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An alternative way of CD4 and CD8 association with protein kinases of the Src family

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Abstract The T-lymphocyte co-receptors of MHC glycoproteins CD4 and CD8 are known to be associated with the protein tyrosine kinase Lck via cysteine-containing sequences in the cytoplasmic domains of CD4 and CD8 and in the N-terminal domain of Lck. Here we demonstrate that a fraction of CD4 and CD8 molecules are associated with very large, detergent-resistant complexes containing several glycosylphosphatidylinositol-anchored proteins, (glyco)lipids, and protein tyrosine kinases Lck and Fyn but apparently no other major transmembrane proteins. Association of Lck and Fyn with these large complexes is, in contrast to simple CD4/CD8-Lck complexes, not sensitive to alkylation with iodoacetamide. These large complexes therefore represent an alternative way of association of CD4 and CD8 with the protein tyrosine kinases, which may play a role in signaling through these receptors.

Introduction

Cytoplasmic domains of the T-lymphocyte co-receptors CD4 and CD8 are known to be noncovalently associated with the Src-family protein-tyrosine kinase (PTK) Lck (Veillette et al. 1988; Rudd et al. 1988). This direct association is dependent on specific sequences in the membrane-proximal part of the CD4/CD8 cytoplasmic domains and in the N-terminal domain of Lck (Turner et al. 1990; Shaw et al. 1990). Lck, as well as a related kinase Fyn, appears to be associated also with the T-cell receptor complex independently of CD4 or CD8 (Gassmann et al. 1992). In our laboratory we have described the existence of very large noncovalent detergent-resistant complexes containing several glycosylphosphatidylinositol (GPI)-anchored leukocyte surface glycoproteins, (glyco)lipids, protein kinases, and probably some of their intracellular protein substrates (Štefanová and Hořejší 1991; Štefanová et al. 1991; Cinek and Hořejší 1992; Bohuslav et al. 1993). Similar complexes were observed also by others in leukocytes and other cell types (Thomas and Samelson 1992; Dráberová and Dráber 1993; Fiedler et al. 1993; Garnett et al. 1993; Arreaza et al. 1994). These complexes may be involved in the well-known phenomenon of cell activation through crosslinking of the GPI-anchored surface receptors (Robinson 1991). In the present study we examined whether these "GPI-complexes" of the T-cell line HPB-ALL ($CD4^+$, $CD8^+$) and peripheral blood T cells contain in addition to the GPI-anchored proteins also some transmembrane proteins which might be involved in linking together the extracellular and intracellular components of these complexes.

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Materials and methods

Reagents and cells

Sepharose 4B and M_r standards were purchased from Pharmacia (Uppsala, Sweden), N-bromosuccinimide, autoradiography films, octylglucoside, and chemicals for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) from Sigma-Aldrich (Praha), NP-40 from Fluka (Buchs, Switzerland), Pansorbin (fixed *S. aureus* bacteria) from Calbiochem (La Jolla, CA), anti-immunoglobulin-peroxidase conjugates from Bio-Rad (Vienna, Austria), chemiluminescence-enhanced western blotting kit, [^{125}I]NaI and [γ-^{32}P]ATP from Amersham Buchler (Braunschweig, Germany), nitrocellulose membrane from Schleicher-Schüll (Dassel, Germany), epoxy-activated Eupergit C1Z from Röhm (Weiterstadt, Germany), and phosphatidylinositol-specific phospholipase C (PI-PLC) from ICN (Irvine, Scotland). The HPB-ALL thymoma cell line ($CD45^+$) was originally obtained from the laboratory of J. L. Strominger (Harvard University, Cambridge, MA). Peripheral blood lymphocytes were obtained from the blood of healthy volunteers by a modification of the standard procedure (Bøyum 1968).

Antibodies

Monoclonal antibodies (mAbs) MEM-28 (IgG1; CD45), MEM-31 (IgG2a; CD8), MEM-32 (IgG1; CD5), MEM-43 (IgG2a; CD59), MEM-53 (IgG1; CD53), MEM-57 (IgG2a; CD3), MEM-59 (IgG1; CD43), MEM-97 (IgG1; CD20), MEM-98 (IgG1; CD6), MEM-102

(IgG1; CD48), MEM-111 (IgG2a; CD54), MEM-115 (IgG2a; CD4), MEM-118 (IgM; CD55) and B2M-01 (IgG2a; β_2-microglobulin) were prepared in our laboratory and described earlier (Štefanová et al. 1989; Hořejší et al. 1986; Hořejší et al. 1988). Leu3a (IgG1; CD4) was obtained from Becton Dickinson (Mountain View, CA), 4G10 (IgG1; anti-phosphotyrosine) from UBI (Lake Placid, NY); DH2 (IgM; anti-ganglioside GM3) was kindly provided by S. Hakomori (The Biomembrane Institute, Seattle, WA), rabbit antisera to Lck (Veillette et al. 1988) and Fyn by A. Veillette (McGill University, Montreal, Canada).

