Spectrin Immunofluorescence Distinguishes a Population of Naturally Capped Lymphocytes in Situ

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ABSTRACT Immunofluorescence analysis of mammalian lymphocytes using antiserum directed against chicken erythrocyte α-spectrin revealed a lymphocyte population in which spectrin antigen was arranged in the form of a discrete cap (hereafter referred to as capped lymphocytes). This subset could be easily distinguished from other lymphocytes in which the spectrin antigen was diffusely distributed near the plasma membrane (noncapped lymphocytes). The subset of capped lymphocytes could be visualized in situ and in isolated cells in the absence of added ligand. Using frozen sections of lymphoid organs that were fixed in formaldehyde prior to the immunofluorescence procedure, capped lymphocytes were found in characteristic locations depending on the tissue examined. In the thymus, the major population of medullary lymphocytes were capped whereas cortical lymphocytes were mostly noncapped. In Peyer's patches, capped lymphocytes were interspersed with noncapped lymphocytes throughout the tissue. In the spleen, capped lymphocytes were concentrated in the periarterial lymphoid sheath of the white pulp and in lymph nodes they were found predominantly in the paracortical and cortical regions. Capped lymphocytes were not visible in the thymus until just before birth and did not appear in the spleen until 3 d after birth. When lymphocytes were isolated from lymphoid organs, fixed in formaldehyde and prepared for immunofluorescence, capped and noncapped lymphocytes were still identifiable and present in the same relative proportions as seen in situ. Results identical to those described above are obtained using antisera directed against guinea pig fodrin. Natural capping of proteins previously shown to co-migrate with a variety of cell surface macromolecules after cross-linking may be a new means of identifying various stages of lymphocyte activation or differentiation.

Spectrin has been implicated in the control of mobility of cell surface glycoproteins in the erythrocyte through a linkage with integral membrane proteins (1, 2, 5, 26, 27). Renewed interest in this function of spectrin has occurred since it has been found that there are nonerythroid forms of this protein (see reviews in references 12, 14, and 18), including studies showing the protein fodrin in lymphocytes and its concurrent redistribution with capping of macromolecules on the surface of T and B lymphocytes (23, 25). Fodrin has been recently shown to be one of the forms of spectrin in non-erythroid cells (3, 10, 15–17) and using antiserum directed against chicken erythrocyte α-spectrin, Nelson et al. (28) found a co-redistribution of spectrin with surface receptors of lymphocytes. Using freshly isolated splenic and thymic lymphocytes, we have observed that α-spectrin and fodrin antigens occur in a capped configuration in particular subsets of lymphocytes prior to the addition of ligand and show here that these naturally capped cells are morphologically identifiable in situ.

MATERIALS AND METHODS

All mice used in these experiments were kept in the animal colonies at Roswell Park Memorial Institute. Data depicted in this study was obtained from Balb/c female mice although similar results were obtained using Balb/c male mice, C57Bl/6 and DBA/2 mice, and various other mammals (hamster, gerbil, and cat).

For single cell suspensions, lymphoid organs were minced into small fragments with scissors and the fragments placed into phosphate buffered-saline (137 mM NaCl, 3 mM KCl, 2 mM KH_2PO_4, and 8 mM Na_2HPO_4, pH 7.4). After 1 min of gentle resuspension of the fragments with a pasteur pipette, the large clumps of cells and connective tissue were allowed to settle out by gravity and the suspension of single cells was washed two times. Cells in PBS were then allowed to settle for 5 min onto coverslips that had been treated with Alcian blue, which promotes the adherence of cells (30). Coverslips with adherent cells were placed into 2% formaldehyde in PBS for 10 min and then were washed two times before incubation with antisera or Triton-containing buffers.

For imprint preparations, spleens and thymuses were removed and placed on a clean glass surface. A new razor blade was used to cut each organ in half; the exposed surface of cells was affixed to a clean, nonalcian blue-treated coverslip by gently pressing the piece of tissue onto the coverslip, cut-side down, and then quickly pulling it away. Approximately five imprints per cut were

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made on a given coverslip. The coverslip was then placed into formaldehyde fixative for 10 min and prepared for immunofluorescence. The time from the death of the animal to the insertion of the coverslips into fixative never exceeded 1 min while the duration of time between cutting the organs in half and affixing them onto the coverslip was only a few seconds.

For preparation of frozen sections, various lymphoid organs were rapidly removed from mice that had been killed by cervical dislocation (or decapitation in the case of embryos or mice <10 d of age.) Organs were placed into O.C.T. compound (Tissue-Tek, Miles Laboratories, Naperville, IL) and frozen at −80°C. Sections 6–8 μm thick were made using an IEC cryostat set at −20°C. Each section was melted onto a glass coverslip which was then immediately placed into formaldehyde fixative. After 10 min, coverslips were rinsed in several changes of PBS before incubation with antisera or Triton-containing buffers.

