Lateral Diffusion of Wild-type and Mutant L\(^d\) Antigens in L Cells

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ABSTRACT We have compared the lateral diffusion of intact transmembrane proteins, wild-type H-2L\(^d\) antigens, with that of mutants truncated in the cytoplasmic domain. Diffusion coefficients and mobile fractions were similar for all molecules examined, from wild-type L\(^d\) antigens with 31 residues on the cytoplasmic side of the plasma membrane to mutants with only four residues in the cytoplasmic domain. This result limits ways in which the lateral diffusion of a major histocompatibility antigen, a transmembrane protein, can be constrained by interactions with other molecules.

The lateral diffusion coefficients measured for membrane proteins range from \(10^{-11}\) cm\(^2\)s\(^{-1}\) to \(5 \times 10^{-9}\) cm\(^2\)s\(^{-1}\) with values for many proteins \(\approx 2 \times 10^{-10}\) cm\(^2\)s\(^{-1}\). This value is 10 to 20 times slower than the fastest diffusion observed, for rhodopsin in disc membranes, (14, 20, 29) and is much lower than expected on the basis of any theory for diffusion in membranes (5, 9, 22). Several lines of evidence suggest that the slow lateral diffusion of many membrane proteins is due to interactions between these proteins and the cytoskeleton: (a) Diffusion of band 3 is 100 times faster in spectrin-deficient mouse erythrocytes than in normal erythrocytes (13, 23). (b) Diffusion of several membrane proteins is increased 100-fold or more in membrane blebs produced on the surface of intact cells. Such blebs appear to lack actin (26, 28, 32). (c) Diffusion of membrane proteins reconstituted into synthetic lipid vesicles is orders of magnitude faster than diffusion of the same proteins in native membranes (17, 27).

Antigens specified by the mammalian major histocompatibility complex (MHC), class I MHC antigens, are expressed on most cells. Lateral diffusion of these antigens, H-2 of mouse and HLA of humans, has been studied in normal and transformed cells, (4, 6, 7, 18, 25) and in liposomes reconstituted with purified antigens (1). MHC antigens are, like other membrane integral proteins, somehow constrained in their lateral diffusion. Diffusion coefficients range from \(\approx 2\) to \(10 \times 10^{-10}\) cm\(^2\)s\(^{-1}\) in lymphocytes and in cultured fibroblasts, while they are \(50-100 \times 10^{-10}\) cm\(^2\)s\(^{-1}\) for MHC antigens in liposomes (1).

A good deal is known about the structure of MHC class I antigens, and complete sequences are available for several different antigens (reviews in 8, 11). All class I antigens consist of a heavy chain of \(\approx 44,000\) d associated with a light chain, beta-2 microglobulin, of 12,000 d. The bulk of the complex lies outside of the cell membrane. A segment of 24 amino acids spans the membrane, and a segment of from 31 to 46 amino acids lies within the cell. This cytoplasmic domain interacts with isolated proteins of the cytoskeleton (19) and other work suggests that the MHC antigens react with the cytoskeleton in intact membranes as well (12, 30). If this is the case, then MHC molecules with modified or truncated cytoplasmic domains ought to diffuse more rapidly than wild-type antigens.

Truncated MHC genes have been produced by two laboratories. Zuniga et al. (33) transfected L cells with mutant H-2 L\(^d\) genes which lacked as many as 24 of the 31 C terminal residues of wild-type L\(^d\). They showed that these mutant antigens were integrated in the membrane and that they effectively presented viral antigens to H-2L\(^d\) restricted cytotoxic T cells. Murre et al. (15) constructed a mutant L\(^d\) in which all but six cytoplasmic residues were deleted and the deleted residues replaced by three amino acids specified by an MHC class II gene. These mutant genes were also expressed in transfected L cells and presented viral antigen to H-2 restricted killer cells. The truncated gene products also капиров нормально when reacted with anti-H-2 antibodies followed by antiglobulins. This implies that metabolically-driven lateral mobility of the truncated antigens is normal, but does not show if their diffusion differs from antigens with full-length cytoplasmic tails.