Cell labeling, solubilization, antigen isolation, and in vitro phosphorylation

Cell surface radioiodination using the N-bromosuccinimide method, immunoprecipitation using a modification of the solid phase immunoisolation technique with antibodies immobilized on plastic surface, in vitro kinase assay, lysate preclearing by means of antibody-coated Pansorbin, gel chromatography on Sepharose 4B, PI-PLC treatment, SDS-PAGE, and western blotting with chemiluminescence-enhanced immunoperoxidase detection were all performed as described in our previous paper (Cinek and Hořejší 1992). For control preclearing (a negative control), Pansorbin-bound mAbs to either CD3 (MEM-57) or CD54 (MEM-111) were used, which yielded similar results and caused only minimal non-specific adsorption as compared with non-precleared lysate.

The lysates were prepared as follows: cells were lysed for 30 min. at 0 °C in a lysis buffer containing 150 mM NaCl, 20 mM Tris-HCl pH 8.2, 1 mM phenylmethyl-sulfonylfluoride and 1% NP-40 with or without 5 mM iodoacetamide (IAA). Nuclei and other insoluble materials were removed by low-speed centrifugation (20000×g, 3 min) and the supernatant was used for further work.

Preparative immunoisolation from the lysates of unlabeled cells was performed using Protein A-purified mAbs covalently attached to the nonporous Eupergit C1Z microparticles (Grässel et al. 1989). Three-hundred microliters of the cell lysate were incubated for 4–6 h with occasional shaking on ice with 10 µl packed volume of the immunosorbent. After washing with the lysis buffer the bound antigens were eluted with 70 µl sample buffer (100 °C, 1 min) and analyzed by SDS PAGE and western blotting.

Co-capping

As a first step, the CD59 red-fluorescent caps were induced as follows: HPB-ALL cells were first incubated with rabbit polyclonal antiserum against CD59 (diluted 1:400; raised in our laboratory by immunization of rabbits with a highly purified human erythrocyte CD59 antigen), then with biotinylated goat anti-rabbit Ig antibody (diluted 1:150; Vector Laboratories, Burlingame, CA) and then with Texas Red-labeled streptavidin (diluted 1:100; Vector Laboratories); all these incubations were done in ice-cold RPMI medium with 10% fetal calf serum for 30 min. The cells were washed three times with ice-cold phosphate-buffered saline (PBS) after each incubation. Cap formation was then induced by incubation of such pretreated cells at 37 °C for 25–35 min. Well-developed red fluorescent CD59 caps were observed in approximately 50% cells. The antigens potentially co-capping with CD59 were then visualized by second staining with appropriate mouse mAbs (used as hybridoma supernatants) followed by fluoresceine-labeled swine anti-mouse Ig (diluted 1:10; ÚSOL, Praha) absorbed with goat and rabbit immunoglobulin; these incubations were also done for 30 min on ice. The washed, double-stained cells were then stored at 4 °C in RPMI medium containing 1% paraformaldehyde and observed in fluorescence microscope (Leitz-Fluovert) at wavelengths corresponding to Texas Red and fluoresceine emission. The mAbs (all prepared in our laboratory) used for detection of the molecules potentially co-capping with CD59 were: B2M-01 (against β_2-microglobulin), MEM-59 (CD43), MEM-97 (CD20; negative control), MEM-57 (CD3), MEM-118 (CD55), MEM-102 (CD48), MEM-115 (CD4), MEM-31 (CD8). It was necessary to use hybridoma supernatants and not diluted ascitic fluids, as the latter often gave false positive results (staining of the CD59-cap) even in the case of various irrelevant negative controls, probably due to the presence of contaminating natural anti-carbohydrate antibodies. In each case 100 cells with well-developed CD59 red cap were examined for distribution of the other potentially co-capping molecule. The percentages reported in Results represent mean values of three such independent experiments.