Immunofluorescence procedure using anti-chicken erythrocyte α-spectrin antiserum was performed as described previously (29). Antifodrin antiserum (24) was the generous gift of Dr. Mark Willard and was used at a dilution of 1 to 50. The same immunofluorescence procedure as used for spectrin was used for fodrin. Cells were photographed using Kodak Tri-X film on a Zeiss Photomicroscope II equipped with an automatic camera. Zeiss fluoroscein filter set #17 was used for observation and photography. Conditions for photography and printing were optimized for portrayal of the cells with the caps of spectrin antigen. However, in some cases, (particularly the higher magnification photographs) the staining intensity of the caps was so bright that printing conditions led to the overdevelopment of other regions, resulting in an artifactually dim representation of the noncapped cells (e.g., Fig. 2*f*).

SDS PAGE was based on the discontinuous Tris-glycine system of Laemmli (22) as modified and described previously (21). Isolated splenic cells were placed into 0.17 M ammonium chloride to lyse red blood cells, washed two times, and the final pellet of approximately 3×10^6 cells was resuspended in 1% SDS-sample buffer. The sample was boiled for 1 min before loading it onto a gel. Preparation of mouse erythrocyte ghosts and chicken erythrocyte membranes was as described previously (20, 29). Immunoautoradiography (9) was performed as described previously (19, 29), except that only 1 μCi of radioiodinated protein A (Amersham Corp., Arlington Heights, IL) was used per gel. Exposure time for Fig. 4 was 2 d with a Dupont Cronex intensifying screen. Coomassie blue-stained gels were photographed prior to drying and exposure to X-ray film.

Several control experiments were run to test the specificity of the immunofluorescent staining of spectrin. Using preimmune rabbit serum followed by fluoroscein-conjugated goat anti-rabbit antiserum, or fluorescein-conjugated goat anti-rabbit antiserum alone, negligible fluorescence was obtained. As previously described (29), if the antiserum is adsorbed with α-spectrin antigen, specific fluorescence is eliminated. Finally, if intact lymphocytes were not extracted with Triton X-100 prior to the addition of antisera, only background levels of fluorescence are seen indicating that the antibody must enter the cell to bind antigen. These results indicate that the fluorescence depicted in this study is specific for α-spectrin antigen and is not due to nonspecific binding of antiserum to lymphocytes.

RESULTS

When freshly-isolated lymphocytes from 1 mo-old mice were allowed to adhere to coverslips, formaldehyde-fixed, and prepared for immunofluorescence using α-spectrin antiserum, we consistently observed that the antigen was in a capped configuration in a large number of cells. Fig. 1, *a* and *b* depict lymphocytes isolated from spleen and thymus, respectively. Among splenic lymphocytes, ~35% of the lymphocytes were capped whereas in the thymus ~45% were capped. Other lymphocytes in which the antigen was diffusely distributed (noncapped) as well as occasional cells with several smaller aggregates of antigen (patched) could be observed. The large number of capped lymphocytes that we observed was an unexpected finding since no attempt was made to cross-link any surface macromolecule prior to fixing the cell with formaldehyde. To minimize the possibility that spectrin antigen was being induced to cap during the adherence of cells to coverslips (usually an interval of ~5 min), touch imprint preparations were used, since the time of removal of cells from the lymphoid organ to their adherence and fixation on coverslips was negligible. As shown in Fig. 1, *c* and *d*, which are touch imprints of spleen and thymus, numerous capped lymphocytes could be observed. Therefore, it appeared as though lymphocytes with a pre-existing cap of spectrin antigen were being isolated from lymphoid organs. To further examine this phenomenon, frozen sections of mouse lymphoid organs were examined by immunofluorescence to determine if capped cells could be visualized in situ.

