CIS- AND TRANS- MEMBRANE CONTROL OF CELL SURFACE TOPOGRAPHY

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The topographic distributions of cell surface components can be modified by perturbations from the cell membrane exterior (cis-membrane effect) or by perturbations from the cell interior that are transferred across the membrane (trans-membrane effect). Using the human erythrocyte ghost as a model system, cis-membrane effects on the topography of anionic sites were produced in B^+ ghosts with anti-B sera (but not with anti-A), R. communis agglutinin and concanavalin A (but not with D. biflorus agglutinin). Cis-membrane linkage was monitored by the state of aggregation of membrane-bound colloidal iron hydroxide particles which bind almost exclusively to neuraminidase-sensitive N-acetylneuraminic acid residues on the outer surface. Trans-membrane effects were observed when purified antibodies against an inner surface membrane protein, spectrin, were sequestered inside the ghosts. The sequestered antispectrin bound to spectrin at inner membrane surface and caused aggregation of the anionic sites on the outer membrane surface. The trans-membrane effects of antispectrin required intact γG antibodies (Fab would not substitute) and was time- and concentration-dependent.

INTRODUCTION

The cell surface appears to be an important structure in the organization and social behavior of multicellular organisms (1). The surface is delineated by a fluid plasma membrane composed of complex proteins, lipids, and saccharides arranged in a highly asymmetric structure (2). For example, in the human erythrocyte membrane saccharides (2–5), virus receptor sites (6), etc., are displayed only on the outer extracellular surface while certain protein components such as the protein spectrin (7–9) are displayed only at the inner surface (4, 10). The "display" of specific membrane components on the outer surface of the cell may determine or influence cell-to-cell interactions through intercellular contacts. Membrane component display may be altered by quantitative changes in the expression of a particular component (either by incorporation of new components or structural alterations of existing components), by spatial changes in the distribution of that component, or by changes in neighboring membrane components that affect structure or accessibility.

This report will deal with changes in the spatial display of certain anionic cell membrane groups on the outer surface of the human erythrocyte and how this spatial display can be affected by perturbations occurring at the outer surface (cis-membrane effect) or by perturbations occurring at the inner surface that are translated across the membrane to the outer surface (trans-membrane effect) (2) (see illustration). The particular components that will be monitored for spatial or distributional changes are the N-acetylneuraminic

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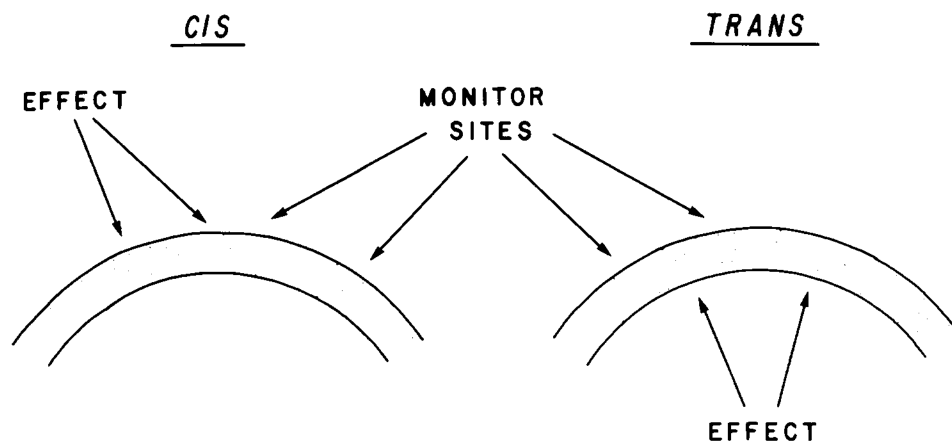

Cis- and trans-membrane effects on the topography of specific sites at the cell surface. The distribution of a specific membrane component is monitored at the outer surface after perturbation of certain sites at the outer surface (cis-effect) or at the inner surface (trans-effect).

acid (NANA) residues present extracellularly (11) at the termini of membrane glycoprotein oligosaccharide chains. NANA surface distribution will be monitored by a colloidal iron hydroxide (CIH) labeling technique adapted to label NANA residues on mounted fixed human erythrocyte ghosts for observation by transmission electron microscopy (12, 13). Antibodies recognizing specific surface antigens and lectins recognizing specific saccharide determinants will be used to probe for cis-membrane effects, while antibodies directed against the inner surface component spectrin (4, 7–10) will be used to probe for trans-membrane effects.

METHODS

Methods generally follow previous accounts of this work (11–13). Ghosts were prepared from fresh human blood (B^+) by a modification (12) of the procedures of Dodge et al. (14). The ghosts were suspended overnight in 300 mosmol phosphate buffer before use.