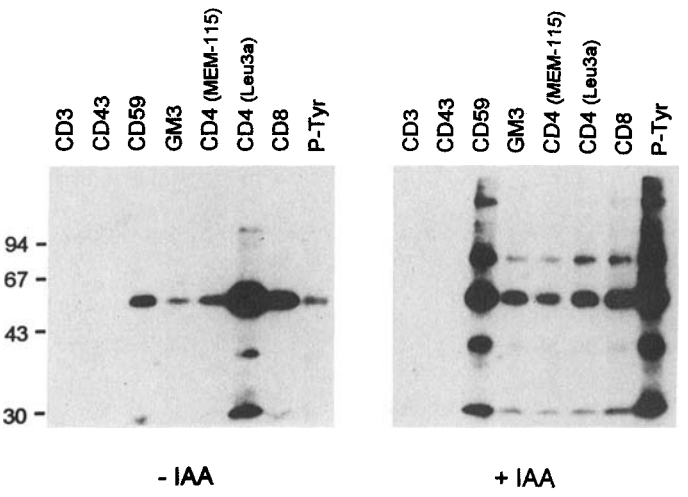


Fig. 1 In vitro autophosphorylation of immunoprecipitated HPB-ALL cell surface proteins. Detergent (1% NP-40) lysates were prepared either in the presence or absence of 5 mM iodoacetamide. MAbs to the indicated antigens were used for immunoprecipitation, the immunoprecipitates incubated with [^{32}P]-γATP and analyzed by SDS-PAGE and autoradiography. Similarly positive as CD59 were also immunoprecipitates of other GPI-anchored proteins (CD48, CDw52, CD55, CDw108); similarly negative as CD3 or CD43 were also MHC class I, CD2, CD5, CD6, CD11a, CD18, CD45 and CD53 (not shown). Positions of M_r standards are indicated ($\times 10^{-3}$)

Results

In order to examine whether in addition to the GPI-anchored proteins some transmembrane proteins are also associated with the GPI-PTK-complexes, we performed immunoprecipitation on immobilized mAbs to various surface antigens of the HPB-ALL cell line, followed by an in vitro kinase assay. Because the association of CD4 and CD8 with the kinase Lck is known to be dependent on a motif containing cysteines, we performed the assay using detergent (1% NP-40) lysates with or without iodoacetamide, which alkylates cysteine thiol groups and disrupts the CD4/CD8-Lck interaction. We performed the immunoprecipitation on mAbs to CD59 (the major GPI-anchored glycoprotein of the HPB-ALL cells), ganglioside GM3 (which was recently demonstrated to be the major glycolipid associated with the GPI-PTK-complexes (Kniep et al. 1994), and several transmembrane proteins abundantly expressed on HPB-ALL cells (MHC class I, CD2, 3, 4, 5, 6, 8, CD11a/CD18, 43, 45, and 53). For comparison, we also performed immunoprecipitation and the in vitro kinase assay on a mAb to phosphotyrosine. As expected from our previous experiments (Kniep et al. 1994), the patterns of in vitro phosphorylated zones were essentially identical for CD59 and GM3 (and also for other GPI-anchored antigens CD55, CD48, and CDw108; not shown), the intensity being

