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Involvement of spectrin in cell-surface receptor capping in lymphocytes

(immunofluorescence/cytoskeleton/plasma membrane/concanavalin A)

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ABSTRACT **Human and mouse lymphocytes of T- and B-cell lineages express a protein (M_r, 240,000) that crossreacts with antibodies raised against chicken erythrocyte α-spectrin as judged by immunofluorescence, immunoprecipitation, and immunoautoradiography; by the same criteria, antibodies raised against chicken erythrocyte β-spectrin do not react with any lymphocyte polypeptide. In all T and B cells analyzed, before surface-directed ligand challenge with concanavalin A and surface immunoglobulins the polypeptide antigenically related to erythrocyte α-spectrin is distributed diffusely at the plasma membrane. Upon challenge, the redistribution of this polypeptide is concurrent with that of the cell-surface receptors initially in patches and then in a cap. Immunoprecipitation of $NaDodSO_4$-solubilized lymphocytes with erythrocyte α-spectrin antiserum shows that in all cases a polypeptide with the same apparent molecular weight as erythrocyte α-spectrin is precipitated. Variable amounts of another polypeptide (M_r, 235,000) are also coimmunoprecipitated. Immunoprecipitations and subsequent immunoautoradiography show that the lymphocyte polypeptide doublet has a composition similar to that of (brain) fodrin, a polypeptide doublet that previously has been found mainly in the cells of nervous tissue.**

The redistribution of cell-surface receptors with ligands and immunoglobulins initially involves the aggregation of the receptors into patches, presumably by the diffusion of the crosslinked receptors in the plane of the membrane, followed by the movement of these patches into a polar cap (1–3). The latter is an energy-dependent nondiffusional process thought to result from a transmembrane association of the receptors with cytoskeletal elements in the cell cortex (4–9). Despite extensive investigations, however, the role of the cytoskeleton in the patching and capping of cell-surface receptors in lymphocytes (for a review, see ref. 9) and the problem of how the cytoskeleton interacts with the plasma membrane to a great extent remains unknown. This interaction in the erythrocyte, on the other hand, is well understood and has been shown to be mediated by spectrin, a protein composed of two nonidentical polypeptide subunits (α-spectrin, M_r 240,000; β-spectrin, M_r 220,000) (for a review, see ref. 10).

Recently, it has been shown that nonerythroid cells also express polypeptides that appear to be biochemically and antigenically related to erythrocyte spectrin (ref. 11–18; see also ref. 19). Some cells (e.g., adult cardiac and skeletal muscle cells) express equimolar amounts of polypeptides antigenically related to erythrocyte α- and β-spectrin (18), whereas other cells (e.g., in nervous tissue) express a polypeptide doublet [termed "fodrin" (13)] composed of a 240,000-dalton component that is antigenically related to erythrocyte α-spectrin and a smaller (235,000) component that does not crossreact with either erythrocyte α- or β-spectrin antiserum (18).

In view of the dynamic role of the cytoskeleton during the patching and capping of cell-surface receptors, we have investigated the composition of polypeptides antigenically related to erythrocyte spectrin in lymphocytes. Our results show that a polypeptide doublet, similar to fodrin in nervous tissue, is immunoprecipitated with erythrocyte α-spectrin antibodies. Upon challenge with cell-surface-directed ligands, the redistribution of the polypeptide antigenically related to erythrocyte α-spectrin follows that of the cell-surface receptors, suggesting that in these cells this erythrocyte spectrin-related polypeptide complex may function to integrate the cytoskeleton with the transmembrane protein–cell surface receptor complex.

MATERIALS AND METHODS

Cells. The following lymphoid cell lines were cultured *in vitro*: human T-cell lineage, 8402 and CEM; human B-cell lineage, Raji and Daudi; human null cell lineage, Nalm 6; human mastocytoma, P815; mouse T-cell lineage, EL4 and Yac-1; and mouse B-cell lineage, Sp2 and Ns1 hybrids. Short-term cultures were grown to a density of 5×10^6 cells per ml in RPM1 1640 medium (GIBCO) supplemented with 1% glutamine and 10% fetal calf serum.