In frozen sections of spleen, capped cells were found in colonies of various sizes that occurred in the white pulp (Fig. 2, *a–c*). The central artery of the white pulp could also be visualized within most colonies (Fig. 2, *b* and *c*) indicating that histologically, capped lymphocytes comprised part of the periarterial lymphoid sheath. Although an occasional capped lymphocyte could be seen in other parts of the spleen (i.e., the red pulp) usually these regions contain lymphocytes that had a noncapped configuration of spectrin antigen. Fig. 2*b* shows the adjoining of capped and noncapped regions of the spleen, whereas Fig. 2, *a* and *c* indicate the anatomical discreteness of the capped lymphocyte colonies seen in transverse section. We have previously shown that this antiserum does not cross-react with mouse erythrocyte α-spectrin (29; see also below); therefore, regions of the red pulp containing aggregates of erythrocytes did not stain by immunofluorescence and appear dim in these figures. The thymus also exhibited a disparate localization of capped and noncapped lymphocytes with the former restricted to the medullary region whereas the latter (as well as cells with a patched configuration of antigen) occupied the cortex of this organ (Fig. 2, *d–f*). In peri-aurical, axillary, and intestinal lymph nodes of adult mice, (Fig. 3*a*; data shown for axial lymph node), capped lymphocytes were found interspersed throughout the cortical and paracortical regions with other lymphocytes that were noncapped. When lymphoid organs from 15 and 18 d-old mouse embryos were examined, capped cells were not seen although noncapped cells were evident. By day 19, capped lymphocytes appeared in the medulla of the thymus but were not seen in the spleen until 2–3 d after birth (not shown).

We frequently observed that lymph nodes (as well as Peyer's patches) were highly variable within the same animal with respect to the percentage of capped lymphocytes. The appearance of the thymus and spleen however was relatively constant among animals of the same age with respect to the number and distribution of capped cells. But, age-dependent differences exist between spleen and thymus since the number of capped cells seen within the medulla of the thymus appeared to decrease with age whereas the number of capped lymphocytes in the spleen remained relatively unaffected by age (not shown). These data may reflect differences in the physiology of lymphocytes in various organs of the immune system.

Using antiserum directed against fodrin, a spectrin-like protein found in neuronal tissues, (24), we obtained identical morphological results on frozen sections and isolated cells to that described above using avian α-spectrin antiserum. Fig. 3*b* is a frozen section of a Peyer's patch found in the ileum of the small intestine of a 1 mo-old mouse stained with antifodrin, in which numerous capped lymphocytes interspersed among noncapped lymphocytes could be seen.

In addition to control experiments described in Materials and Methods, the specificity of both α-spectrin and fodrin antisera used in this study was further analyzed by immunoautoradiography (Fig. 4). As expected from previous reports (25, 28), both antisera reacted with the same 240-kd lympho-

