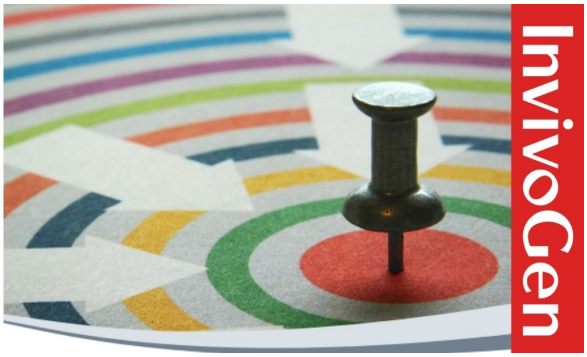

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CELLULAR AND SUBCELLULAR DISTRIBUTION OF PBP72/74, A PEPTIDE-BINDING PROTEIN THAT PLAYS A ROLE IN ANTIGEN PROCESSING*[1]

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A 72/74-kDa peptide binding protein (PBP72/74) was previously described which plays a role in the processing and/or presentation of Ag, possibly by facilitating the association of processed Ag with the MHC class II molecules. PBP72/74 was recently shown to be related to the 70-kDa family of heat shock proteins (hsp70), whose members show the general characteristic of binding to denatured or inappropriately folded proteins. Here we describe the cellular and subcellular distribution of PBP72/74. By flow cytometry with PBP72/74-specific rabbit antisera, PBP72/74 is detected on the surfaces of mouse Ig^+ B cells and MAC-1^+ macrophages. PBP72/74 was not detected on the surfaces of Thy-1^+ T cells or NK1.1^+ NK cells. The cell surface expression of PBP72/74 does not require MHC class II expression. Indeed, the Ia^- variant B cell lymphoma cell line, M12.C3, expresses PBP72/74 at levels equivalent to that of the Ia^+ parent cell line, M12.4.1, from which it was derived. Furthermore, the fibroblast L cell line, DAP.3, shows no cell surface expression of PBP72/74, nor do DAP.3 lines transfected with and expressing genes encoding the α- and β-chain of the I-A^d and I-E^d molecules. Moreover, treatment of B cells with either IL-4 or LPS, which increases Ia expression severalfold, does not affect PBP72/74 expression. Thus, PBP72/74 cell surface expression appears to be a property of B cells and macrophages, independent of Ia expression. In addition, the B cell surface expression of PBP72/74 is not altered by stress in the form of heat shock. Thus, PBP72/74 appears to be a constitutive noninducible member of the hsp70 family. By immunoelectron microscopy, PBP72/74 is detected in approximately 36% of early endocytic vesicles into which surface Ig is internalized after binding to anti-Ig antibodies. This compartment was previously shown to contain class II en route to the cell surface associated with invariant chain and the proteases cathepsin B and D and is suggested to be a subcellular site of antigen processing. PBP72/74 is also found associated with the plasma membrane, endoplasmic reticulum, and membranes proximal to the Golgi stacks. The cellular and subcellular distribution of PBP72/74 is consistent with its playing a role in the processing or presentation of Ag with the MHC class II molecules.

Th cells recognize Ag after processing and presentation by MHC class II-expressing APC. This involves the internalization of the Ag into acidic intracellular compartments, proteolysis of the Ag, and association of the resulting peptides with MHC class II molecules (reviewed in Ref. 1). At present, the molecular details of Ag processing remain to be delineated, including the mechanism of assembly of peptide-MHC complexes within the APC. Synthetic peptides representing T cell antigenic determinants have been demonstrated to bind to MHC class II molecules in vitro in detergent solution and in lipid bilayers (2–4). However, the kinetics of binding are unusual and are not completely consistent with the kinetics of Ag processing and presentation by APC. Indeed, the binding of peptide to MHC shows an extraordinarily slow association ($t_{1/2}$ = 8 h) and dissociation rate ($t_{1/2}$ = 30 h) in vitro (3). However, APC rapidly assemble stimulatory complexes when provided with antigenic peptides, and these are lost from the cell within several hours (5, 6). Thus, APC appear to have the ability to assemble and disassemble peptide-Ia complexes more rapidly than the rates measured in vitro would indicate. Although there may be several explanations for this, one possibility is that APC have mechanisms which facilitate the loading and unloading of MHC class II molecules with processed Ag. We have described one protein whose characteristics suggest it as a candidate to participate in such processes. This is PBP72/74[4] (7) which is a member of the hsp family (8).

PBP72/74 was isolated by its ability to bind to a known antigenic peptide of a soluble globular protein pigeon cytochrome *c*, residues 81–104 (7). PBP72/74 binds to this peptide but not to the corresponding region within the native protein. PBP72/74 binds to other peptides related to cytochrome *c*, as well as to peptides which show no sequence similarity. Thus, although not discriminating of primary amino acid sequence, PBP72/74 appears to be specific for some feature of the peptide not found in the native protein. PBP72/74-specific antisera block the processing and/or presentation of Ag to Ag-specific T cells and thus PBP72/74 appears to function at some step in Ag processing (7). PBP72/74 was shown to be serologically related to the hsp70 family (8), which

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[4] Abbreviations used in this paper: PBP72/74, peptide-binding protein of 72/74 kDa; Pc, pigeon cytochrome *c*; Pc 81-104, C-terminal fragment of Pc, residues 81–104; PE, phycoerythrin; hsp, heat shock protein; hsp70, 70-kDa hsp; BiP, Ig-binding protein.

consists of abundant cellular proteins, some of which are constitutively expressed, others of which are induced by stress (9). Several hsp members have been shown to play a role in the assembly of newly synthesized proteins and their transport to appropriate sites in the cell and in the housekeeping of inappropriately folded or denatured proteins (10, 11). So far, all members of the hsp70 family have been shown to bind ATP which in certain cases results in release of their polypeptide substrate. Similarly, PBP72/74 binds ATP which causes the release of bound peptide. Based on the shared properties of PBP72/74 and the hsp, it is reasonable to suggest that PBP72/74 may function at the site of Ag processing to capture newly processed antigenic peptide and facilitate its association with Ia and/or transport peptide to the cell surface for subsequent association with Ia. In this regard, it is of interest to define the cellular and subcellular distribution of PBP72/74.