Antibodies. Antibodies raised against electrophoretically purified chicken erythrocyte α-spectrin (11) and β-spectrin (18) have been characterized in detail elsewhere.

Immunofluorescence. Surface-receptor capping and the distribution of polypeptides antigenically related to erythrocyte spectrin in lymphocytes were followed in the same cell. Lymphocytes were induced to cap in the presence of either rhodamine-labeled concanavalin A (ConA) (50 μg/ml; Polysciences) or goat anti-mouse Ig antiserum (Miles) in the culture medium at room temperature. Control cells were incubated in the absence of ligand. At various times after the addition of Con A or Ig, the cells were pelleted and fixed in phosphate-buffered saline (P_i/NaCl) containing 2% formaldehyde and 0.5% Triton X-100 for 5 min at room temperature. The cells were then washed in the same buffer but without formaldehyde and incubated with preimmune serum or with erythrocyte α- or β-spectrin antiserum at a dilution of 1:100 in P_i/NaCl for 30 min at room temperature. The cells were either in suspension or attached to polylysine-coated coverslips. After extensive washing with P_i/NaCl, the cells were incubated with fluorescein isothiocyante-labeled goat anti-rabbit Ig antisera (1:150 dilution; Miles) for 30 min at room temperature. The cells were again washed extensively in P_i/NaCl and examined in a Leitz phase-contrast/epifluorescence microscope.

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Abbreviations: ConA, concanavalin A; P_i/NaCl, phosphate-buffered saline.

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Immunoprecipitation and Immunoautoradiography. Immunoprecipitation of polypeptides from lymphocytes and brain tissue was performed according to the method of Lingappa *et al.* (20) as modified by Levine and Willard (13). We used 5–20 $\times 10^6$ lymphocytes for each immunoprecipitation with 1:100–1:500 dilution of the appropriate antiserum. Immunoprecipitated proteins were separated by $NaDodSO_4$/10% polyacrylamide gel electrophoresis based on the system of Laemmli (21) as modified (22). Immunoautoradiography (23) was performed as described (24).

RESULTS

Immunofluorescence. Fig. 1 shows the patching and capping of the ConA surface receptors of Ns1 hybrid cells in suspension. Similar results were also obtained with Raji and EL4 cells and with surface immunoglobulin capping in Ns1 hybrids (not shown). Phase-contrast microscopy of the Ns1 hybrid cells showed that the overall cell morphology and, in particular, the plasma membrane were well preserved under the incubation and fixation/permeabilization conditions used in this study (Fig. 1 *c*, *f*, and *g*). At time 0, the ConA receptors were evenly distributed over the entire surface of the plasma membrane (Fig. 1*a*). Indirect immunofluorescence microscopy of the same cell after incubation with erythrocyte α-spectrin antiserum showed that the crossreacting antigen(s) is also diffusely distributed over the inner surface of the plasma membrane (Fig. 1*b*). Cells not permeabilized with Triton X-100 showed no fluorescence with α-spectrin antiserum, demonstrating that the crossreacting antigen(s) is distributed on the cytoplasmic side of the plasma membrane (not shown).

