TWO DIMENSIONAL DISTRIBUTION OF CONCANAVALIN-A RECEPTOR MOLECULES ON FIBROBLAST AND LYMPHOCYTE PLASMA MEMBRANES

Paolo M. COMOGLIO* and Renzo GUGLIELMONE
Dept. of Human Anatomy, University of Torino, 10126 Torino, Italy

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1. Introduction

Several models have been proposed for the structural organisation of the plasma membrane. Singer et al. [1,2] recently suggested a fluid as opposed to a crystalline double layer for the phospholipid matrix in which the protein molecules are embedded. This "fluid mosaic" model pictures the cell membrane as similar to a glyco- or lipo-protein solution in a viscous, lipid solvent. Its macromolecules might therefore be expected to move on the outer surface of the cell as result of both intrinsic and extrinsic membrane interactions.

Certain carbohydrate radicals found on the surface membrane are recognised by the plant lectin concanavalin-A (Con-A) [3–5]. This agglutinin binds a group of membrane glycoproteins [6] and under appropriate conditions this may induce their redistribution on lyphocyte plasma membranes [7]. Moreover other lymphocyte surface molecules (i.e. immunoglobulins and Theta antigen [8], H-2 transplantation antigens [9], receptors for antigen–antibody complexes [10]) may undergo similar redistribution when bound by their corresponding antibodies.

It is not known at present if surface movements of macromolecules can be induced on cells other than lymphocytes. This paper reports a comparative study of glycoprotein movements on mouse lymphocyte and fibroblast cell surfaces.

* Mailing address: Paolo Comoglio, Dept. of Human Anatomy and Biology, C.so Massimo d'Azeglio 52, 10126 Torino, Italy

2. Material and methods

Normal Balb/C mice spleen cells were isolated by gentle teasing in ice-cold t8 (Difco) complete tissue culture medium. CCL1 mouse fibroblasts (kindly provided by Dr. G. Forni) were grown in Dulbecco's modified Eagle's medium supplemented with 10% calf serum; for labelling experiments confluent fibroblasts were removed from the flask by scraping with a rubber tipped spatula and were suspended in saline. Experiments on fibroblast coverslip monolayers were also run.

Direct and indirect immunofluorescence (using Fluorescein isothiocyanate (FITC) labelled Con-A, or unlabelled Con-A and fluorescent rabbit anti Con-A antibodies, respectively) gave similar results, though the second was preferred for its much greater sensitivity. All the experiments reported in this paper were performed by indirect labelling. Antisera were raised in rabbits by three injections of twice crystallized Con-A (Miles) in Freund's complete adjuvant. The immunoglobulin fraction (Ig) was isolated by elution of a DEAE-cellulose column with a 0.0175 M phosphate buffer pH 6.3. FITC labelling was carried out with the dialysis technique [12], followed by a second chromatography on DEAE-cellulose as described above. The immunological staining procedure outlined by Taylor et al. [8] was employed. The cells were suspended in 0.15 M NaCl, 0.01 M phosphate buffer pH 7.2 and incubated for 30 min at 4° in presence of excess Con-A. After repeated washings, the cells were incubated for a further 30 min at 4° with various concentrations of the FITC-labelled Ig fraction of anti Con-A antiserum. After further washings cells were resuspended in the culture medium and

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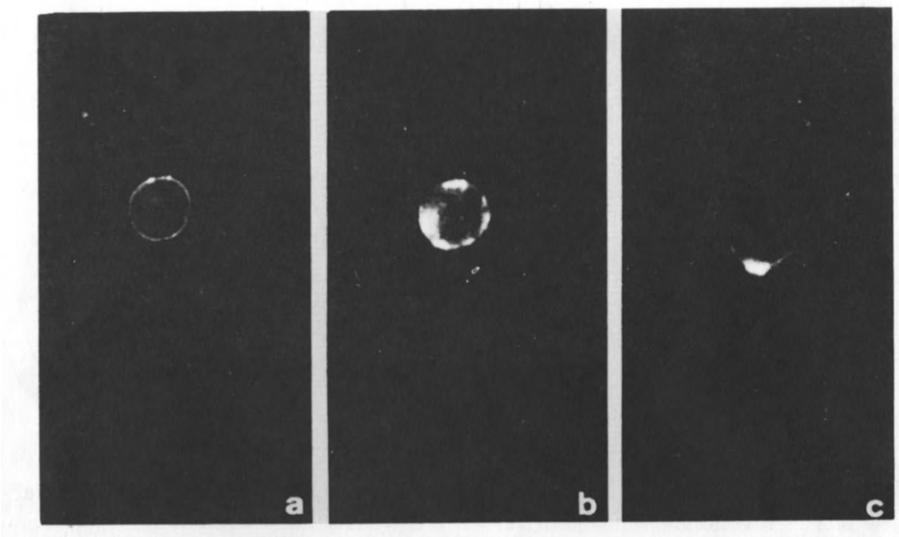

Fig. 1. Immunofluorescence patterns displayed by mouse spleen cells following incubation with Con-A and FITC-labelled rabbit anti Con-A antiserum under different experimental conditions (see text): a) ring b) spots c) single polar cap.

incubated for 15 min to 60 min at 37°. A final concentration of 10^{-3} M sodium azide was then added and the cells were observed under a UV microscope at room temperature. Controls included cells without Con-A in the first incubation and with 0.25 M sucrose or 0.05 M methyl-α-D-glucopyranoside to inhibit binding.