Recent progress has been made in identifying the subcellular site of Ag processing. By using immunoelectron microscopy, we have identified an endocytic compartment in B cells into which surface Ig is internalized following binding to Ig-specific antibodies, acting as surrogate Ag (12). This compartment contains much of the machinery believed to be required for Ag processing, including MHC class II molecules en route to the cell surface associated with invariant chain and the proteases cathepsin B and D. By studying the biosynthetic pathway of MHC class II molecules, it has been shown that class II molecules intersect the endocytic route in transport from the *trans*-Golgi to the cell surface (13, 14). Class II is delayed at this site of intersection for 1 to 3 h and it was suggested that processed Ag could associate with class II during this time. To investigate whether PBP72/74 has the potential to participate in the Ag binding by MHC class II in the compartment described (12) we have investigated the cellular and subcellular distribution of PBP72/74. By flow cytometry, PBP72/74 is detected on B cell and macrophage surfaces and appears to be expressed by these cells independently of MHC class II. Analysis by immunoelectron microscopy shows that PBP72/74 is associated with approximately 36% of endocytic vesicles into which Ig internalizes Ag, with plasma membranes and with endoplasmic reticulum membranes. This distribution is consistent with the role of PBP72/74 in Ag processing and presentation.

MATERIALS AND METHODS

Mice. CBA/J and C57BL/6 mice were obtained from The Jackson Laboratories, Bar Harbor, ME.

Preparation of splenic cells and cell lines. B cells were prepared by treatment of RBC-depleted spleen with T cell-specific antibodies and C as previously described (15). B cell-depleted populations were prepared by treatment with the mAb Jlld (16) and C. The cell lines DAP.3, RT10.3C1, RT2.3.3H-D6 (17, 18), M12.4.1 (19), and M12.C3 (20) were kindly provided by Dr. R. Germain (NIH). LK35.2 (21) and CTLL (22) were obtained from ATCC. The B cell lymphoma CH27 (23) was characterized by and kindly provided by Dr. G. Haughton (University of North Carolina). IM-9 cells are maintained in Dr. F. Brodsky's laboratory (12). Where indicated, B cells were incubated 18 to 20 h at a density of 6×10^6 cells/ml with IL-4 (200 U/ml), LPS (50 μg/ml), or medium alone, harvested and washed, and dead cells removed by Ficoll-Hypaque centrifugation. To induce a heat shock response (24), B cells or LK35.2 cells were incubated at a density of 2×10^7 cells/ml for 30 min at 42°C. Cells were washed, incubated with [^{35}S]-methionine for 30 min, washed, incubated for various times, washed, lysed, and subjected to SDS-PAGE (7) to assess hsp synthesis.

Ag presentation assay. Presentation of Pc to the Pc-specific T cell hybrid, TPc, was determined by the ability of APC, provided with Pc, to activate TPc cells to secrete IL-2 measured by the ability of the culture supernatants to support the growth of the IL-2 dependent cell line CTLL, as previously described (15).

Antibodies. Hybridomas secreting the following mAb were obtained from ATCC: mAb GK1.5 specific for the T cell marker CD4 (25), mAb Jlld specific for Jlld (16), mAb M1/70.115.2 specific for the macrophage marker Mac-1 (26), and mAb 14.4.4s specific for I-E^k and I-E^d (27). All cell lines are routinely monitored for *Mycoplasma* and are free of this infection. Hybridomas were cultured in complete media (15) containing 10% FCS. The mAb p136, specific for NK1.1 (28) was kindly provided as an ascites fluid by Dr. G. Koo (Merck Sharp and Dohme). Culture supernatant from hybridoma MKD6 specific for I-A^d (29) was the gift of Dr. J. Miller (University of Chicago). mAb M5/49 specific for Thy-1 (30) was purchased from Boehringer Mannheim (Indianapolis, IN). Rabbit antiserum specific for PBP72/74 was prepared as previously described (7, 8). Serum obtained from rabbits immunized with Pc-keyhole limpet hemocyanin as described elsewhere (31), was used as a source of nonspecific immune sera. Rabbit antisera specific for mouse IgG was prepared as previously described (15). PE-coupled goat antibodies specific for rabbit Ig was purchased from Sigma Chemical Co. (St. Louis, MO). FITC-coupled goat antibodies specific for mouse IgG was purchased from Boehringer Mannheim and FITC-coupled goat antibodies specific for rat IgG was purchased form Cappel Laboratories (Malvern, PA).

Flow cytometry. Cells were washed three times in cold PBS, pH 7.2, containing 0.02% NaN_3 and 5% FCS (PBS-FCS). Cells (1 to 2×10^6) were resuspended in 50 μl of clarified primary antibody diluted in PBS-FCS on ice. Cells were washed three times in cold PBS-FCS and once in PBS containing 0.02% NaN_3 and 5% goat serum Sigma Chemical (St. Louis, MO), resuspended in 50 μl PE- and/or FITC-coupled secondary antibodies diluted in PBS, 0.02% NaN_3 and 5% goat serum and incubated for 25 min on ice. Cells were washed three times in PBS-FCS, and fixed by incubation in 0.5 ml 1% formaldehyde (Polysciences, Warrington, PA) in PBS-FCS. Cells were analyzed on a FACSCAN flow cytometer (Becton Dickinson, Mountain View, CA).

Immunoelectron microscopy. IM-9 cells were prepared for electron microscopy as previously described (12). Briefly, IM-9 cells were incubated sequentially with goat antibodies specific for human Ig and rabbit antibodies specific for goat Ig conjugated to 15-nm gold beads for 60 min at 4°C, warmed to 37°C, and incubated for 2 min. Cells were fixed and then stained with reduced osmium and 2% aqueous uranyl acetate and embedded in LR White Resin. Ultrathin sections were cut and mounted on 150-mesh nickel grids. Sections were incubated with either PBP72/74-specific rabbit serum or nonspecific immune serum diluted 1/50 in PBS containing 0.8% BSA and 0.1% gelatin (PBS-BSA) for 1 h at 25°C, washed, and incubated for 2 min at 25°C with protein A conjugated to 5-nm gold beads (E-Y Labs, Inc.) diluted 1/4 in PBS-BSA. Labeled grids were viewed on a JEOL 100C transmission electron microscope at 60 kV.