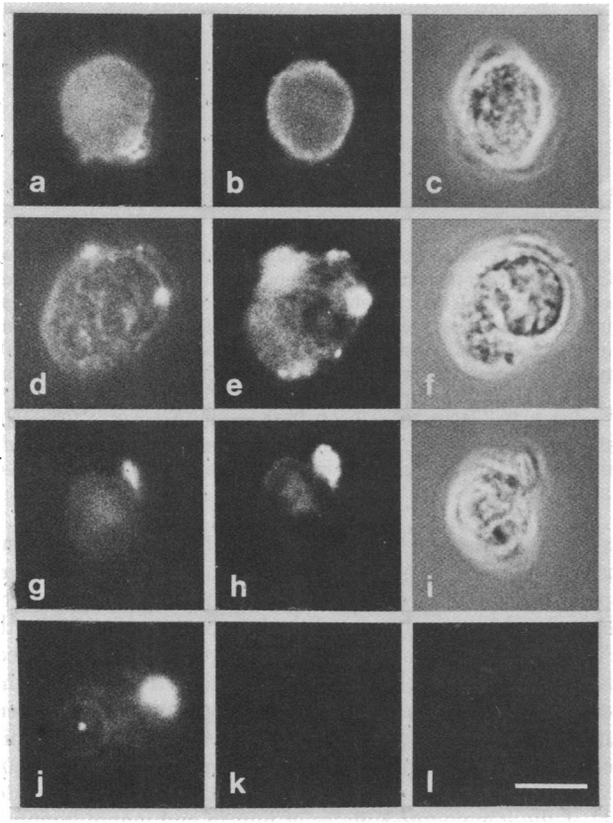

FIG. 1. Fluorescence and phase-contrast microscopy of Ns1 hybrid cells at various stages of capping of ConA cell-surface receptors: time 0, *a*, *b*, and *c*; 15 min, *d*, *e*, and *f*; 30 min, *g*, *h*, *i*, *j*, *k*, and *l*. Fluorescence micrographs of the same cell at each time point show the distribution of ConA (*a*, *d*, *g*, and *j*), α-spectrin (*b*, *e*, and *h*), and β-spectrin (*k*); *l* shows the control with preimmune serum. The cell-surface receptors were directly labeled with rhodamine-conjugated ConA and the distribution of α- and β-spectrin was visualized by using indirect immunofluorescence with fluorescein isothiocynate-labeled goat anti-rabbit antisera. *c*, *f*, and *i* show the phase-contrast images of the same cell shown in the fluorescence micrographs at each point. (Bar = 5 μm.)

After 15 min of incubation, the ConA fluorescence revealed the redistribution of surface receptors into several patches (Fig. 1*d*). The distribution of the crossreacting antigen(s) was coincidental with that of the ConA receptors, with intense fluorescence at the site of each patch (Fig. 1*e*). After 30 min of incubation, all the patches containing ConA receptors had coalesced into a single cap (Fig. 1g); the erythrocyte α-spectrin antibody fluorescence also was concentrated exclusively at the polar cap (Fig. 1 *h* and *j*). In parallel experiments using erythrocyte β-spectrin antiserum, fluorescence staining was not observed at any stage of the capping process (Fig. 1*k*). The preimmune serum also gave negligible fluorescence (Fig. 1*l*).

Immunoprecipitation and Immunoautoradiography. Fig. 2*a* shows the polypeptides immunoprecipitated from Yac-1 mouse T cells with preimmune serum and erythrocyte α- and β-spectrin antisera, respectively. A polypeptide with an apparent molecular weight similar to that of human erythrocyte α-spectrin (M_r 240,000; Fig. 2*b*, lane 1) was immunoprecipitated with α-spectrin antiserum (Fig. 2*a*, lane 2). A minor protein (black dot) also was immunoprecipitated with the preimmune serum and thus represents a nonspecific reactant with erythrocyte α-spectrin antiserum (see also Fig. 3). The erythrocyte β-spectrin antiserum did not immunoprecipitate any polypeptide as determined by Coomassie blue-staining (Fig. 2*a*, lane 3). In the other cell lines analyzed, a complex of two polypeptides was specifically immunoprecipitated with erythrocyte α-spectrin antiserum and was composed of a major polypeptide (apparent M_r 240,000) and another polypeptide (apparent M_r about 235,000) (Fig. 2*b*). The amount of the lower component varied between different cell lines and between experiments (compare Yac-1 immunoprecipitates in Fig. 2*a*, lane 2 and Fig. 2*b*, lane 8), indicating that the complex between the M_r 240,000 and 235,000 polypeptides is relatively labile under these immunoprecipitation conditions. Interestingly, the immunoprecipitates of lymphocytes of T-cell lineage have a relatively higher concentration of this doublet than do those of B-cell lineage, null cells, and the mastocytoma (Fig. 2*b*).