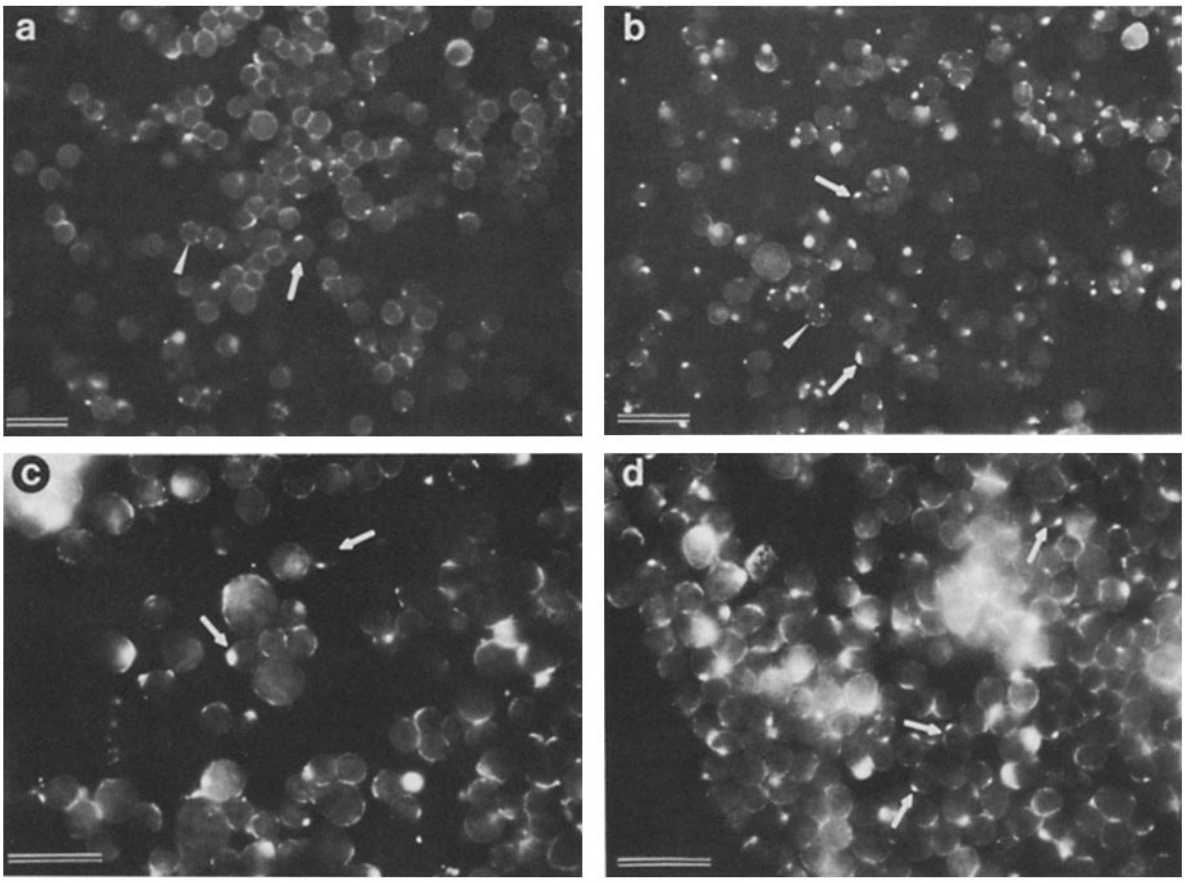

FIGURE 1 Immunofluorescence of mouse lymphocytes with anti-α-spectrin. Isolated lymphocytes from (*a*) spleen and (*b*) thymus of a one mo-old mouse stained with anti-α-spectrin. Numerous lymphocytes in which the spectrin antigen is localized in the form of a cap can be observed (arrows) interspersed among other lymphocytes in which the antigen is more uniformly distributed. Occasional cells with smaller aggregates of antigen (patched cells) can also be observed (arrowheads). Imprint preparations of (*c*) spleen and (*d*) thymus also contain lymphocytes in which spectrin antigen is aggregated in one pole of the cell (arrows). Bars, 20 μm.

cyte protein that co-migrates with chicken and mouse erythrocyte α-spectrin and both reacted with chicken erythrocyte α-spectrin, although the cross-reactivity of antifodrin with the chicken protein was considerably less than that of the anti-spectrin. However, at the exposure duration used here, neither antiserum showed cross-reactivity with mouse erythrocyte α-spectrin indicating that the reaction obtained in the lymphocyte lane and by immunofluorescence was not due to contaminating erythrocytes. Although some biochemical differences have been reported between the antigens recognized by antifodrin and anti-α-spectrin (3, 10, 16, 17), the immunoautoradiographic results indicate that in lymphocytes, the two antisera were cross-reacting with the same protein complex. In addition, double immunofluorescence experiments using both antisera on the same capped and noncapped lymphocytes show that the two antisera have identical distributions (not shown).

DISCUSSION

The phenomenon of capping (31) is a segregation of specific plasma membrane-associated macromolecules to one discrete region of the membrane. Usually associated with this energy-requiring activity are (*a*) cross-linking by ligands, which appears to initiate capping and (*b*) coordinated movement of both surface macromolecules and several submembraneous cytoskeletal proteins (see reviews in references 6, 13, 14). This latter observation has led to the general assumption that the forces required for capping are generated by these membrane-associated cytoskeletal proteins; yet, the mechanism of linkage between the cytoskeleton and cell surface groups in the lymphocyte has never been established. Alternative hypotheses regarding cap formation have also been proposed (4, 8). Although capping has provided a useful model system for determining interrelationships between various membrane proteins, the physiological significance of redistribution of these groups is unknown.

In this report, we have presented our morphological investigations concerning lymphocytes that have a naturally-occurring capped configuration of α-spectrin, a protein previously reported to co-cap with various cell surface macromolecules on T and B cells only after the addition of cross-linking ligands (28). However, all of the experiments described in that study were done on various lymphocyte cell lines that apparently do not exhibit natural capping of spectrin. Therefore, a physiologic factor may be required to maintain lymphocyte spectrin in a capped configuration, or capped lymphocytes may only be seen in a specific phase of differentiation. Our results also differ from previous reports of "spontaneous" capping of various cytoskeletal proteins, such as myosin, since spontaneous capping is dependent upon (and subsequent to)