RESULTS

Expression of PBP72/74 on spleen cell surfaces. RBC-depleted spleen cells from CBA/J mice were analyzed for the expression of PBP72/74 by two-color flow cytometry. Spleen cells were stained with either nonspecific immune rabbit serum or PBP72/74-specific rabbit serum and antibodies specific for either Ig, Jlld, Mac-1, Thy-1, CD4, or NK1.1. The majority of Ig^+ spleen cells express PBP72/74 (Fig. 1). Approximately 53% of spleen cells stain positively for both Ig and PBP72/74 as compared to 4% which stain using Ig-specific antibodies and control nonspecific immune rabbit serum. Similarly, the majority of $Jlld^+$ cells stain positively for PBP72/74 (Fig. 1). Jlld is a marker present on the majority of nonimmune B cells (16), or approximately 35 to 40% of total spleen cells. The percent of spleen cells positive for both Jlld and for PBP72/74 is approximately 30%, while 5 to 10% of $Jlld^+$ cells show no PBP72/74 expression. Mac-1 positive cells also show staining with PBP72/74 specific serum (Fig. 1). Approximately 16% of spleen cells stain positively for Mac-1 and the majority of these are PBP72/74 positive. Thus, both splenic B cells and macrophages show cell surface expression of PBP72/74. In contrast, T cells and natural killer cells appear negative for PBP72/74 surface

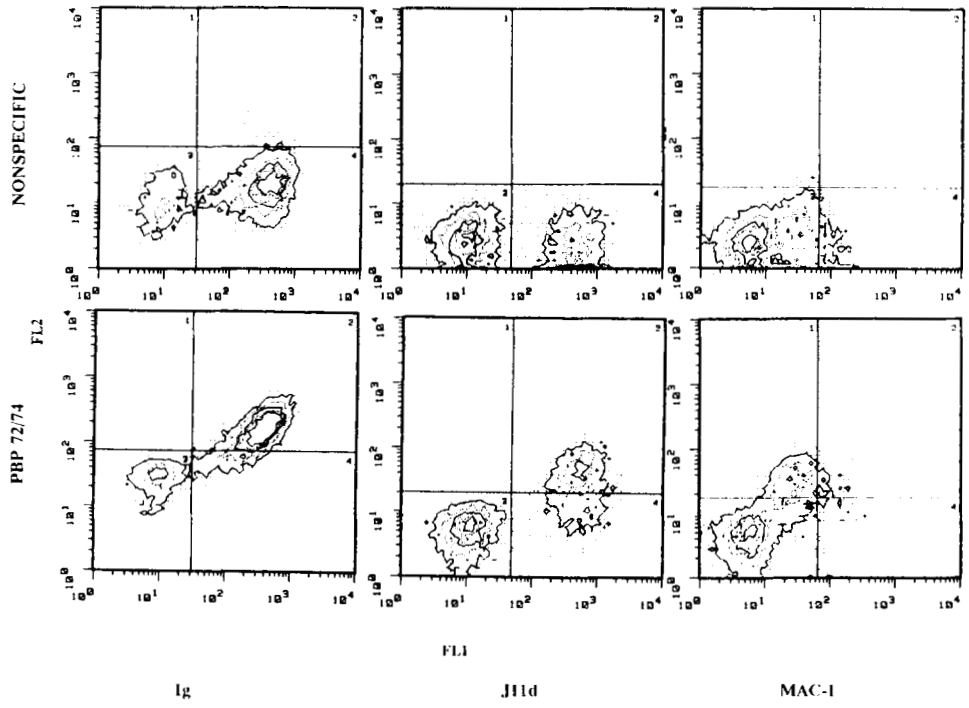

Figure 1. PBP72/74 is expressed on the surfaces of splenic B cells and macrophages. RBC-depleted spleen cells from CBA/J mice were stained, with either nonspecific immune rabbit serum or PBP72/74-specific rabbit serum detected by PE-conjugated goat anti-rabbit Ig (FL2) and with either FITC-conjugated goat anti-mouse Ig or with rat mAb specific for either Mac-1 or JIId detected by FITC-conjugated goat-anti-rat Ig (FL1).

expression (Figs. 2 and 3). Spleen cells staining strongly for Thy-1 or CD4 are negative for PBP72/74 (Fig. 2). Natural killer cells, identified by a monoclonal antibody specific for the 136,000 M_r marker NK1.1 in C57Bl/6 mice, show no PBP72/74 staining (Fig. 3).

PBP72/74 expression does not correlate with MHC class II expression. The above analysis of the surface expression of PBP72/74 on spleen cells shows that the majority of MHC class II expressing cells, namely B cells and macrophages, express PBP72/74. Previous studies

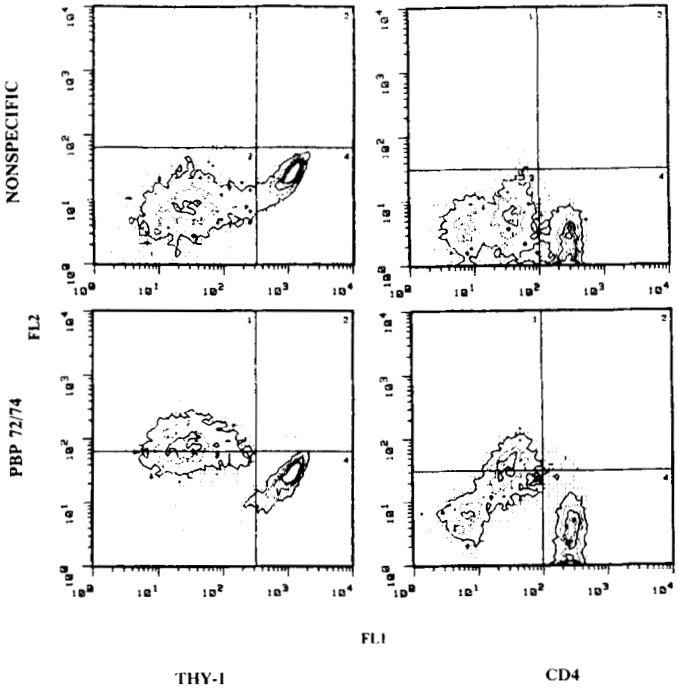

Figure 2. Splenic T cells show no cell surface expression of PBP72/74. RBC-depleted spleen cells from CBA/J mice were stained, with either nonspecific immune rabbit serum or PBP72/74-specific rabbit serum detected by PE-goat-anti-rabbit Ig (FL2) and with either a rat mAb specific for Thy-1 or a rat mAb specific for the CD4 molecule detected by FITC-goat-anti-rat Ig (FL1).