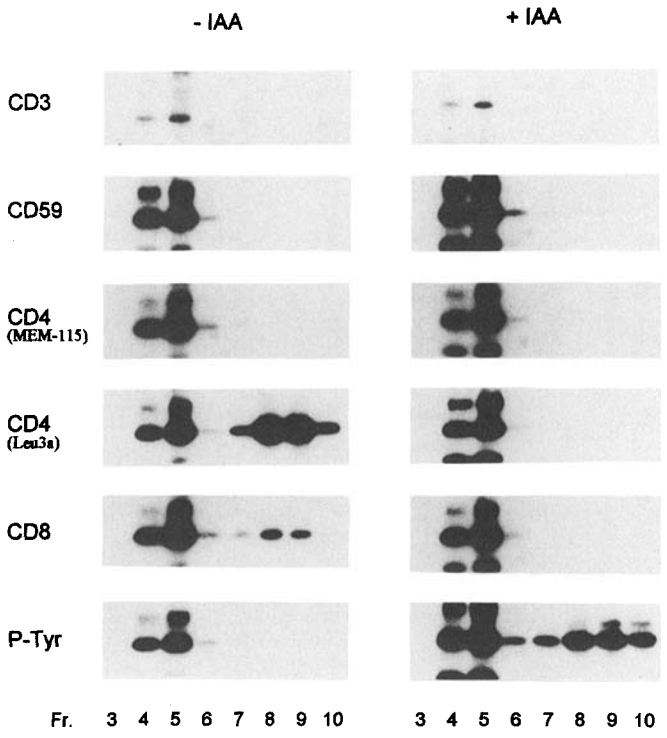


Fig. 2 The results of the in vitro kinase assay on immunoprecipitates obtained from Sepharose 4B fractions. The columns used were calibrated by size standards (erythrocytes, IgM, IgG), the elution maxima of which were in the fractions 4, 7, and 9, respectively. Fraction 4 corresponds therefore to the void volume, fraction 10 to the total volume. HPB-ALL detergent lysates prepared in the absence (*left column*) or presence (*right column*) of iodoacetamide were used. The antigens immunoprecipitated are indicated; two mAbs to CD4 (MEM-115 and Leu3a) were used. CD3 is a negative control. Only the relevant parts of the gels are shown

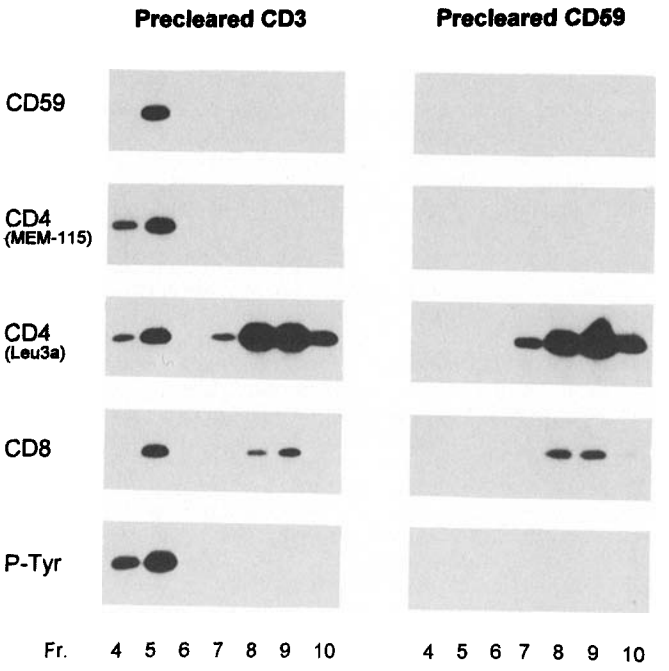


Fig. 3 Preclearing of the HPB-ALL cell detergent lysate (without iodoacetamide) by immobilized mAbs to CD3 (a negative control) or CD59. The precleared lysates were used for Sepharose 4B gel filtration, immunoprecipitation and the in vitro kinase assay as in Figure 2. Similar results were obtained with the alkylated lysate and also when immobilized mAbs to CD4 or CD8 were used for preclearing instead of the mAb to CD59 (not shown). Only the relevant parts of the gels are shown

stronger in the case of samples obtained from the lysate containing iodoacetamide (Fig. 1). As shown in previous papers (Štefanová et al. 1991; Cinek and Hořejší 1992), the major 55–60000 M_r in vitro phosphorylated zone corresponds mainly to the kinase Lck; the identity of the other zones (presumably substrates of the kinases) is presently unknown. A very similar pattern of phosphorylated zones was also observed in the case of anti-phosphotyrosine, CD4, and CD8 immunoprecipitates, while all other immunoprecipitates were negative (Fig. 1). Similarly negative under these conditions were also mAbs to a number of other molecules, such as CD7, 27, 28, 96, 98, 99, 100, and 101 (Cinek et al. 1994). In the case of CD4 the intensity of the phosphorylated zones was stronger in samples immunoprecipitated from the lysates without iodoacetamide (Fig. 1).

A characteristic feature of the GPI-complexes observed previously by us was their large size as demonstrated by gel filtration on Sepharose 4B. Therefore, we next performed immunoprecipitation and the in vitro kinase assay on the lysate fractions from Sepharose 4B column. The results were essentially identical for CD59, CD4, CD8, and anti-phosphotyrosine when an alkylated lysate was used (i.e., the associated protein-kinase activity was detectable only in very large complexes), but different when a non-alkylated lysate was used: in the latter case, the kinase activity associated with CD4 and CD8 was present both in fractions corresponding to very large complexes (the void-volume fractions) and in fractions corresponding to much smaller molecules or complexes (Fig. 2). Interestingly, one of the CD4 mAbs used did not precipitate the low M_r CD4-kinase complexes, but did immunoprecipitate the large complexes (Fig. 2).