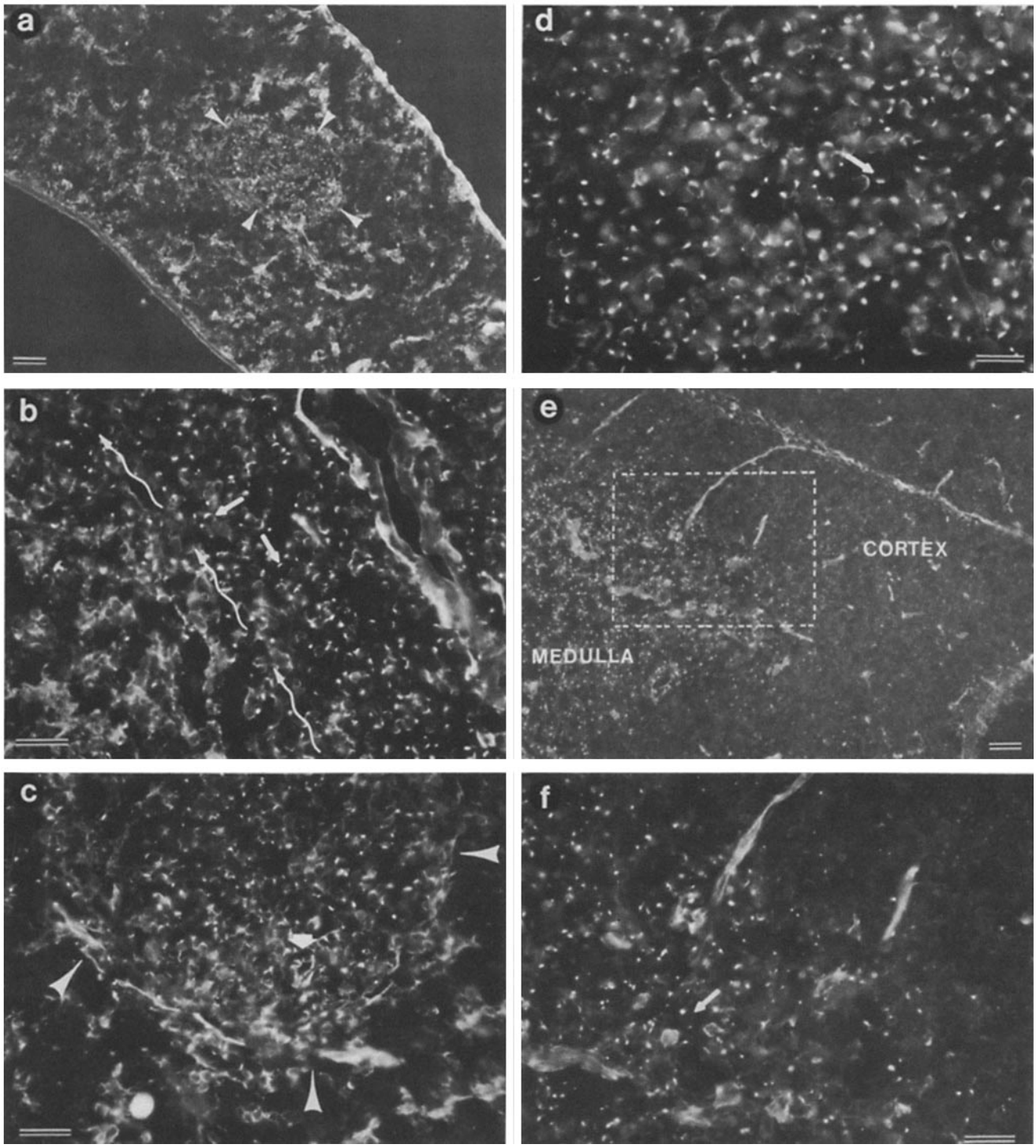

FIGURE 2 Anti-α-spectrin immunofluorescence of frozen sections of mouse spleen and thymus. (a) Low magnification view of a section of 2 wk-old mouse spleen with a colony of capped lymphocytes (colony indicated by arrowheads). Capped lymphocytes can be better visualized in *b*, which is a higher magnification view of a colony of capped cells (arrows) in the white pulp of the spleen of a 1 mo-old mouse. The border between capped lymphocytes and other noncapped lymphocytes is approximately delineated by the curved arrows. The artery of the white pulp is shown in longitudinal section in the upper right portion of the figure. A similar colony (indicated by arrowheads) cut in transverse section is shown in *c*; artery is indicated by short arrow. (*d*) Large expanse of capped lymphocytes (arrow) seen in the thymus of a 2 wk-old mouse. In the thymus, capped lymphocytes are found primarily in the medulla as shown in a low magnification view in e. The border area between cortex and medulla delineated by the rectangle in e is shown in higher magnification in *f*. Capped cell is indicated by arrow. Bars, 20 μm.

the onset of lymphocyte motility in vitro (7) and is not seen in rounded (i.e., nonmotile) freshly isolated cells as are shown in Fig. 1 of this paper. Using another antiserum that reacts with the same 240-kd protein as does anti-α-spectrin in lymphocytes, i.e., anti-guinea pig fodrin, we have also observed the occurrence of capped cells in situ. This protein has also been previously reported to co-cap with either antibody cross-linked surface immunoglobulin or histocompatibility antigens