demonstrated that PBP-specific serum has no cross-reactivity with MHC class II molecules (7). The coincident expression of MHC class II and PBP72/74 may indicate a molecular association of MHC class II and PBP72/74. To determine if MHC class II expression is required for PBP72/74 expression, the B cell lymphoma (M12.4.1) and an Ia^- variant cell line derived from it (M12.C3) were analyzed for PBP72/74 expression (Fig. 4). M12.C3 cells generated and characterized by Glimcher and coworkers (20), do not express mRNA for I-A α and β chains and although they express α- and β-I-E chains in the cytoplasm they fail to transport these to the cell surface. Thus, while the parent M12.4.1 cells express significant amounts of both I-A and I-E molecules on the cell surface, M12.C3 cells show no staining over background (Fig. 4). However, both M12.4.1 and M12.C3 show cell surface staining for PBP72/74 (Fig. 4). Thus, PBP72/74 expression is independent of MHC class II expression. Moreover, treatments of B cells which result in increased expression of MHC-class II also have no effect on PBP72/74 expression (Table I). Splenic B cells were incubated with either IL-4 or LPS or medium alone for 18 hr, washed and analyzed for $I\text{-}E^k$ and PBP72/74 expression. Both treatments with IL-4 and with LPS result in a substantial increase in $I\text{-}E^k$ expression but with no significant increase in PBP72/74 expression (Table I). Thus, PBP72/74 surface expression is not under the same regulatory controls as MHC class II.

To determine if MHC class II expression can result in PBP72/74 surface expression, mouse L cell fibroblasts transfected with the genes encoding the α- and β-chains of MHC class II were analyzed for PBP72/74 surface expression. The MHC class II negative parental L cell line DAP.3 and the DAP.3 cell line transfected with and expressing the α and β-chains of $I\text{-}A^d$ (RT2.3.3H-D6) and the α- and β-chains of $I\text{-}E^d$ (RT10.3C1) were analyzed. Neither the MHC class II negative parental cell line nor the $I\text{-}A^d$ nor $I\text{-}E^d$ expressing transfected cell lines show positive staining for PBP72/74 (Fig. 5). Thus, PBP72/74 cell surface expression is not dependent on or induced by

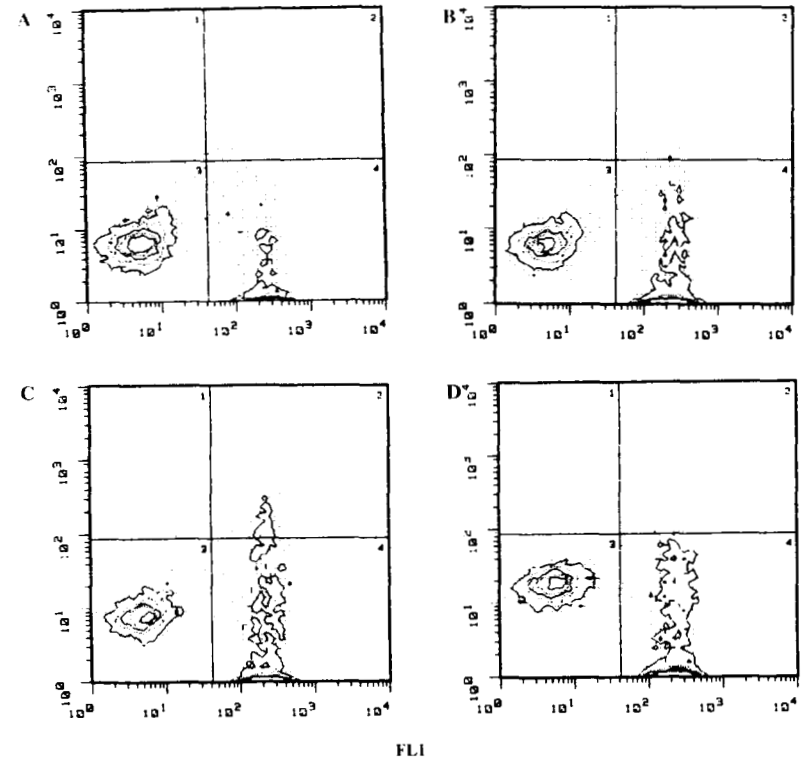

Figure 3. Splenic NK cells show no PBP72/74 cell surface expression. RBC-depleted spleen cells from C57BL/6 mice were depleted of B cells by treatment with the mAb Jlid and C. Cells were stained by using either nonspecific immune rabbit serum (*A* and *B*) or PBP-specific rabbit serum (*C* and *D*), detected by using PE-goat-anti-Rb Ig (FL2) and with a mAb specific for NK1.1 (*B* and *D*) or with no primary antibody (*A* and *C*) followed by FITC-goat-anti-mouse Ig (FL1).

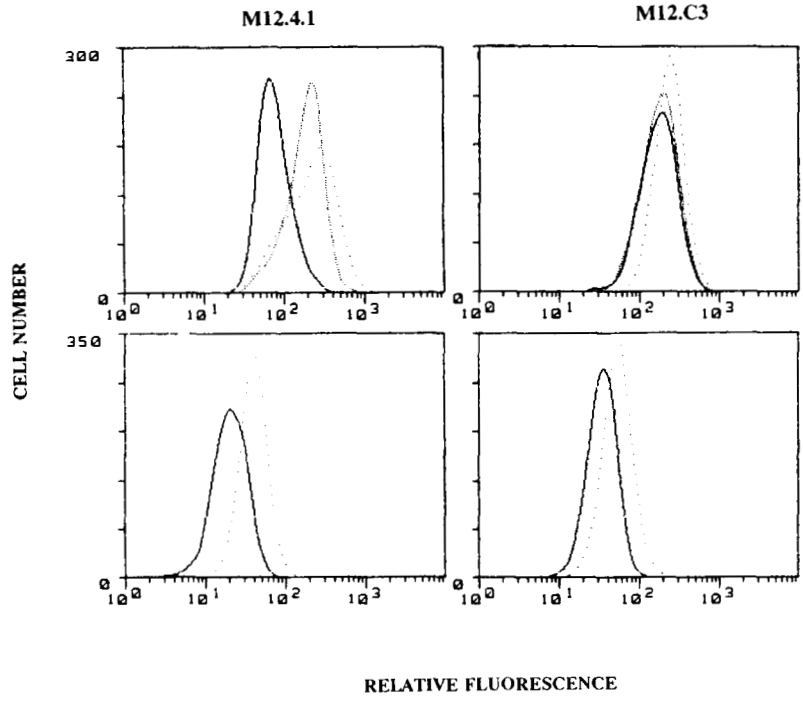

Figure 4. PBP72/74 cell-surface expression does not correlate with MHC class II expression in B cell lymphomas. The MHC class II-positive B cell lymphomas M12.4.1 and M12.C3 were stained with mAb specific for I-A^d (*dashed line*) or mAb specific for I-$E^{k/d}$ (*dotted line*) or with no primary antibody (*solid line*) followed by FITC-coupled secondary antibodies (*top panels*) or with PBP72/74 specific antiserum (*dotted line*) or a nonspecific immune sera (*solid line*) detected using PE-goat-anti-rabbit Ig (*bottom panel*).