These results appeared to indicate that at least some CD4 and CD8 molecules (but not other transmembrane proteins) were present in the GPI-complexes and that these large, protein-kinase-containing complexes were also specifically recognized by antibodies to phosphotyrosine.

In order to determine whether all these molecules reside in a single type of a complex, we performed quantitative preclearing of the lysate by immobilized mAb to CD59. As shown in Figure 3, the large kinase-containing complexes precipitable by mAb to CD59, CD4, CD8, and to phosphotyrosine all disappeared from the lysate precleared by immobilized mAb to CD59. Similarly, these mAbs precipitated very small kinase-containing complexes after preclearing with immobilized mAbs to CD4 or CD8 (not shown). This indicates that there was basically a single type of complex containing CD59, CD4, CD8, phosphotyrosine-bearing proteins, and protein kinases rather than several distinct large complexes of different composition. However, we were not able to address in this way the question of possible quantitative heterogeneity of these

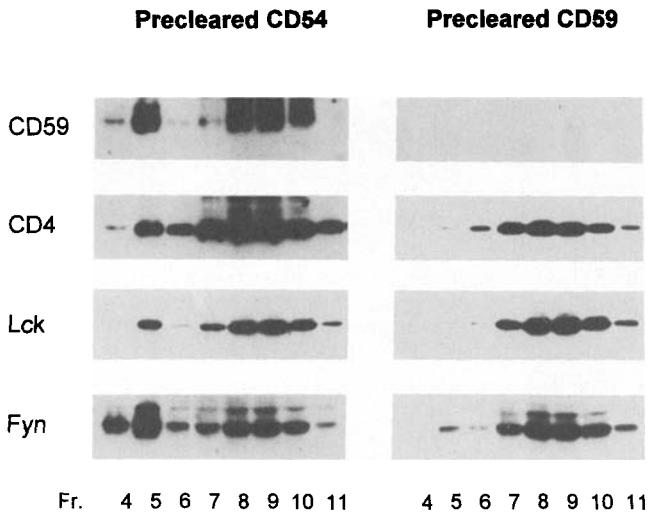


Fig. 4 Distribution of the relevant molecules in the Sepharose 4B gel filtration fractions as detected by western blotting. The detergent lysate of HPB-ALL cells (without iodoacetamide) was precleared by Pansorbin coated with the mAb to CD54 (negative control) or to CD59, subjected to gel filtration of Sepharose 4B (conditions as in Figure 2) and the fractions analyzed by SDS-PAGE and western blotting (non-reduced samples). The replicas were immunostained with mAbs to the indicated antigens. Only the relevant portions of the gels are shown

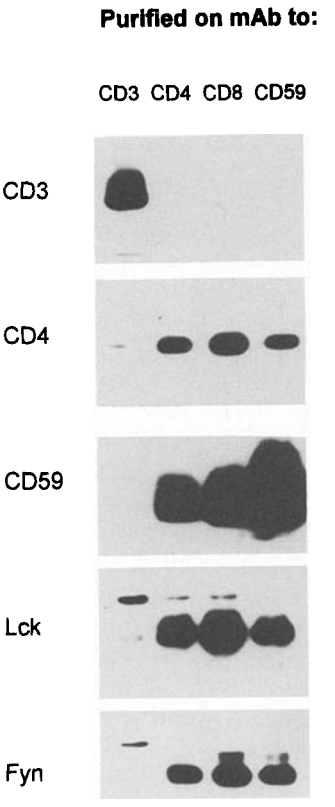


Fig. 5 Co-isolation of relevant molecules from the detergent whole cell lysate of HPB-ALL cells on immobilized mAbs to CD3, CD4, CD8, and CD59. Non-reduced SDS-eluates of the immunosorbents were analyzed by western blotting, using antibodies to the indicated molecules. Only the relevant parts of the gels are shown

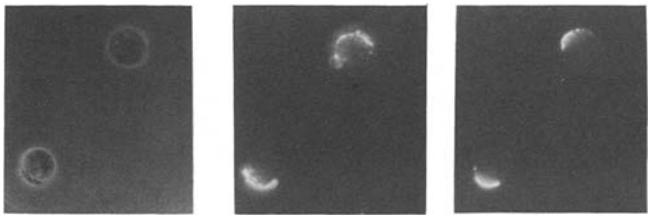
complexes (i.e., some complexes may possibly contain high, others low ratio of CD59/CD4, etc.).