By immunoautoradiography of the gel shown in Fig. 2*b* with erythrocyte α-spectrin antiserum, it was found that the upper polypeptide (M_r 240,000) of the doublet crossreacted with α-spectrin antiserum but the lower component (M_r 235,000) did not (Fig. 2*c*, lanes 4–6); shorter exposures of the immunoautoradiogram than those shown here unambiguously demonstrate that in the remaining cell lines only the upper component crossreacted with the erythrocyte α-spectrin antiserum (data not shown). The labeled polypeptides smaller than the crossreacting polypeptide represent degradation products from this protein (Fig. 2*c*) (for comparison see ref. 11). Neither component of the doublet crossreacted with erythrocyte β-spectrin antiserum (not shown). Fig. 2*d* shows the immunoprecipitates of $NaDodSO_4$-solubilized lymphocytes with erythrocyte β-spectrin antiserum. By Coomassie blue staining, there does not appear to be any polypeptide specifically immunoprecipitated. This was also confirmed by immunoautoradiography with erythrocyte β-spectrin antiserum of the gel shown in Fig. 2*d* (see Fig. 2*e*).

These results show that the composition of polypeptides related to erythrocyte spectrin in the lymphocyte is different from that described in adult cardiac and skeletal muscle cells (18) but appears to be similar to that found in nervous tissue (13, 15–17).