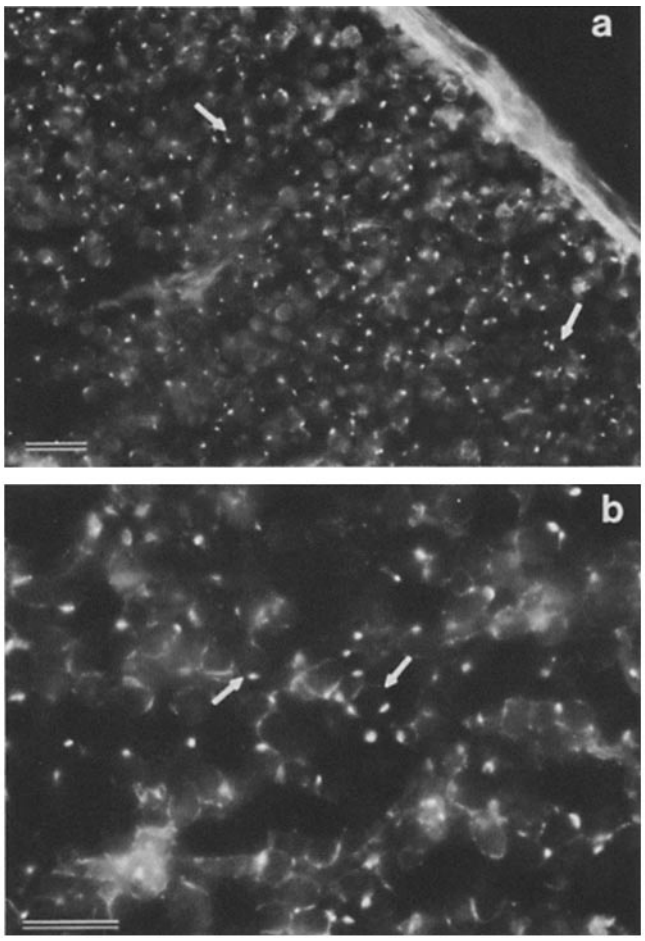

FIGURE 3 Immunofluorescence of frozen sections of mouse lymphoid organs stained with anti-α-spectrin and anti-fodrin. (*a*) Section of axial lymph node (cortical and paracortical regions) from a 1-mo-old mouse stained with anti-α-spectrin; numerous capped lymphocytes (arrows) can be seen interspersed among other noncapped lymphocytes. (*b*) Higher magnification view of a section of Peyer's patch tissue from 1-mo-old mouse ileum stained with antifodrin. Capped cells (arrows) are seen among other noncapped cells. Bars, 20 μm.

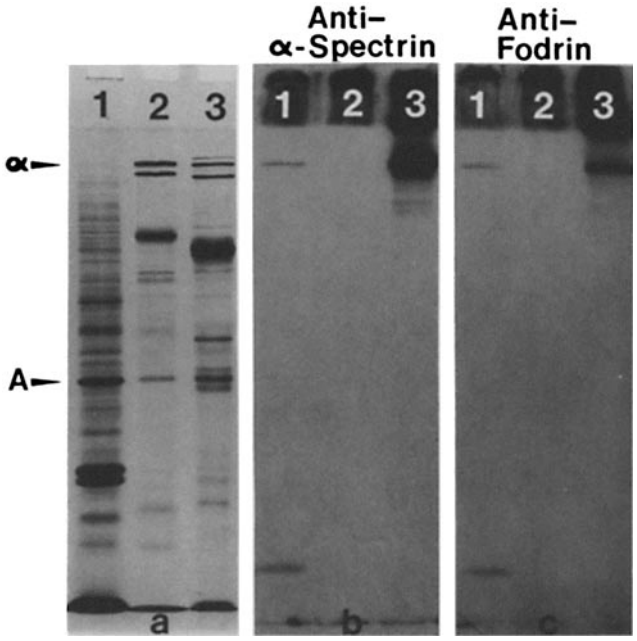

FIGURE 4 Immunoautoradiography of mouse lymphocytes and erythrocytes and chicken erythrocytes using anti-α-spectrin and anti-fodrin. (*a*) Coomassie-blue-stained gel; (*b*) corresponding autoradiogram prepared by incubating the gel sequentially with anti-α-spectrin and iodinated Protein A; (*c*) Autoradiogram prepared by reacting a gel similar to that shown in *a* with antifodrin followed by iodinated protein A. Lane *1*, mouse lymphocytes isolated from spleen; lane *2*, erythrocyte ghosts prepared from the blood of the same mouse; lane *3*, chicken erythrocyte membranes. α indicates position of α-spectrin in both erythrocyte lanes; the position of actin is indicated by *A*. The identity of the low molecular weight band that appears on the autoradiograms of the lymphocyte lane using both antisera is unknown; however, it appears to be a nonspecific reaction due to binding of some portion of the protein A molecule since it appears on autoradiograms prepared by reacting gels of lymphocytes with iodinated protein A alone (our unpublished observations). This band has also been observed previously with other antisera on gels of buffy coat cells (20).