It was of further interest to determine what fractions of total cellular CD59, CD4, CD8, and of the associated kinase(s) were present in these complexes. It was determined previously that the GPI-complexes in HPB-ALL cells contain the kinase Lck (Štefanová et al. 1991; Cinek and Hořejší 1992) but no information has been available so far concerning the other major potentially relevant kinase Fyn. To this aim we performed gel filtration of the lysate on Sepharose 4B and subjected the fractions to SDS-PAGE and western blotting. As shown in Figure 4, a clearly detectable fraction of total CD59, CD4, Lck, and Fyn and only a very minor fraction of CD8 (undetectable in this way, not shown) was found in the elution volume corresponding to the large complexes. This pattern of distribution between the high M_r and low M_r fractions was not significantly different whether the lysate with or without iodoacetamide was used (not shown). As expected from the previous in vitro phosphorylation experiments, CD4, Lck, and Fyn disappeared from the void volume fraction if the lysate was first quantitatively precleared with immobilized mAb to CD59 (Fig. 4). Similarly, nearly all CD59 present in the void volume fractions disappeared after preclearing of the lysate with immobilized mAbs to CD4 and CD8 (not shown). In a complementary experiment, CD59, CD4, Lck, and Fyn could be unambiguously demonstrated in the materials eluted from the CD59, CD4, and CD8 but not CD3 immunosorbents (Fig. 5). CD8 could not be demonstrated in this way, obviously because only a small amount of this component was associated with the complexes. No CD3 was detectable in these materials (except for the eluate from the CD3 immunosorbent), ruling out a nonspecific adsorption as a possible cause of the observed results.

In agreement with these biochemical data, partial association of CD4 and CD8 with GPI-anchored proteins was also confirmed by co-capping on intact cells as described under Materials and methods. The caps induced by cross-linking of CD59 were clearly immunostained by antibodies to the GPI-anchored glycoproteins CDw108 (35% of cases), CD55 (30%) and CD48 (31%), but also to CD4 (16%) and CD8 (15%). In contrast, the CD59-caps were not stained (i.e., less than 2%) by antibodies to β_2-microglobulin, CD3, CD45, CD43, CD18, and CD5 (Fig. 6).

To approach more directly the question of whether some other transmembrane proteins are associated with the

Fig. 6 An example of co-capping of CD4 with the GPI-anchored glycoprotein CD59 on HPB-ALL cells. Similar co-modulation with CD59 was observed also for three other GPI-anchored antigens (CDw108, CD55, CD48) and for CD8 as described in the text. In the cases of non-cocapping antigens (e.g., β_2-microglobulin, CD3, CD45, CD43, CD18, CD5) the green fluorescence was evenly distributed over the cell surface (not shown)



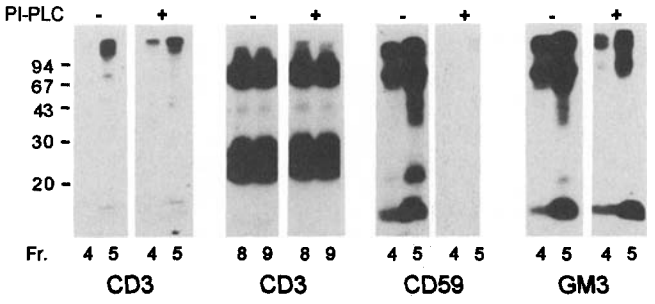


Fig. 7 Immunoprecipitation of the large complexes from surface radioiodinated HPB-ALL cells (untreated or PI-PLC treated). The detergent lysates were subjected to gel filtration on Sepharose 4B and the void volume fractions (No. 4 and 5) corresponding to the very large complexes were used for immunoprecipitation. CD3 was used as a negative control in these fractions. CD3 was precipitated equally well from the low M_r fractions (Fr. 8 and 9) of both untreated and PI-PLC-treated cell lysates (*second panel*). Nonreduced samples were analyzed by SDS PAGE and autoradiography. Similar results as with the mAb to GM3 were obtained also with mAbs to CD4 and CD8 (not shown)

