complex mixtures of phospholipids as is the case in the plasma membrane (19, 20). They are therefore broadly active and do not necessarily implicate particular lipids in this phenomenon.

What is the basis for the effects of these lipases on cell surface antigen? The native antigens we have studied, including *in vitro* synthesized copolymers, are not lipidated. Therefore, these results suggest that processed antigen may be associated with lipids of the APC membrane. The precise nature of this association is not defined by our data. It could be noncovalent, or a covalent lipid modification, as has been described for several cell membrane proteins.

It is well established that most soluble proteins require processing for cell surface display to T cells. For at least some antigens (including OVA), *in vitro* denaturation and/or proteolysis is sufficient to bypass processing (5–7). *In vivo* processed antigen has not been isolated, and its structure is solely inferred from these reconstitution experiments. Therefore, we do not know whether *in vivo* and *in vitro* processed antigen are identical structurally, nor whether they associate with cells in a similar manner. These points are currently under study. Precisely why processing is required is also not understood. Our results raise the possibility that processing is important for antigen–membrane association.

Recently, Delisi and Berzofsky have analyzed several immunogenic peptides and have identified a common amphipathic structure (8). They suggested that this might be of importance to antigenic interaction with the APC/Ia and/or the T-cell receptor. This structural feature could allow noncovalent interaction of peptides with lipid. Although this is the simplest model for our results, recent experiments raise the possibility of additional complexity. Thus, we have recently observed that the antibiotic cerulenin, which inhibits lipid modification of proteins, specifically blocks antigen processing (unpublished data).

The association of processed antigen with membrane lipids could have several implications. First, it provides a means of stable association of antigen on the APC surface independent of Ia molecules. Second, it would provide sites that are essentially nonsaturable, consistent with the failure of irrelevant proteins to inhibit APC antigen presentation (data not shown). Finally, the associated antigen should be highly mobile in the plane of the membrane, which should facilitate reversible interaction with other membrane structures such as the Ia molecule.

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