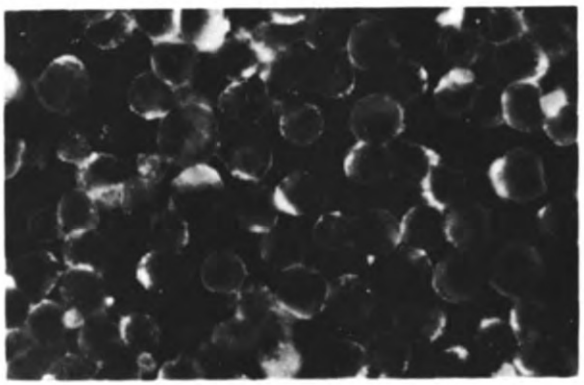

Fig. 2. Mouse fibroblasts labelled with Con-A and anti Con-A antibodies and incubated at 37° for 60 min. The Con-A binding sites are redistributed in large patches or single polar caps over the cell surface.

3. Results and discussion

Most lymphocytes displayed a uniform fluorescence pattern with dots of varying intensity distributed over the whole surface after cold incubation (fig. 1a). Further incubation for 15–20 min at 37°, however, was usually accompanied by the appearance of numerous spots or, occasionally, patches (fig. 1b). With optimum anti Con-A antibody concentrations, both spot and patches tended to merge into a single polar cap (fig. 1c). The distribution of the fluorescent tracer was nearly homogeneous on the fibroblast cell surface stained at 4° and further incubation at 37° for more than 30 min led, as in the case of lyphocytes, to the formation of surface fluorescent patches or single polar caps (fig. 2).

Both fibroblast and lymphocyte cell surface cap formation (table 1) was temperature-dependent and inhibited by anti Con-A present at 50 times the optimum concentration and by the addition of 10^{-3} M sodium azide before the 37° incubation. Patch formation was also inhibited by NaN_3, though some cells still displayed widely separated spots. In both cell types clustering was suppressed in favour of a ring-like fluorescence uniformly distributed over the whole surface when the first 30 min incubation at 4° was done in presence of 2×10^{-2} M glutaraldehyde.

Table 1
Relative frequency of fluorescence patterns in different cells.

Incubation after staining Time (min)	Temp. (°)	Ring	Spots	Patches	Cap
2	at 4	++	(+)–	–	–
60	4	++	+ –	–	–
2	37	++	+ –	–	–
15	37	(+)–	++	++	(+)–
30	37	(+)–	+	++	+
30	37*	(+)–	++	+	–
30	37▲	+	++	–	–
30	37■	+++	–	–	–

*) Staining performed by excess antibodies (50-fold optimal concentration of rabbit anti-Con-A antiserum Ig fraction).
▲) Sodium azide 10^{-3} M added to the medium before incubation.
■) Glutaraldehyde 2×10^{-2} M added before staining.

The formation of spots, patches and caps under different experimental conditions is indicative of topographical redistribution of concanavalin-A receptor molecules and demonstrates their transverse movement within the plasma membrane of the fibroblasts and lymphocytes.

Clustering of this kind is probably due to divalent bonding of anti Con-A antibodies, leading to lattice formation on the cell surface: a graphic interpretation of this view is shown in fig. 3. The fact that clusters are not formed in excess antibody offers supporting evidence.

Patches and the total congregation of Con-A receptors into polar caps require extensive transverse movement, which is clearly dependent on temperature and active metabolism of the cell concerned. Spot formation is also triggered by increased temperature, but, unlike patches and caps, it is not suppressed by sodium azide. If, as postulated [1, 2], the plasma membrane lipid matrix is fluid and if only weak protein to protein bonds exist, the limited clustering which is responsible for spot formation may be attributable to slight surface movements due, for example, merely to the kinetic energy possessed by the membrane molecules. The addition of glutaraldehyde forms stable bridging links between these molecules [11] thus totally preventing even the limited translation movements required to form spots.

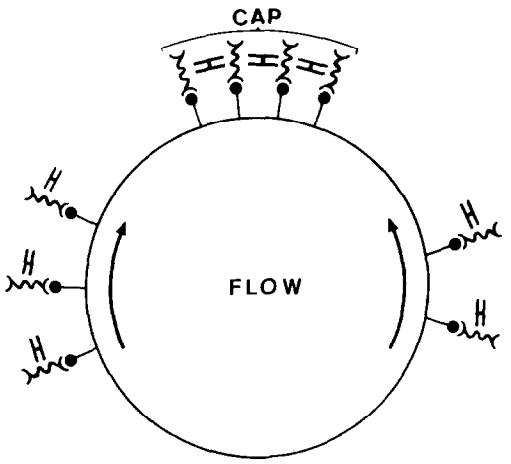

Fig. 3. Cap formation may be due to immobilisation of Con-A bound molecules as a result of cross-linking of anti-Con-A antibodies at a single point of the cell. A metabolically-dependent flow of Con-A bound molecules might then build up around this primary cluster.

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References

[1] S. Singer, in: Structure and Function of Biological Membranes, ed. L. Rothfield (Acad. Press, New York, 1971) p. 145.
[2] S. Singer and G. Nicolson, Science 175 (1972) 720.
[3] M. Inbar and L. Sachs, Proc. Natl. Acad. Sci. U.S. 63 (1969) 1418.
[4] L. Mallucci, Nature New Biol. 233 (1971) 241.
[5] G. Nicolson, Nature New Biol. 233 (1971) 244.
[6] D. Allan, J. Auger and M. Crumpton, Nature New Biol. 263 (1972) 23.
[7] C. Smith and J. Hollers, J. Reticuloendoth. Soc. 8 (1970) 458.
[8] R. Taylor, P. Duffus, M. Raff and S. DePetris, Nature New Biol. 233 (1971) 225.
[9] W. Davis, Science 175 (1972) 1006.
[10] C. Bianco, personal communication 1972.
[11] D. Sabatini, K. Bensch and R. Barnett, J. Cell Biol. 17 (1963) 19.
[12] H. Clark and C. Shepard, Virology 20 (1963) 642.