of isolated murine splenic lymphocytes (25). It is apparent therefore, that there may be two populations of lymphocytes with respect to the distribution of spectrin or fodrin: one in which the spectrin/fodrin antigen is uniformly distributed (and therefore available for induced capping with ligand as previously described) and a second subset in which the spectrin/fodrin is already capped prior to the addition of ligand. Both of these lymphocyte subsets (capped and non-capped) can be visualized in Fig. 1 using isolated cells and in various locations in situ as shown in Figs. 2 and 3 of this paper. Our observation of naturally capped cells does not preclude the possibility that these lymphocytes are responding to cross-linking ligands in their physiological environment, which stimulates capping of membrane-associated proteins. In this case, perhaps certain surface macromolecules will be found in a capped configuration, which is coincident to that of spectrin.

The differentiation state of lymphocytes may be important with regard to whether or not the cells are capped since in the thymus, (see Fig. 2, *e* and *f*), capped lymphocytes were restricted to the medulla where lymphocytes differ developmentally in several respects from cortical lymphocytes (11). During erythropoiesis, it has been shown that membrane regions of increased spectrin concentration are associated with decreased deformability and endocytic activity (32, 33). Perhaps similar modifications of membrane deformability due to spectrin capping will be observed during lymphocyte maturation. A possible pre-existing polarity with respect to membrane deformability could fulfill the constraints of the model of capping proposed by Berlin and Oliver (4) in which interaction of a particular receptor or ligand-receptor complex with a region of membrane that is altered in its association with underlying cytoskeletal elements could either arrest passage of the complex (resulting in formation of a cap) or facilitate its passage to another region of the membrane. The close association of spectrin with integral membrane proteins, and its function in modulation of membrane integrity and deformability as demonstrated in the erythrocyte, makes this protein a good candidate for local reconstruction of membrane properties in the lymphocyte. A pre-existing polarity of membrane-associated cytoskeletal proteins (which could significantly alter the deformability of certain regions of the membrane) could also provide experimental support for the "membrane-flow" hypothesis of cap formation proposed by Bretcher (8).

Analysis of spectrin capping (or other as yet undescribed naturally capped proteins) may help us to recognize the physiological significance of in situ capping with regard to lymphocyte activation and/or differentiation. This work is currently being extended to determine whether or not any exogenously or endogenously derived macromolecules on the cell surface exist in a capped configuration that is coincident to that of spectrin and to characterize the surface phenotype of the subset of capped lymphocytes in various lymphoid tissues.