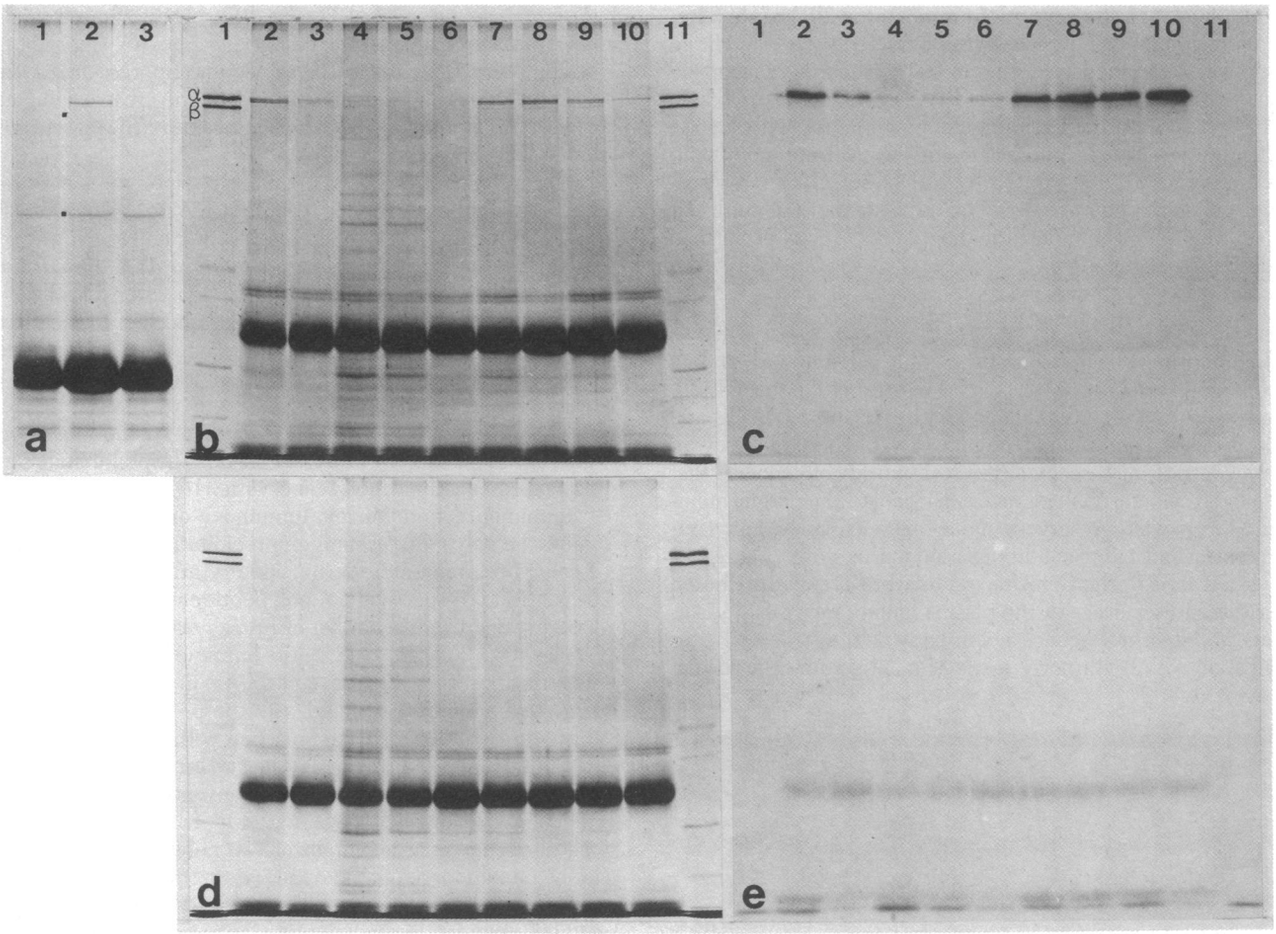

FIG. 2. $NaDodSO_4$/10% polyacrylamide gels of immunoprecipitates, and their corresponding immunoautoradiograms, of human and mouse lymphocytes of T- and B-cell lineage with α- and β-spectrin antisera. $NaDodSO_4$-solubilized proteins from lymphocytes were incubated with α- and β-spectrin antisera, respectively, and the immune complex was precipitated with protein A-bearing staphylococci. The resulting immunoprecipitates were analyzed on $NaDodSO_4$/10% polyacrylamide gels followed by immunoautoradiography. (*a*) Coomassie blue-stained gel of immunoprecipitates of Yac-1 cells with preimmune (lane 1), α-spectrin (lane 2), and β-spectrin (lane 3) antisera (13×10^6 cells per immunoprecipitate). (*b* and *c*) Coomassie blue-stained gel (*b*) of immunoprecipitates of lymphocytes with α-spectrin antiserum and its corresponding immunoautoradiogram with α-spectrin antiserum (*c*)—lanes: 1 and 11, human erythrocyte ghosts as markers for α-spectrin (M_r 240,000) and β-spectrin (M_r 220,000); 2, CEM (10×10^6 cells); 3, Nalm 6 (5×10^6 cells); 4, Daudi (10×10^6 cells); 5, Raji (10×10^6 cells); 6, P815 mastocytoma (6×10^6 cells); 7, EL4 (10×10^6 cells); 8, Yac-1 (20×10^6 cells); 9, Sp2 (15×10^6 cells); 10 Ns1 hybrids (20×10^6 cells). (*d* and *e*) Coomassie blue-stained gel (*d*) of immunoprecipitates of lymphocytes with β-spectrin antiserum and its corresponding immunoautoradiogram with β-spectrin antiserum (*e*); the lane order and numbers of cells used for each immunoprecipitate are exactly as in *b* and *c*.

To examine this further, we immunoprecipitated $NaDodSO_4$-solubilized whole 10-day chicken embryo brain and adult mouse brain with chicken erythrocyte α- and β-spectrin antisera. Erythrocyte α-spectrin antiserum immunoprecipitated a polypeptide doublet from both chicken and mouse brain (Fig. 3*a*, lanes 2 and 3, respectively), the upper component of which had an apparent M_r of 240,000, similar to that of chicken and human erythrocyte α-spectrin (lanes 6 and 5, respectively); the lower component had an apparent M_r of ≈235,000 and showed a slight difference in electrophoretic mobility between chicken and mouse brain. However, the polypeptide doublet from mouse brain appeared to be similar in electrophoretic mobility to that immunoprecipitated from mouse (and human) lymphocytes with erythrocyte α-spectrin antiserum (compare with Fig. 2*b*). The amount of the M_r 235,000 component from mouse brain varied between preparations, as in the case of the lymphocyte polypeptide (see above). The parallel immunoprecipitations with erythrocyte β-spectrin antiserum show that comparatively much lower concentrations (if any) of serologically crossreactive forms of erythrocyte β-spectrin are present in brain.