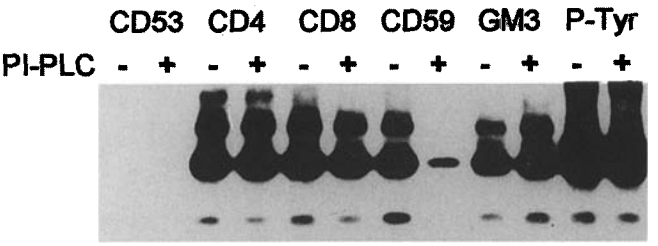


Fig. 8 An in vitro kinase assay on the immunoprecipitates obtained from alkylated detergent lysates of untreated and PI-PLC-treated HPB-ALL cells. The antigens immunoprecipitated are indicated. Only the relevant part of the gel (35–110000 M_r) is shown

complexes, we surface-radioiodinated HPB-ALL cells, treated them with PI-PLC and immunoprecipitated with mAbs to some of their previously identified components. The assumption was that iodinatable transmembrane proteins potentially present in these complexes should not be affected by PI-PLC treatment and should be recovered by immunoprecipitation of the complexes by mAbs to any components insensitive to this enzyme, such as glycolipids (GM3), CD4, or CD8. On the other hand, the GPI-anchored components should largely disappear from these complexes obtained from the PI-PLC-treated cells.

Major radioiodinated proteins present in the complexes obtained from untreated cells had approximately 80000, 70000, and 20000 M_r. These proteins largely disappeared after the PI-PLC treatment (as reasoned above, it was necessary to use mAbs to GM3, CD4, or CD8 for precipitation from the void volume fractions because most of CD59 was lost by the enzyme treatment; Fig. 7). Comparison of M_r of the proteins immunoprecipitated from the low M_r fractions and the western blotting data indicate that the 20000 M_r PI-PLC-sensitive zone corresponds to CD59. A very small amount of a protein corresponding apparently to CD8 (32 kD) was detectable in the complexes when reduced samples were analyzed (not shown). The PI-PLC-sensitive 70000 M_r zone is the GPI-anchored protein CD55 (Cinek and Hořejší 1992) and the major PI-PLC sensitive 80000 M_r protein is the recently defined novel GPI-anchored antigen CDw108 (P. Angelisová and co-workers, manuscript in preparation). CD4 is known to be poorly iodinated and therefore could not be detected in this way. It should be noted that the only major zone which did not decrease in intensity after PI-PLC treatment and immunoprecipitation by the anti-GM3 antibody was that one migrating with the dye-front and corresponding to radioiodinated (glyco)lipids (Štefanová and Hořejší 1991). Therefore, the large complexes do not contain any additional major transmembrane (PI-PLC-resistant) proteins.

MAbs to CD4, CD8, GM3, and to phosphotyrosine but not mAbs to CD59 and to other GPI-anchored molecules apparently immunoprecipitated the same large PTK-containing complexes detected by the in vitro kinase assay whether or not the cells were pretreated by PI-PLC (Fig. 8), confirming in another cell type our previous conclusion (Cinek and Hořejší 1992) that the GPI-anchored proteins are not critically important in maintaining the integrity of these supramolecular structures.

These results demonstrate that there are no major PI-PLC-resistant (transmembrane) proteins in the GPI-PTK-complexes, although minor amounts of such molecules or proteins poorly radioiodinated (such as CD4) may have not been detected. However, the absence of a number of major transmembrane proteins in the GPI-complexes of HPB-ALL cells is indicated by the negative results of in vitro kinase assays on their immunoprecipitates.

Finally, we tested whether these complexes (microdomains), containing in addition to the GPI-anchored proteins also CD4 or CD8, exist as well in peripheral blood T cells. Indeed, results were obtained similar to those in HPB-ALL cells, as indicated both by the results of in vitro kinase assays and western blotting on the Sepharose 4B gel filtration fractions of the NP-40 lysates (data not shown).

Discussion

In this communication we demonstrate that small amounts of the transmembrane proteins CD4 and CD8 are components of the previously described large, detergent-resistant complexes in the membranes of HPB-ALL cells, composed mainly of (glyco)lipids such as GM3, GPI-anchored glycoproteins (CD59, CDw52, CD55, CDw108, CD48) and containing protein tyrosine kinases Lck and Fyn. CD4 and CD8 seem to be the only transmembrane proteins present in these complexes demonstrable also in peripheral blood T cells. Therefore, CD4 and CD8 are able to associate with the Lck kinase in two different modes: first, through the well-known simple direct complexes dependent on cysteine-containing sequences, and second through the presently described large GPI-PTK-complexes. While the first type of small complexes is disrupted by the alkylating agent iodoacetamide, the other is not. This probably means that the CD4/CD8 association with the kinases in the latter