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REFERENCES

1. Bennett, V. 1982. The molecular basis for membrane-cytoskeletal association in human erythrocytes. *J. Cell Biochem.* 18:49–65.
2. Bennett, V., and P. J. Stenbuck. 1979. The membrane attachment protein for spectrin is associated with band 3 in human erythrocyte membranes. *Nature (Lond.).* 280:468–473.
3. Bennett, V., J. Davis, and W. E. Fowler. 1982. Brain spectrin, a membrane-associated protein related in structure and function to erythrocyte spectrin. *Nature (Lond.).* 299:126–131.
4. Berlin, R. D., and J. M. Oliver. 1982. The movement of bound ligands over cell surfaces. *J. Theor. Biol.* 99:69–80.
5. Branton, D., C. M. Cohen, and J. Tyler. 1981. Interaction of cytoskeletal proteins on the human erythrocyte membrane. *Cell.* 24:24–32.
6. Braun, J. 1983. Receptor-cytoskeleton interactions in the lymphocyte. *Immunol. Today.* 4:21–25.
7. Braun, J., K. Fujiwara, T. D. Pollard, and E. Unanue. 1978. Two distinct mechanisms for redistribution of lymphocyte surface macromolecules. I. Relationship to cytoplasmic myosin. *J. Cell Biol.* 79:409–418.
8. Bretcher, M. S. 1976. Directed lipid flow in cell membranes. *Nature (Lond.).* 260:21–23.
9. Burridge, K. 1978. Direct identification of specific glycoproteins and antigens in sodium dodecyl sulfate gels. *Methods Enzymol.* 50:54–64.
10. Burridge, K., T. Kelley, and P. Mangeat. 1982. Nonerythroid spectrins: actin-membrane attachment proteins occurring in many cell types. *J. Cell Biol.* 95:478–486.
11. Cantor, H., and I. Weissman. 1976. Development and function of subpopulations of thymocytes and T lymphocytes. *Prog. Allergy.* 20:1–64.
12. Cohen, C. M. 1983. The molecular organization of the red cell membrane cytoskeleton. *Semin. Hematol.* 20:141–158.
13. Edelman, G. M. 1976. Surface modulation in cell recognition and cell growth. *Science (Wash. DC).* 192:218–226.
14. Geiger, B. 1983. Membrane-cytoskeleton interaction. *Biochim. Biophys. Acta.* 737:305–341.
15. Glenney, J. R., P. Glenney, M. Osborn, and K. Weber. 1982. An F-actin and calmodulin-binding protein from isolated brush borders has a morphology related to spectrin. *Cell.* 28:843–854.
16. Glenney, J. R., P. Glenney, and K. Weber. 1982. F-actin binding and crosslinking properties of porcine brain fodrin, a spectrin-related molecule. *J. Biol. Chem.* 257:9781–9787.
17. Glenney, J. R., P. Glenney, and K. Weber. 1982. Erythroid spectrin, brain fodrin and intestinal brush border proteins (TW-260/240) are related molecules containing a common calmodulin-binding subunit bound to a varient cell type-specific subunit. *Proc. Natl. Acad. Sci. USA.* 79:4002–4005.
18. Goodman, S. R., and K. Shiffer. 1983. The spectrin membrane skeleton of normal and abnormal human erythrocytes: a review. *Am. J. Physiol.* 244 (*Cell Physiol.* 13): C121–C141.
19. Granger, B. L., and E. Lazarides. 1980. Synemin: a new high molecular weight protein associated with desmin and vimentin filaments in muscle. *Cell.* 22:727–738.
20. Granger, B. L., E. A. Repasky, and E. Lazarides. 1982. Synemin and vimentin are components of intermediate filaments in avian erythrocytes. *J. Cell Biol.* 92:299–312.
21. Hubbard, B. D., and E. Lazarides. 1979. Co-purification of actin and desmin from chicken smooth muscle and their co-polymerization in vitro to intermediate filaments. *J. Cell Biol.* 80:166–182.
22. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.
23. Levine J., J. H. P. Skene, and M. Willard. 1981. GAPs and fodrin: novel axonally transported proteins. *Trends Neurosci.* 4:273–277.
24. Levine, J., and M. Willard. 1981. Fodrin: axonally transported polypeptides associated with the internal periphery of many cells. *J. Cell Biol.* 90:631–643.
25. Levine, J., and M. Willard. 1983. Redistribution of fodrin (a component of the cortical cytoplasm) accompanying capping of cell surface molecules. *Proc. Natl. Acad. Sci. USA.* 80:191–195.
26. Marchesi, V. T. 1983. The red cell membrane cytoskeleton: recent progress. *Blood.* 61:1–11.
27. Nicolson, G. L., and R. G. Painter. 1973. Anionic sites of human erythrocyte membranes. II. Antispectrin-induced transmembrane aggregation of the binding sites for positively charged colloidal particles. *J. Cell Biol.* 59:395–406.
28. Nelson, W. J., C. A. L. S. Calaco, and E. Lazarides. 1983. Involvement of spectrin in cell-surface receptor capping in lymphocytes. *Proc. Natl. Acad. Sci. USA.* 80:1626–1630.
29. Repasky, E. A., B. L. Granger, and E. Lazarides. 1982. Widespread occurrence of avian spectrin in nonerythroid cells. *Cell.* 29:821–833.
30. Sommer, J. R. 1977. To cationize glass. *J. Cell Biol.* 75(2, Pt. 2):745*a*. (Abstr.)
31. Taylor, R. B., P. H. Duffus, M. C. Raff, and S. de Petris. 1971. Redistribution and pinocytosis of lymphocyte surface immunoglobulin molecules induced by anti-immunoglobulin antibody. *Nat. New Biol.* 233:225–229.
32. Tokuyasu, K. T., R. Schekman, and S. J. Singer. 1979. Domains of receptor mobility and endocytosis in the membranes of neonatal human erythrocytes and reticulocytes are deficient in spectrin. *J. Cell Biol.* 80:481–486.
33. Zweig, S. E., K. T. Tokuyasu, and S. J. Singer. 1981. Membrane-associated changes during erythropoiesis. On the mechanism of maturation of reticulocytes to erythrocytes. *J. Supramol. Struct.* 17:163–181.