TABLE I

Effect of IL-4 and LPS treatment on PBP 72/74 expression[a]

Addition	Mean Fluorescence Intensity	
	I-E^k	PBP72/74
No treatment	40	260
IL-4	590	250
LPS	300	160

[a] Splenic B cells were incubated with either LPS or IL-4 and compared with untreated cells for expression of I-E^k or PBP72/74 as described in *Materials and Methods*.

MHC class II expression but rather appears to be an independent function of B cells and macrophages.

Heat shock does not increase the cell surface expression of PBP72/74. The expression of several members of the heat shock family increases dramatically following exposure to various forms of stress, including heat shock (9). Although PBP72/74 appears to be constitutively expressed, it was of interest to determine if stress increases the level of cell surface expression of PBP72/74. To induce the heat shock response, LK35.2 cells were incubated for 30 min at 42°C, returned to 37°C and cultured for varying lengths of time. Analysis of the lysates of cells which had been incubated with [^{35}S]methionine for 30 min after heat shock showed a characteristic reduction in overall protein synthesis but with a significant increase in proteins in the 60,000 to 80,000 M_r range.

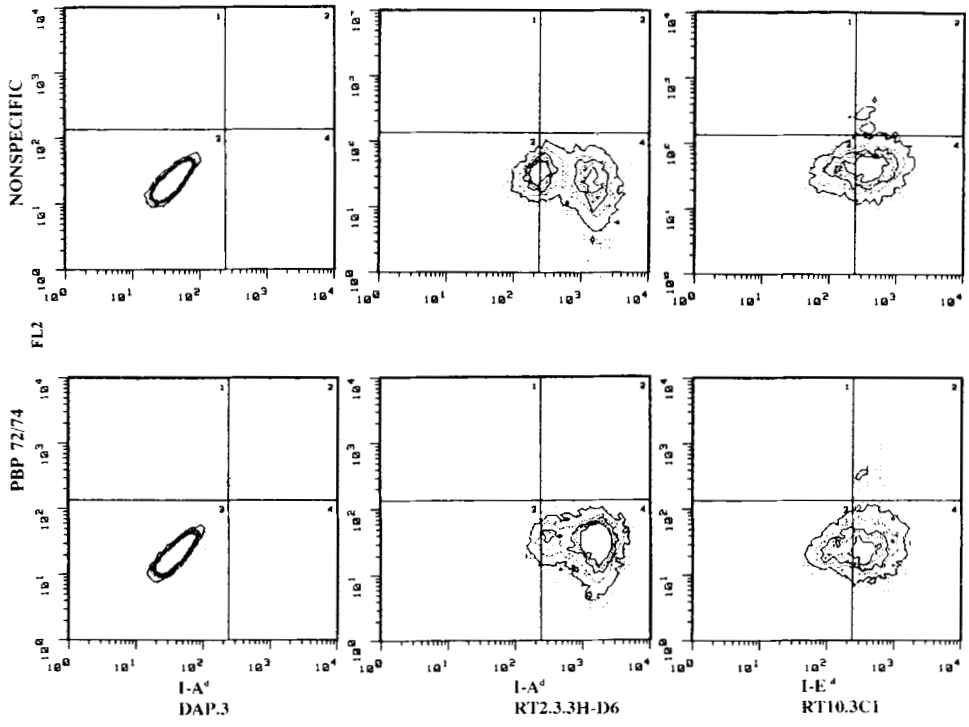

Figure 5. MHC class II expression in fibroblast cell lines does not result in PBP72/74 expression. The L cell fibroblast line DAP.3 and the DAP.3 line transfected with α and β chains of I-A^d (RT2.3.3H-D6) or I-E^d (Rt10.3C1) were stained with either PBP72/74 specific serum (*bottom panels*) or nonspecific immune rabbit serum (*top panels*) detected using PE-goat-anti-rabbit Ig (FL2) and either mAb specific for I-A^d or I-E$^{k/d}$ detected by using FITC-labeled goat-anti-mouse Ig.

Analysis of PBP72/74 surface expression showed no change after heat shock (Table II). In addition, heat shocked cells are indistinguishable from untreated cells in their APC function (Table II).

Analysis of the subcellular distribution of PBP72/74 by immunoelectron microscopy. Immunoelectron microscopy was used to identify the subcellular location of PBP72/74. Of particular interest was the colocalization of PBP72/74 in early endocytic vesicles into which surface Ig transports bound Ag for subsequent processing. Previous analysis (12) showed that after 2 min surface Ig is localized in vesicles which contain MHC class II en route to the surface associated with invariant chain and the proteases cathepsin B and D. The B cell lymphoma IM-9 cells were labeled sequentially with goat antibodies specific for human Ig and rabbit antibodies specific for goat IgG conjugated to 15-nm gold beads. Cells were incubated for 2 min at 37°C to allow cross-linked surface Ig to internalize. Cells were processed for electron microscopy and ultrathin sections were labeled with either PBP72/74-specific antisera or nonspecific immune sera and protein A conjugated with 5-nm gold beads. PBP72/74-specific antisera stained approximately 36% of endocytic vesicles into which cross-linked Ig is internalized (Fig. 6) (67 endosomes were counted) compared with 5% labeled with nonspecific immune sera. The presence of PBP72/74 in a large proportion of these early endosomes is consistent with a role in Ag processing. PBP72/74 is also observed on the endoplasmic reticulum membrane in vesicles near the Golgi, on the plasma membrane, and in the cytoplasm in what may be small vesicles (Fig. 6).