complexes is indirect. In agreement with the results of others we hypothesize that the GPI-complexes are primarily (glyco)lipid-based structures in which the GPI-anchored proteins are inserted through their lipid tails (i.e., they behave in a glycolipid-like manner) and the PTKs are analogously inserted in the inner leaflet of the lipidic bilayer via their attached myristic and palmitic acid residues (Shenoy-Scaria et al. 1994). Very few transmembrane proteins such as CD4 and CD8 can be also selectively present in such leukocyte membrane microdomains, while most of them are for unknown reasons excluded from these areas. We cannot rule out that small amounts of transmembrane molecules not tested in the immunoprecipitation/in vitro kinase test are also present, but at least those 18 tested were absent. In our experience the in vitro kinase test is much more sensitive than direct methods (immunoprecipitation from surface labeled cells), as it can obviously detect even minor components of the kinase-containing complexes. The results of our experiments on immunoprecipitation of the complexes from surface radioiodinated cells are also in agreement with the idea that no major transmembrane proteins are present, as all major zones were greatly reduced when the complexes were precipitated by the mAb to GM3 (and similarly by mAbs to CD8 and CD4) from the PI-PLC treated cells (Fig. 7). Of course, in this way we could have missed any proteins poorly labeled by radioiodination. To determine definitively the protein composition of the complexes, it will be necessary to purify them in amounts sufficient for direct staining and identification by microsequencing.

It is possible that at least some of the Lck molecules present in the GPI-PTK-complexes do have a direct contact with the CD4/CD8 molecules in addition to their anchorage in the lipidic structure via the fatty acid residues. It is conceivable that even after blocking of this direct, SH-dependent interaction by iodoacetamide, Lck can still remain associated with the GPI-PTK-complex via the fatty acid anchoring and therefore in close contact with CD4/CD8. On the other hand, the CD4/CD8 association with Lck (and also Fyn) within these large complexes may be only indirect and entirely independent of the cysteine-containing sequences. This alternative type of Lck association with CD4 and CD8 might be relevant to the recently described functionality of the CD4 and Lck variants incapable of mutual direct interaction (Levin et al. 1993; Killeen and Littman 1993). Significantly, the GPI-anchored proteins do not seem to be essentially important components of the large complexes, as they can be largely removed by PI-PLC without an effect on the core containing CD4, CD8, the kinases, and presumably certain (glyco)lipids. Our co-capping results confirm that the associations between the GPI-anchored proteins, CD4, and CD8 do exist on the surface of intact cells.

Two other points of the present work should be mentioned: 1) remarkably, mAb to phosphotyrosine immunoprecipitated the GPI-PTK-complexes but apparently no other large kinase-containing complexes. This indicates that the GPI-PTK-complexes are unique structures containing phosphotyrosine residues (presumably those in the Src kinases) and protein kinases. A potentially interesting point is that the mAb to phosphotyrosine apparently did not precipitate any protein kinase activity from the low M_r fractions of the non-alkylated lysate. This could be due to the action of a phosphatase in the iodoacetamide-free lysate; some of the phosphoproteins associated with the large complexes may be less susceptible to the dephosphorylation. Another possibility is that partial alkylation may change the conformation of PTKs in the low M_r fractions in such a way that some critical phosphotyrosine residues become better available to the antibody. It may be speculated that such a potential phosphorylation difference between the kinases present in the large and small complexes could be relevant in signalling through these different complexes; 2) We observed the existence of two types of CD4 mAbs, one of which precipitated under the conditions used in the absence of iodoacetamide both the large GPI-PTK-complexes and the conventional, small CD4-Lck complexes, while the other precipitated only the large complexes. Both these mAbs immunoprecipitated only the large complexes from the alkylated lysates. It seems likely that these differences reflect different affinities of the mAbs toward CD4; the immobilized mAbs with lower affinity effectively bind (due to multivalent attachment) the large complexes, while small, presumably monomeric CD4-Lck complexes may bind too weakly and are lost during washing. However, it is also possible that some CD4 epitopes are better preserved in the large complexes but partially lost or modified in the simple small CD4-Lck complexes.

In conclusion, the functionally important receptors of T cells CD4 and CD8 can be associated with the protein kinases Lck and Fyn in an alternative, indirect way, owing to their presence in the large GPI-PTK-complexes. This type of association can possibly contribute to some aspects of signaling through CD4 and CD8.

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