The corresponding immunoautoradiogram with erythrocyte α-spectrin antiserum (Fig. 3*b*) shows that only the upper component of the polypeptide doublet immunoprecipitated with α-spectrin antiserum from chicken and mouse brain crossreacts (lanes 2 and 3), as in the case of the lymphocytes (compare with Fig. 2*c*). However, this sensitive technique also demonstrated the presence of a polypeptide antigenically related to erythrocyte α-spectrin in the β-spectrin immunoprecipitates of chicken and mouse brain (Fig. 3*b*, lanes 1 and 4, respectively). This technique was also applied to immunoprecipitates of lymphocytes. Fig. 3*c* shows a Coomassie blue-stained polyacrylamide gel of the immunoprecipitates of human and mouse lymphocytes of T- and B-cell lineage, respectively, with β-spectrin antiserum. Fig. 3*d* shows the corresponding immunoautoradiogram with erythrocyte α-spectrin antiserum. The polypeptide doublet immunoprecipitated with α-spectrin (see Fig. 2*b*) was not immunoprecipitated with β-spectrin antiserum (see also ref. 18). The results of use of this sensitive indirect technique to probe for the presence of β-spectrin in lymphocytes substantiates the results obtained with direct approaches—that a serologically crossreacting form of erythrocyte β-spectrin is not present.