TABLE II

Heat shock does not alter PBP72/74 expression or APC function[a]

Treatment	Mean Fluorescence Intensity		APC Function
	I-E^k	PBP72/74	
Control	280	58	2.0
Heat shock	220	58	2.0

[a] LK35.2 cells were incubated at 42°C (heat shock) or 37°C (control) for 30 min, then incubated at 37°C for varying lengths of time (2, 4, and 8 h) and analyzed for I-E^k and PBP72/74 expression by flow cytometry. Fluorescence intensity of staining is shown for cells 4 h after heat shock and similar results were obtained at other time points. APC function was measured 8 h after heat shock. Cells were incubated with the Pc-specific T cell hybrid TPc 9.1 and graded concentrations of Pc. IL-2 was measured in the supernatant 24 h later. The concentration of Pc (μM) required for half-maximal TPc 9.1 IL-2 responses is shown.

DISCUSSION

A peptide-binding protein, PBP72/74, was previously described which plays a role in Ag processing and/or presentation as indicated by the ability of antisera raised against the purified protein to block the processing and presentation of Ag to MHC class II-restricted Th cells (7). It was hypothesized that PBP72/74 may function to bind to newly processed antigenic peptides in the APC and facilitate their interaction with the MHC class II molecules and/or the TCR (8). PBP72/74 was subsequently shown to be serologically related to the hsp70 family of proteins and to share a characteristic feature of members of this family, namely ATP binding, which for PBP72/74 causes a release of bound peptide. The observation of a peptide-binding hsp70 family member having a role in Ag processing and presentation is novel. However, this type of function for a hsp70 protein is not unexpected, because several members of the hsp family have been shown to bind to newly synthesized polypeptides before their folding or assembly into native conformations or to denatured or inappropriately folded proteins in the cell (reviewed in Refs. 10 and 11). In certain cases, hsp have been shown to transport newly synthesized proteins to the subcellular site where they function. In all cases studied, ATP causes a release of hsp from their substrate. At present it is not known if PBP72/74 is a new member of the hsp70 family. Previous studies (8) have shown that it is distinct from BiP (32), as a mAb specific for BiP did not recognize purified PBP72/74 in immunoprecipitation. PBP72/74 is recognized in Western blot by mAb N27 which is specific for a constitutive member of the hsp70 family but not by mAb N15 which recognizes a stress

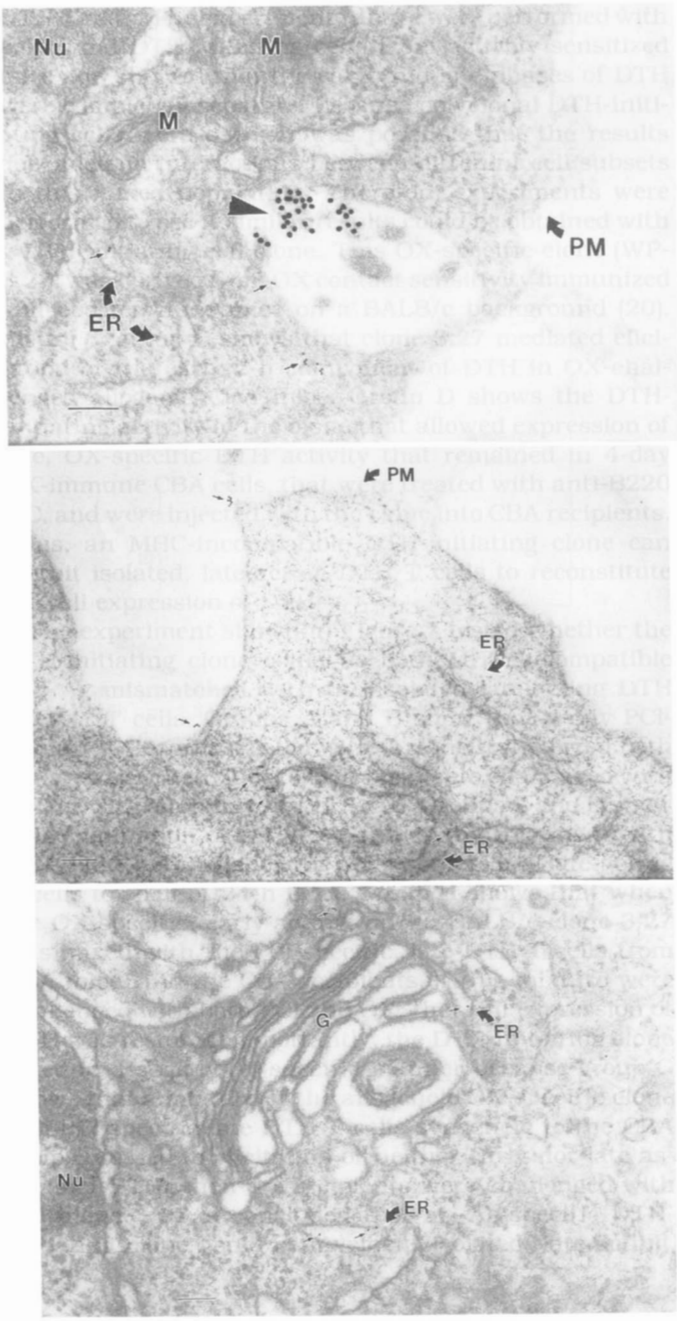

Figure 6. Intracellular colocalization of internalized immunoglobulin and PBP72/74. *Small arrows* designate staining with anti-PBP72/74 antisera and protein A (5-nm gold), *large arrowheads* indicate anti-human Ig and anti-goat Ig conjugated to 15-nm gold and *curved arrows* indicate the endoplasmic reticulum. Final magnification on all micrographs is 84,000 ×; the *bar* represents 0.119 μm. Nu, nucleus; G, golgi stacks; ER, endoplasmic reticulum; M, mitochondria; PM, plasma membrane. After a 2-min internalization of Ig, anti-PBP72/74 staining is seen on the endosomal membrane, in the cytoplasm, and in the ER (*top panel*), in the ER and on the plasma membrane (*middle panel*), and in the ER near Golgi stacks, but not in the Golgi stacks themselves (*bottom panel*).

induced hsp70 family member. Studies in progress to isolate the genes encoding PBP72/74 in mice should resolve this question and lend further insight into the relationship between PBP72/74 and other members of the hsp family.