For the experiments on cis-membrane effects, these ghosts were treated with subagglutinating concentrations of human anti-A or human anti-B (Ortho), Ricinus communis agglutinin (15), Dolichos biflorus agglutinin (16), or concanavalin A (17) for 30 min at 37°C. The concentrations of antibodies and lectins varied from concentrations that gave slightly less than half-maximal agglutination at the end of the 37°C incubation to concentrations that gave no detectable agglutination. The ghosts were washed 1–2 times by centrifugation and fixed in 1.5% buffered glutaraldehyde for 30–60 min at room temperature. They were washed again and stored at 0°C for CIH labeling.

For the trans-membrane experiments affinity-purified antispectrin γG antibodies at various concentrations (from 0.05–10 μg/ml per 0.05 ml ghost pellet) were sequestered inside resealed ghosts by hypotonic lysis (11). The ghosts were incubated for 30 min at 37°C, washed, and fixed in 1.5% glutaraldehyde for 30–60 min, as above. Antispectrin Fab fragments were made and purified by the method of Porter (18).

Fixed ghosts were prepared for CIH labeling by first floating them at an air-buffer interface and then mounting them on electron microscope grids coated with thin support

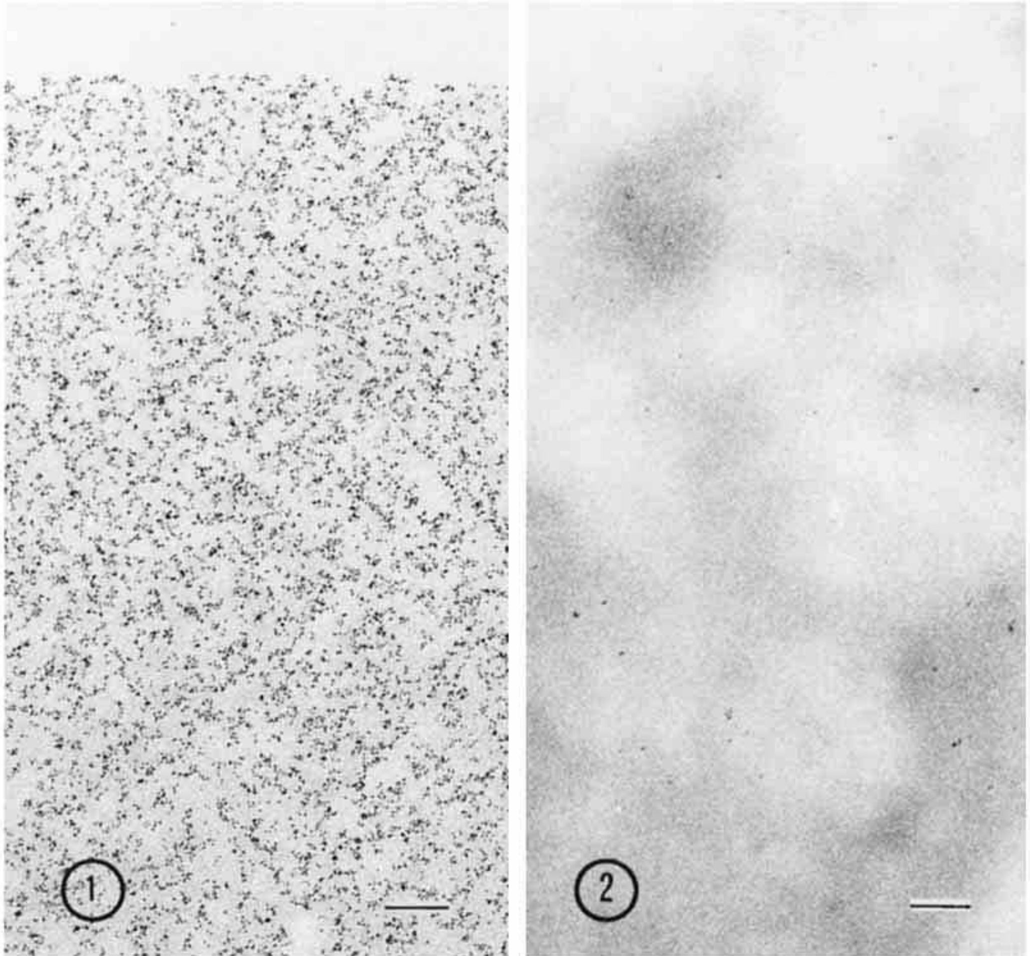

Fig. 1. The surface of a glutaraldehyde-fixed human erythrocyte ghost mounted on a thin support film and labeled with colloidal iron hydroxide. The colloidal iron particles are present in small clusters randomly expressed across the membrane surface. The edge of the membrane is at the top of the figure. Bar equals 0.1 μm. (X 75,600).