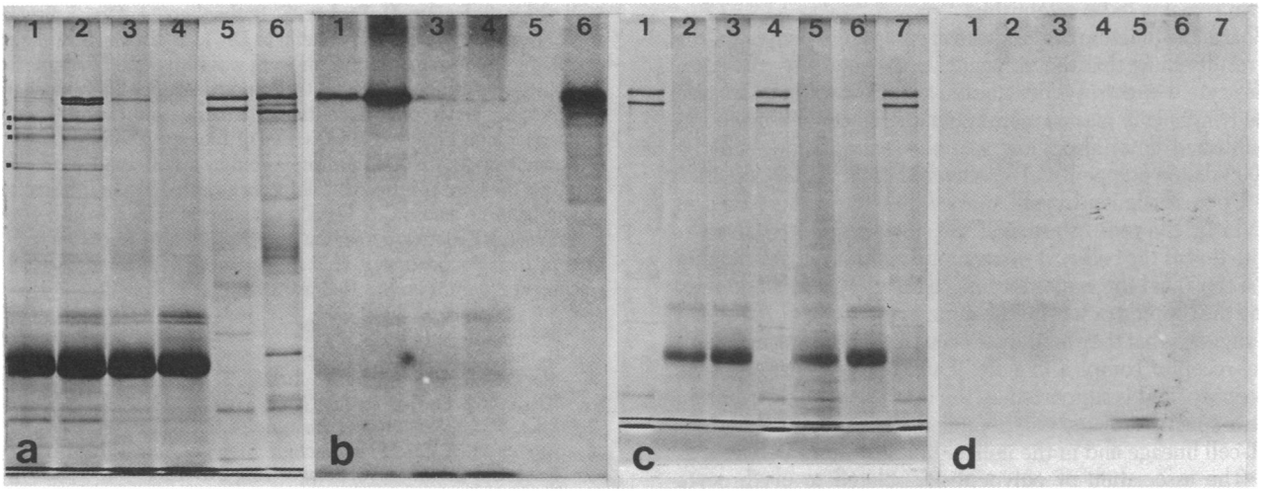

FIG. 3. Coomassie blue-stained $NaDodSO_4$/10% polyacrylamide gels of immunoprecipitates of chicken and mouse brain and lymphocyte polypeptides and their corresponding immunoautoradiograms with α- and β- spectrin antisera. For details of the experimental procedure see the legend to Fig. 2. (*a*) Coomassie blue-stained gel of immunoprecipitates of $NaDodSO_4$-solubilized whole 10-day chicken embryo brain (lanes 1 and 2) and adult mouse brain (lanes 3 and 4) with α-spectrin (lanes 2 and 3) and β-spectrin (lanes 1 and 4) antisera; lane 5, human erythrocyte ghosts; lane 6, purified chicken erythrocyte membranes. (*b*) Corresponding immunoautoradiogram of the gel in *a* with α-spectrin antiserum. (*c*) Coomassie blue-stained gel of immunoprecipitates of lymphocytes of CEM (lane 2), Raji (lane 3), EL4 (lane 5), and Ns1 hybrid (lane 6) cell lines with β-spectrin antiserum; lanes 1, 4, 7, human erythrocyte ghosts. (*d*) Corresponding immunoautoradiogram of *c* with α-spectrin antiserum.

DISCUSSION

The results of this study demonstrate the presence of a polypeptide antigenically related to chicken erythrocyte α-spectrin in human and mouse lymphocytes of T- and B-cell lineage, as judged by immunofluorescence, immunoprecipitation, and immunoautoradiography. We have also demonstrated by the same criteria that a serologically crossreactive form of chicken erythrocyte β-spectrin is not present in the same cells. The failure to detect polypeptides antigenically related to erythrocyte β-spectrin in mammalian lymphocytes does not appear to be due to the inability of the antiserum to crossreact with the corresponding non-avian antigens because we could demonstrate their presence indirectly in mouse brain. Furthermore, it was previously shown that these antisera, raised against electrophoretically purified α and β subunits of chicken erythrocyte spectrin, do crossreact with mammalian erythrocyte α- and β-spectrin, respectively, although the crossreactivity is relatively weak compared to that with the corresponding antigens in nonerythroid cells (11, 18).

Although polypeptides antigenically related to erythrocyte β-spectrin were not detected in lymphocytes, the polypeptide that crossreacts with erythrocyte α-spectrin antiserum is specifically associated, as a doublet, with a 235,000-dalton polypeptide. This doublet appears to be similar in polypeptide composition to that immunoprecipitated from adult mouse brain, which has been termed "fodrin" (13). In this respect, it is interesting to note that a preliminary immunofluorescence study has recently shown that antibodies raised against both components of brain fodrin crossreact with a component of the lymphocyte plasma membrane, although the crossreacting polypeptides were not analyzed (25). Several studies have shown that fodrin has many biochemical properties in common with erythrocyte spectrin (15–17). It forms tetramers composed of equimolar amounts of the 240,000- and 235,000-dalton polypeptides, binds to actin filaments, and is found in association with the plasma membrane (15–17). However, the two components of brain fodrin exhibit peptide maps that are different from each other as well as from mammalian erythrocyte α- and β-spectrin (16, 18). Although it remains to be shown whether the polypeptides expressed in brain and lymphocytes are the same gene products, the results of this study indicate that a polypeptide similar to the lower component of brain fodrin is also expressed in association with an α-spectrin-related polypeptide in lymphocytes.

The fact that the fodrin polypeptide doublet in different cell types all have a 240,000-dalton polypeptide related to erythrocyte α-spectrin as a common constituent and that the doublet, at least in nervous tissue, forms a complex biochemically similar to the erythrocyte spectrin tetramer indicates that the 235,000-dalton polypeptide component is in fact a member of the spectrin family of the polypeptides and as such may be referred to as γ-spectrin. Thus, although several polypeptides in nonerythroid cells have biochemical properties similar to those of mammalian erythrocyte spectrin, they appear to be different gene products. This suggests that the genes coding for these polypeptides comprise a group of multigene families (see also ref. 17) whose expression is tailored to the functional requirements of the plasma membrane in different cells (19).