The results presented here show a cellular and subcellular distribution of PBP72/74 which have not been described for other hsp70 family members and which is consistent with PBP72/74 having a function in antigen processing and/or presentation. PBP72/74 is expressed on the surfaces of B cells and macrophages but not T cells, NK cells, or fibroblasts. This is despite the fact that PBP72/74 can be purified from cell lysates of both T cells and 3T3 fibroblasts (7). Thus, the surface expression of PBP72/74 on B cells and macrophages is presumably a differentiated function of these two APC. How PBP72/74 becomes cell-surface associated is not known. PBP72/74 could have a membrane anchoring domain, which, if so, would be unique among the known hsp70 proteins. Alternatively, PBP72/74 could be associated via a fatty acid tail. However, preliminary studies indicate that lipase treatment of APC, which, as described by Falo et al. (33) abrogates APC function, does not alter PBP72/74 cell-surface expression. Last, PBP72/74 may become associated with another membrane protein. Precedence for this comes from the observation that a hsp70 family member is bound to membrane vesicles containing terminal transferrin receptors during reticulocyte maturation (34). In this case, the hsp may function as a chaperone for the receptor. One interesting possibility is that PBP72/74 functions as a chaperone for the MHC class II molecule during its transport through the cell and its peptide loading. However, the results presented here indicate that PBP72/74 is not associated with MHC class II. Indeed, the surface expression of PBP72/74 is not dependent on MHC class II expression and moreover, is not induced with MHC class II expression or regulated by inducers of class II expression, namely IL-4 and LPS.

The results presented here show that PBP72/74 has a restricted subcellular distribution showing a predominant lumenal staining. Of considerable interest is the observation of PBP72/74 in early endocytic vesicles into which surface Ig transports bound Ag. Previous studies (12) showed that MHC class II en route to the cell surface associated with invariant chain and the proteases cathepsin B and D colocalize to these vesicles. On this basis these vesicles have been proposed to be a site of Ag processing and peptide-MHC class II association. The presence of PBP72/74 in these vesicles is consistent with its having a role in these processes. The immunoelectron microscopy studies also demonstrated PBP72/74 is present on plasma membranes, verifying its surface expression as detected by flow cytometry. In addition, PBP72/74 is observed associated with endoplasmic reticulum vesicles near the Golgi, and with very small cytoplasmic structures which cannot be resolved, due to the minimal fixation used, but may be small membrane vesicles. Whether PBP72/74 functions in each of these distinct locations remains to be determined.

As stated above many of the hsp appear to have similar functions, namely binding to unfolded proteins and facilitating their appropriate folding or transport in the cell. What appears to distinguish the members is their site of function in the cell. For example, BiP appears restricted to the endoplasmic reticulum (10). It is attractive to speculate that PBP72/74 may represent an endosomal member of the hsp family and has become associated with Ag processing by virtue of its peptide binding ability. PBP72/74 may function to simply scavenger antigenic peptides at the site of processing, concentrating these for subsequent association with MHC. In addition, PBP72/74 could play a more fundamental role in what may prove to be a complex process of invariant chain-MHC class II dissociation and peptide binding during antigen processing. In this regard, it is of interest that in humans, the genes for the major heat shock protein hsp70 have been mapped

to the MHC (35).

Finally, the results presented here show that the cell surface expression of PBP72/74 is not affected in B cells by heat shock. Kaufmann and Wand-Wurttenberger (36) have reported staining of hsp on the surfaces of stressed mouse macrophages but not on untreated macrophages using the mAb N27. We have previously shown that mAb N27 recognizes PBP72/74 in Western blot; however, it is not known if it reacts with native, surface PBP72/74. Moreover, it is not known if PBP72/74 is regulated similarly in B cells and in macrophages. The observation of cell-surface expression of hsp has been given added significance recently by the finding that bacterial 65-kDa hsp-specific T cells kill stressed macrophages, presumably due to their cross-reaction with macrophage hsp (37). Such immunologic cross-reactivities have been implicated to play a role in autoimmune disease (reviewed in Refs. 38 and 39). Whether PBP72/74 is a target of hsp-specific T cells is not known. Future studies on the mechanism of hsp surface expression may help clarify this issue.

Note added in proof. As determined by N-terminal amino acid micro-sequencing, 74-kDa PBP appears to be a new member of the hsp70 family.