Fig. 2. The legend is the same as in Fig. 1 except that the ghosts were treated with neuraminidase prior to fixation and colloidal iron labeling. Bar equals 0.1 μm. (X 75,600)

films (4). After a brief treatment with 5% bovine serum albumin solution, the mounted fixed ghosts were treated for 2 min with a CIH solution (pH 1.8) prepared by a modification (12) of the methods of Gasic et al. (19). After the labeling with CIH, the specimens were washed for 1 min by floating them on 4–5 consecutive drops of 12% acetic acid (pH 2) and two drops of distilled water (11–13). The specimens were observed after drying in a Hitachi Model HU–12.

Specificity of CIH labeling to NANA residues was checked by neuraminidase treatment of human ghosts. Ghosts were treated with V. cholerae neuraminidase (Sigma) (1 U/10^7 ghosts) in 0.01 M Tris · HC1 buffer (pH 6.0) containing 0.01 M $CaC1_2$ for 60 min at 37°C. The neuraminidase-treated ghosts were washed, fixed, and labeled as above.

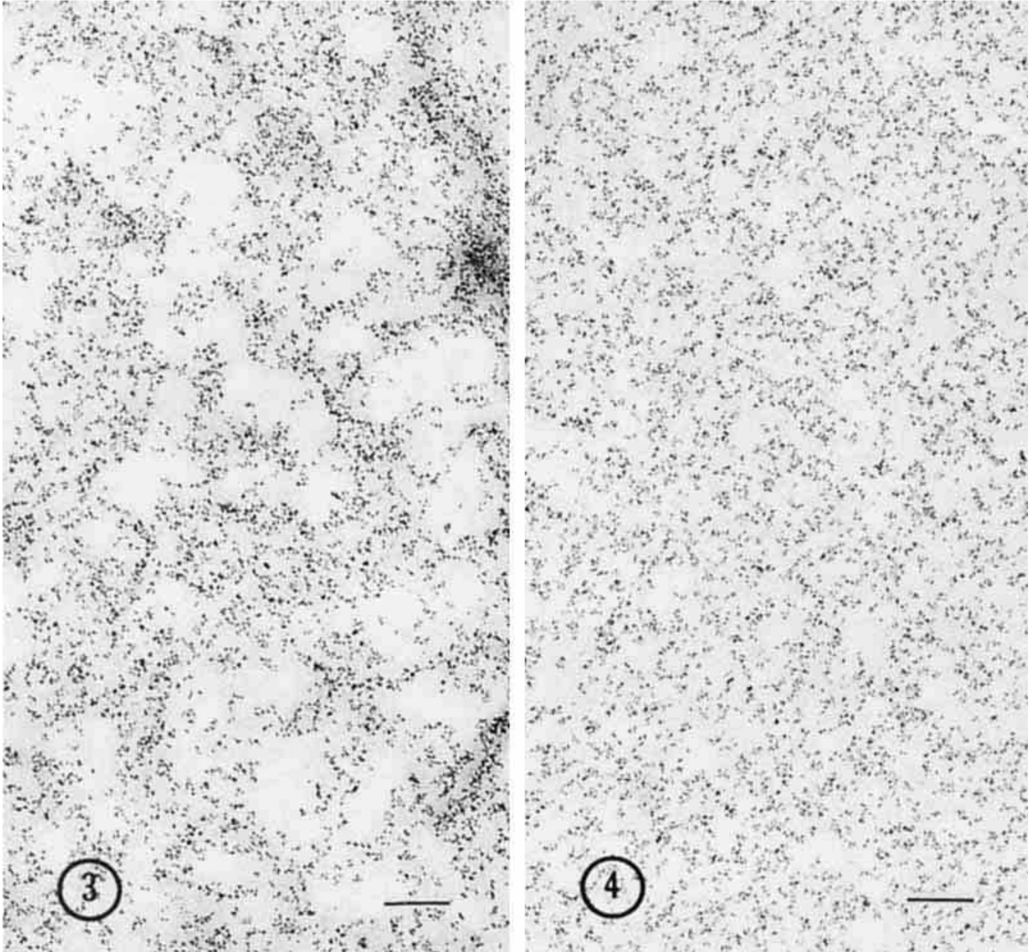

Fig. 3. The legend is the same as in Fig. 1 except that the ghosts (B^+) were treated with a solution of anti-B sera prior to fixation and colloidal iron labeling. The colloidal iron clusters are present in an aggregated state. Bar equals 0.1 μm. (X 75,600)