We have used fluorescence photobleaching to measure the lateral diffusion of wild-type and three mutant H-2 L\(^d\) antigens expressed in L cells transfected with the appropriate genes. Three of these genes, the wild-type L\(^d\) (27.5), a mutant (BAL907) in which 25 of the 31 residues of the cytoplasmic domain are replaced by 19 residues, not derived from the L\(^d\) gene, and a mutant (BAL911) truncated to a seven residue cytoplasmic domain, have been described previously (33). A third mutant, C48, is further truncated to four residues of the wild-type cytoplasmic domain (ly-s-arg-ser-glu). The cytoplasmic sequences predicted for all 4 L\(^d\) antigens are shown in Fig. 1.

Here we show that the diffusion coefficients of the four
types of antigens differ less than twofold from one-another, and that the mobility of the mutant antigens appears to be restricted to the same extent and by the same factors as the mobility of wild-type H-2L and H-2K antigens.

MATERIALS AND METHODS

Construction of mutants, transfection of mouse L cells, characterization and maintenance of transformants have been described previously (33). Mutant C48 was constructed in a similar manner: details of its construction will be published elsewhere (Zuniga, in preparation). Two clones of each transfectant were used in our experiments: 27.5.27 5-E-11 and 27.5.27 4-B-5, 911 B-6 and 911 D-6, 907 B-4 and 907 C-4, C48 I-1-F and C48 I-1-C. No difference was found when diffusion coefficients were compared between clones expressing the same L~ antigen and the results for each antigen are pooled for measurements on both clones.

Monoclonal Antibodies and Antibody Fragments: The cell line HB27, producing a monoclonal anti-H-2L, anti-H-2D~ antibody, 28-14-8 (16) was obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in 600-ml flasks, and the IgG2= antibody was purified from spent culture medium. Medium, clarified by centrifugation at 300 g for 10 min, was adjusted to pH 8.1 with NaOH, and slowly passed over a column of protein A Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) at 4°C. The column was washed with 5 vol of pH 8.1 0.1 M phosphate buffer and then of protein A Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) at 4°C.

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labeled MHC antigens is greatly reduced in several types of human and mouse cells, including L cells, grown to high density (Wier, M., and M. Edidin, unpublished). BAL191 antigens, bearing only 7/31 residues of the cytoplasmic domain are similarly affected by culture density. An average of 34% of labeled antigens were mobile on 71 individual log phase cells, while an average of 11% were mobile on 43 individual high density cells. Indeed, in two experiments, on 18 cells, no recovery of fluorescence (practically, R < 10%) could be observed, though diffusion was detectable if the measured spot was bleached a second time.

Deletion of up to 27 of 31 residues of the cytoplasmic domain of H-2Ld antigens does not affect either the lateral diffusion coefficient measured for these antigens or the fraction of all antigens free to diffuse in the plane of the membrane. This result is similar to that obtained for lymphocyte membrane Ig. This molecule has only three cytoplasmic residues, lys-val-lys (21), but diffuses at ca. 2 × 10^{-10} cm^2s^{-1} (3) and appears to interact with the lymphocyte cytoskeleton (30).

We may consider three plausible mechanisms for regulation of lateral diffusion: (a) The small cytoplasmic tail remaining on the truncated antigens may contain sufficient residues, in particular basic amino acids, interact with the cytoskeleton. (b) The MHC antigens may interact with other membrane integral proteins, which in turn interact with the cytoskeleton. (c) Diffusion may be constrained by the effects of the total, high, concentration of membrane proteins on the viscosity of the lipid bilayer (17, 24). This concentration would not change appreciably in transfected cells. There are precedents for all three possibilities, though the first might be ruled out if a completely truncated Ld gene could be constructed and expressed in transfected cells. The second possibility is suggested by observations that stearoylated dextrins, integrated into the cell membrane bilayer by fatty acid tails, diffuse at ~3 × 10^{-10} cm^2s^{-1} (31). This 10 to 20 times slower than observed for lipid-soluble dyes and 5–6 times slower than the diffusion of stearoyl dextrins in synthetic membranes. The result strongly suggests that stearoyl dextran molecules interact with one another and with membrane proteins and that these interactions retard diffusion. The third possibility is raised by recent reports from Reis, Corn, and W. Weis. 1981. Two mRNAs with different 3' ends encode membrane-bound and secreted forms of immunoglobulin a polypeptide. Cell 20:303-312.


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