This hypothesis is supported by comparison of the composition of the polypeptides serologically related to erythrocyte spectrin that are expressed in lymphocytes and in adult cardiac muscle cells (18). In the lymphocyte, the cytoskeleton is thought to play a dynamic role in the mobility of the transmembrane protein–cell surface receptor complex (see below), whereas in cardiac muscle cells the cytoskeleton has a more static, structural role linking the contractile apparatus to the sarcolemma. Both cell types express a common polypeptide antigenically related to erythrocyte α-spectrin (this study and ref. 11). However, whereas adult cardiac muscle cells express polypeptides antigenically related to erythrocyte β-spectrin, lymphocytes express a 235,000-dalton polypeptide (γ-spectrin) that is antigenically distinct from erythrocyte β-spectrin. Thus, it appears that the main difference in the combination of spectrin-related polypeptides expressed in different cells is in the polypeptides that are associated with the α-spectrin-related molecule. This is particularly interesting because, in the erythrocyte, β-spectrin associates with the transmembrane anion transporter through ankyrin (for a review, see ref. 10). Whether γ-spectrin can also associate with a polypeptide in lymphocytes that is analogous to

ankyrin remains to be established.

What is the function of the spectrin complex in lymphocytes? Our results show that the polypeptide antigenically related to erythrocyte α-spectrin is distributed at the cytoplasmic surface of the lymphocyte plasma membrane; cell fractionation studies (unpublished data) also show that this polypeptide (and the 235,000-dalton polypeptide) is recovered in the membrane fraction. Upon challenge by cell surface-directed ligands such as ConA or Ig, the redistribution of this polypeptide is coincidental with that of the cell-surface receptors: initially in patches and then a cap. That the redistribution of this polypeptide doublet during the capping process is the same irrespective of the ligand used suggests that they may be associated with the plasma membrane–receptor complex in general and not with just one type of receptor. This is supported by the nonrestricted distribution of the α-spectrin-related polypeptide in lymphocytes of both B- and T-cell lineage and in the null and mastocytoma cell lines as well. The association of polypeptides related to erythrocyte spectrin with the lymphocyte plasma membrane and cell-surface receptors is particularly interesting in view of its well-documented interaction with actin in erythrocytes (for a review, see ref. 10). Actin filaments are believed to be involved in the redistribution of patches of cell-surface receptors into a single cap, particularly because cytochalasin B is known to inhibit capping (26) and actin has been shown to be indirectly linked to cell-surface receptors (6, 7). This has led to the hypothesis that there exists a ubiquitous protein X which binds to the actin microfilament network and associates with the cell-surface receptors via transmembrane proteins (27–29). The results of this study, together with the well-documented characteristics of erythrocyte spectrin and the emerging evidence that erythroid and nonerythroid spectrin analogues have similar functional properties (see above) indicate that the role of these spectrin-like polypeptides in the lymphocyte is similar to that proposed for protein X. Klausner *et al.* (29) have extended the original hypothesis to suggest that protein X also binds Ca^{2+}, either directly or indirectly. In this respect, it is interesting to note that one of the characteristic properties of erythrocyte spectrin and spectrin-related polypeptides in nonerythroid cells is their ability to interact with calmodulin (14–16, 30). Furthermore, calmodulin has also been localized at the plasma membrane beneath ligand–cell surface receptor complexes (31). Because calmodulin has been implicated in the mediation of various cellular activities that involve transient fluxes of Ca^{2+} as signals (for a review, see ref. 32), it may also be involved in changing the Ca^{2+} concentration locally in the region of the subcortical actin microfilament network.

The involvement of polypeptides related to erythrocyte spectrin in cell-surface receptor capping in lymphocytes indicates a functional role for these molecules in nonerythroid cells. Further studies of the interaction of integral proteins of the plasma membrane with these molecules and the subcortical actin filament network in lymphocytes will help toward an understanding of the long-standing problem of how the cytoskeleton interacts with the plasma membrane in nonerythroid cells.

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