Fig. 4. The legend is the same as in Fig. 3 except that anti-A sera was used. Bar equals 0.1 μm. (X 75,600)

RESULTS AND DISCUSSION

Human erythrocyte ghosts were treated with various antibodies or lectins, fixed with glutaraldehyde, and mounted and labeled with CIH. As previously found using these techniques (11–13, 21, 22), the CIH particles were distributed in random clusters across the membrane surface of untreated ghosts (Fig. 1); these CIH clusters represent dense zones of anionic residues on the human ghost surface. The anionic residues were overwhelmingly NANA, because the CIH labeling was lowered about 85–90% after neuraminidase treatment was used to cleave off approximately 90% of the surface NANA (Fig. 2) 12, 13, 20).

When human erythrocyte ghosts (B^+) are treated with human anti-B reagents (1–4

HAU) at 37°C for 30 min, washed, fixed, and labeled with CIH, the colloidal iron particles are distributed in a more aggregated topographic distribution (Fig. 3) compared to the same ghosts treated with an identical titer of anti-A antibodies (Fig. 4). Since these reagents act on the side of the membrane where the CIH-binding NANA residues are being monitored, this reaction is an example of a cis-membrane effect on the topographic distribution of CIH sites. Similar results on the cis-membrane aggregation of CIH sites were found when ghosts (B^+) were treated with R. communis agglutinin (specific for *β-D*-galactose-like residues [15] (0.01–0.1 mg/ml) or concanavalin A (specific for *α-D*-mannose-like residues [15] (0.5–2 mg/ml) but not Dolichos biflorus agglutinin (type-A-specific lectin that binds to N-acetyl-*D*-galactosamine-like residues [16] (0.1–5 mg/ml). Since D. biflorus agglutinin is an A-blood-type specific lectin (16), its inability to cause cis-membrane aggregation of CIH sites on B-type cells was expected. The aggregation of CIH sites by R. communis and con A is specific as inclusion of the appropriate saccharide inhibitor (*β*-lactose for R. communis agglutinin; *α* -methyl-*D*-mannoside for con A) during the incubation prevents cis-membrane aggregation of CIH sites.

The fact that certain antibodies and lectins can cause cis-membrane aggregation of CIH binding NANA sites indicates some sort of structural linkage between these sites and the NANA residues. In some of the cases, the linkage is probably covalent. Marchesi et al. (22) have isolated and characterized the major sialoglycoprotein of the human erythrocyte membrane (glycophorin) and have determined that it contains approximately 80% of the erythrocyte NANA residues, the ABO blood group antigens, and some lectin sites. It does not appear to contain concanavalin A sites (V. T. Marchesi, personal communication), so the cis-membrane aggregation of CIH-binding sites by concanavalin A may occur through another glycoprotein molecule(s) that interacts noncovalently with glycophorin in the membrane. The aggregation of surface glycoproteins by antibodies and lectins at 37°C is well known and probably occurs by cross-linking components that are normally mobile in the fluid membrane plane (2, 23–26).

As mentioned above, some type of covalent or noncovalent linkage is necessary for cis-membrane effects. In some systems cis-membrane effects do not occur: surface Ig determinants on certain lymphoid cells can be aggregated without affecting the distribution of con A binding sites (24), and on lymphoid cells carrying multiple histocompatibility alloantigens ($H-2^k$, $H-2^d$), one set of specificities can be aggregated independent of the second set (27).

To study the effect of trans-membrane perturbation on the topography of anionic sites at the ghost outer surface, R. G. Painter (University of California, San Diego) and I used affinity-purified antibodies against an inner-surface protein, spectrin (4, 7–10), and monitored changes in CIH distribution at the outer surface (11). This was done by sequestering antispectrin γG molecules inside ghosts by hemolysis, allowing time to reseal and then incubating for 30 min at 37°C. At low concentrations of antispectrin γG (<0.05 mg/ml) and at high concentrations of antispectrin (>5–10 mg/ml) there was no apparent effect on the topography of CIH particles, but at intermediate concentrations (0.1–5 mg/ml) the CIH sites were aggregated on the membrane outer surface (Fig. 5). When Fab fragments were substituted for γG antibodies, the CIH distribution was similar to the controls (Fig. 6) (11). The antispectrin-induced trans-membrane aggregation of CIH-binding NANA was also found to be temperature- and time-dependent and could be prevented by prior glutaraldehyde fixation. The details of these results will be presented elsewhere (11).