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REFERENCES

1. **Harding, C. V., F. Layva-Cobian, and E. R. Unanue.** 1988. Mechanisms of antigen processing. *Immunol. Rev. 106:77.*
2. **Babbitt, B. P., P. M. Allen, G. Matsueda, E. Haber, and E. R. Unanue.** 1985. Binding of immunogenic peptides to Ia histocompatibility molecules. *Nature 317:359.*
3. **Buus, S., A. Sette, S. M. Colon, D. M. Jenis, and H. M. Grey.** 1986. Isolation and characterization of antigen-Ia complexes involved in T cell recognition. *Cell 47:1071.*
4. **Sadegh-Nasseri, S., and H. M. McConnell.** 1989. A kinetic intermediate in the reaction of an antigenic peptide and I-E^k. *Nature 337:274.*
5. **Lakey, E. K., L. A. Casten, W. L. Niebling, E. Margoliash, and S. K. Pierce.** 1988. Time dependence of B cell processing and presentation of peptide and native protein antigens. *J. Immunol. 140:3309.*
6. **Roosnek, E., S. Demotz, G. Corradin, and A. Lanzavecchia.** 1988. Kinetics of MHC-antigen complex formation on antigen-presenting cells. *J. Immunol. 140:4079.*
7. **Lakey, E. K., E. Margoliash, and S. K. Pierce.** 1987. Identification of a peptide binding protein that plays a role in antigen presentation. *Proc. Natl. Acad. Sci. USA 84:1659.*
8. **VanBuskirk, A., B. L. Crump, E. Margoliash, and S. K. Pierce.** 1989. A peptide binding protein having a role in antigen presentation is a member of the hsp70 heat shock family. *J. Exp. Med. 170:1799.*
9. **Lindquist, S.** 1988. The heat-shock proteins. *Annu. Rev. Genet. 22:631.*
10. **Rothman, J. E.** 1989. Polypeptide chain binding proteins: Catalysts of protein folding and related processes in cells. *Cell 59:591.*
11. **Hartl, F. U., and W. Neupert.** 1990. Protein sorting to mitochondria: evolutionary conservations of folding and assembly. *Science 247:930.*
12. **Guagliardi, L. E., B. Koppelman, J. S. Blum, M. S. Marks, P. Cresswell, and F. M. Brodsky.** 1990. Co-localization of molecules involved in antigen processing and presentation in an early endocytic compartment. *Nature 343:133.*
13. **Cresswell, P.** 1985. Intracellular class II HLA antigens are accessible to transferrin-neuraminidase conjugates internalized by receptor mediated endocytosis. *Proc. Natl. Acad. Sci. USA 82:8188.*
14. **Neefjes, J. J., V. Stollorz, P. J. Peters, H. J. Geuze, and H. L. Ploegh.** 1990. The biosynthetic pathway of MHC class II but not class I molecules intersects the endocytic route. *Cell 61:171.*
15. **Casten, L. A., E. K. Lakey, M. L. Jelachich, E. Margoliash, and S. K. Pierce.** 1985. Anti-immunoglobulin augments B cell antigen presentation independently of internalization of receptor-antigen complex. *Proc. Natl. Acad. Sci. USA 82:5890.*
16. **Bruce, J., F. W. Symington, T. J. McKean, and J. Sprent.** 1981. A monoclonal antibody discriminating between subsets of T and B cells. *J. Immunol. 127:2496.*
17. **Germain, R., and H. Quill.** 1986. Unexpected expression of a unique mixed type class II MHC molecule by transfected L cells. *Nature 320:72.*
18. **Germain, R. N., J. D. Ashwell, R. A. Lechler, D. H. Margulies, K. M. Nickerson, G. Suzuki, and J. Y. L. Tou.** 1985. "Exon-shuffling" maps control of antibody and T cell recognition sites to the NH2 terminal domain of the class II major histocompatibility polypeptide A_β. *Proc. Natl. Acad. Sci. USA 82:2940.*
19. **Kim, K. J., C. Kanellopoulous-Langevin, R. M. Merwin, D. H. Sachs, and P. Asofsky.** 1979. Establishment and characterization of BALB/c lymphoma lines with B cell properties. *J. Immunol. 122:549.*
20. **Glimcher, L. H., D. J. McKean, E. Choi, and J. G. Seidman.** 1985. Complex regulation of class II gene expression analysis with class II mutant cell lines. *J. Immunol. 135:3542.*
21. **Kappler, J., J. White, D. Wegmann, E. Mustain, and P. Marrack.** 1982. Antigen presentation by Ia$^+$ B cell hybridomas to H-2 restricted T cell hybridomas. *Proc. Natl. Acad. Sci. USA 79:3604.*
22. **Gillis, S., M. M. Ferm, W. Ou, and K. Smith.** 1978. T cell growth factor; parameters of production and a microassay for activity. *J. Immunol. 120:2027.*
23. **Pennell, C. A., L. W. Arnold, P. M. Lutz, N. J. LoCasio, P. B. Willoughby, and G. Haughton.** 1985. Cross-reactive idiotypes and common antigen binding specificities expressed by a series of murine B cell lymphomas: etiological implications. *Proc. Natl. Acad. Sci. USA 82:3799.*
24. **Welch, W. J., and J. R. Feramisco.** 1985. Rapid purification of mammalian 70,000-dalton stress proteins: affinity of the proteins for nucleotides. *Mol. Cell. Biol. 5:1229.*
25. **Dialynas, D. P., Z. S. Quan, K. A. Wall, A. Pierres, J. Quintans, M. R. Loken, M. Pierres, and F. W. Fitch.** 1983. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK1.5: similarity of L3T4 to the human Leu-3/T4 molecule. *J. Immunol. 131:2445.*
26. **Springer, T., G. Galfre, D. S. Secher, and C. Milstein.** 1979. MAC-1: a macrophage differentiation antigen identified by monoclonal antibody. *Eur. J. Immunol. 9:301.*
27. **Ozato, K., N. Myer, and D. H. Sachs.** 1980. Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. *J. Immunol. 124:533.*
28. **Koo, G., and J. R. Peppard.** 1984. Establishment of monoclonal anti-NK1.1 antibody. *Hybridoma 3:301.*
29. **Kappler, J. W., B. Skidmore, J. White, and P. Marrack.** 1981. Antigen-inducible, H-2-restricted, interleukin-2-producing T cell hybridomas. *J. Exp. Med. 153:1198.*
30. **Davignon, D., E. Martz, T. Reynolds, K. Kurzinger, and T. A. Springer.** 1981. Lymphocyte function-associated antigen (LFA): a surface antigen distinct from Lyt2, 3 that participates in T lymphocyte-mediated killing. *Proc. Natl. Acad. Sci. USA 78:4535.*
31. **Casten, L., and S. K. Pierce.** 1988. Receptor-mediated antigen processing: Increased antigenicity of a globular protein antigen covalently coupled to antibody specific for B cell surface structures. *J. Immunol. 140:404.*
32. **Hendershot, L., D. Bole, G. Kohler, and J. F. Kearney.** 1987. Assembly and secretion of heavy chains that do not associate post-translationally with immunoglobulin heavy chain-binding protein. *J. Cell Biol. 104:761.*
33. **Falo, J. D., B. Benacerraf, and K. L. Rock.** 1987. Characterization of antigen association with accessory cells: specific removal of processed antigens from the cell surface by phospholipases. *Proc. Natl. Acad. Sci. USA 83:6994.*
34. **Davis, J. Q., A. Dansereau, R. M. Johnstone, and V. Bennett.** 1986. Selective externalization of an ATP-binding protein structurally related to the clathrin-uncoating ATPase/heat shock protein in vesicles containing terminal transferrin receptors during reticulocyte maturation. *J. Biol. Chem. 261:15368.*
35. **Sargent, C. A., I. Dunham, J. Trowsdale, and R. D. Campbell.** 1989. Human major histocompatibility complex contains genes for the major heat shock protein HSP 70. *Proc. Natl. Acad. Sci. USA 86:1968.*
36. **Kaufmann, S. H. E., B. Schoel, and A. Wand-Wurttenberger.** 1990. *Curr. Top. Microbiol. Immunol. In press.*
37. **Koga, T., A. Wand-Wurttenberger, J. DeBruyn, M. E. Munk, B. Schoel, and S. H. E. Kaufmann.** 1989. T cells against a bacterial heat shock protein recognize stressed macrophages. *Science 245:1112.*
38. **Kaufmann, S. H. E.** 1990. Heat shock proteins and the immune response. *Immunol. Today 11:129.*
39. **Young, R. A.** 1990. Stress proteins and immunology. *Annu. Rev. Immunol. 8:401.*