Trans-membrane effects translated to the cell membrane surface may be important for

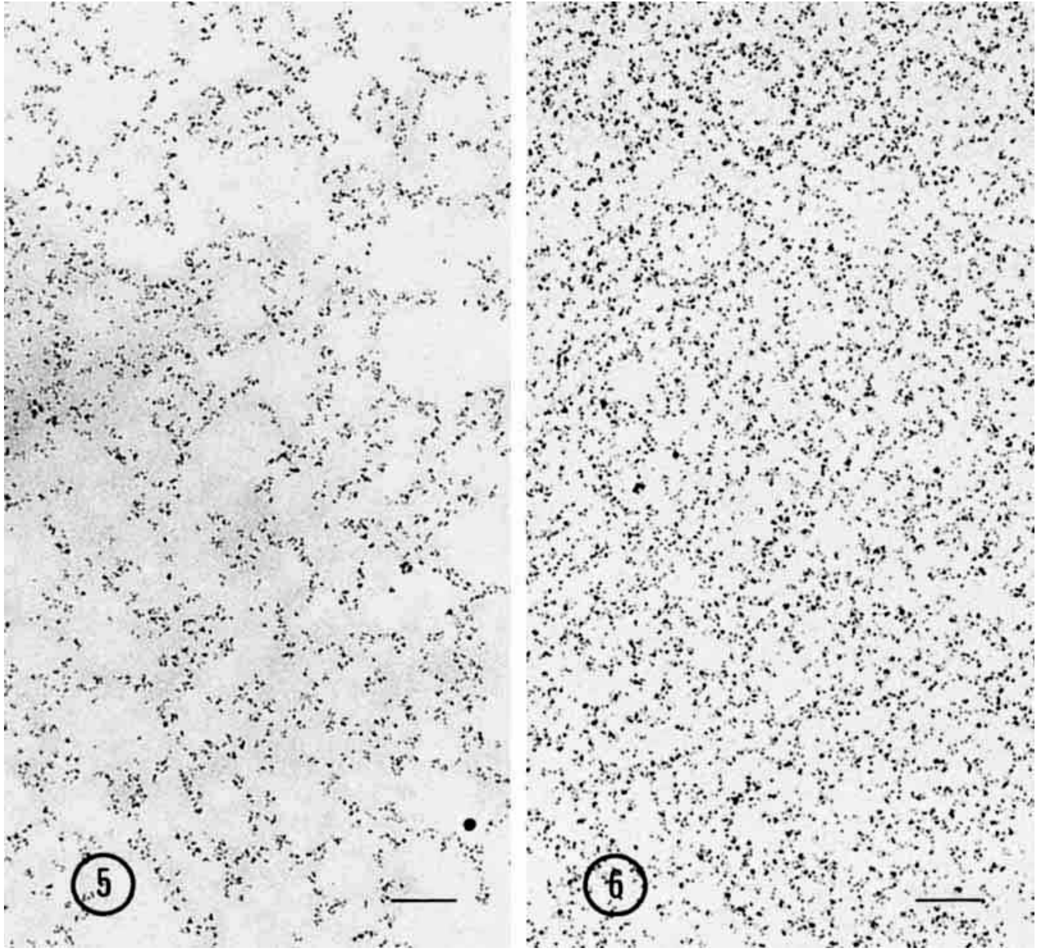

Fig. 5. Antispectrin γG (0.25 mg/ml) was sequestered inside ghosts by hemolysis and the ghosts were incubated for 30 min at 37°C. At that time the ghosts were washed, fixed, and mounted for colloidal iron labeling. The antispectrin induces intense aggregation of the colloidal iron sites. Bar equals 0.1 μm. (X 75,600)

Fig. 6. The legend is the same as in Fig. 5 except that antispectrin Fab fragments were substituted for antispectrin γG molecules. No aggregation of colloidal iron sites occurs. Bar equals 0.1 μm. (X 75,600)

cell motility and for the control of surface function. Microfilament–microtubular structures have been implicated in cell movement (28) and their attachment at the inner membrane surface (29) may be analogous to the results here and elsewhere (11) that proteins inside the cell can exert their influence on integral membrane glycoprotein expression at the cell exterior. Yin et al. (30) have recently shown that the microtubular binding drugs colchicine and vinblastine have dramatic effects on con A-mediated agglutination of transformed-fibroblasts, and Ukena and Berlin (31) have found that these drugs also change the membrane topography of polymorphonuclear lymphocytes during phagocytosis. Trans-membrane linkage may provide the means by which cells can directly or indirectly control their surface topographies.

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