Immunoelectron microscopy on the topographical distribution of the poliovirus receptor

Klaus Mannweiler,1* Peter Nobis,2 Heinz Hohenberg1 and Wolfgang Bohn1

1Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg and
2Abteilung Molekularbiologie der Universität Hamburg, Martinistrasse 52, D 2000 Hamburg 20, F.R.G.

The topographical distribution of the poliovirus receptor on the cell surface was demonstrated by immunoelectron microscopy using monoclonal antibodies and immunogold markers. The receptor appeared in small clusters, which were randomly distributed over the cell surface and along cellular processes. The distribution pattern of the clusters corresponded to that of adsorbed and immunogold-labelled poliovirus particles and suggests a multivalent organization of poliovirus binding sites. Freeze-fracturing and ultrathin sectioning did not reveal any specific ultrastructures within the plasma membrane at labelled receptor areas. Incubation of native cells with anti-receptor antibodies did not remove the receptor molecule from the cell surface nor did it induce ultrastructural alterations within the plasma membrane. The antibody–receptor complexes exhibited lateral mobility within the plasma membrane and were able to aggregate into large immune complexes after incubation with a second ligand.

In picornavirus infection the cell and tissue tropism is determined by specific cell surface receptors (Holland & Hoyer, 1962; Dimmock, 1982; Crowell & Landau, 1983; Colonno, 1986). A characteristic feature is that all three serotypes of poliomyelitis virus (PV) bind to the same receptor (Minor et al., 1984), a transmembrane glycoprotein (Crowell & Landau, 1983; Colonno, 1986) which is supposed to belong to the Ig superfamily (Mendelsohn et al., 1989). The human gene coding for the receptor maps with that for the receptor of the major human rhinovirus group on chromosome 19 (Miller et al., 1974; Shepley et al., 1988). Various monoclonal antibodies (MAbs) against the poliovirus receptor (PVR) have been developed, which selectively block the adsorption of the virus and prevent PV infection of susceptible culture cells (Minor et al., 1984; Nobis et al., 1985; Shepley et al., 1988).

So far, data on the distribution pattern of the PVR on the cell surface have been deduced only indirectly from the adsorption pattern of viral particles identified in ultrathin sections (Dunnebacke et al., 1969; Zeichhardt et al., 1985). They suggest an even distribution of the PVR on the cell surface and a preferential association with microvilli and their bases (Zeichhardt et al., 1985). Direct evidence for the three-dimensional topographical distribution of the receptor molecules on the cell surface is lacking. This information can be obtained with the replica immunogold labelling procedure that allows demonstration of the three-dimensional distribution pattern of cell surface antigens at high resolution (Mannweiler et al., 1982). In addition, the label-fracture procedure (Pinto da Silva & Khan, 1984) should provide a means to correlate ultrastructures within the plasma membrane with antigenic binding sites on its outer surface. This would give information about the macromolecular arrangement within the plasma membrane in labelled receptor areas, especially after binding of ligands.

HeLa or Vero cells were fixed with aldehyde and incubated with the anti-receptor MAb D171 (Nobis et al., 1985) followed by incubation with rabbit anti-mouse antibody and Protein A-coated gold particles (pAg). The cells were post-fixed with aldehyde and prepared for Pt/C replication according to Hohenberg et al. (1985) and Hohenberg (1989). Small clusters of four to seven gold markers were detectable on these Pt/C replicas (Fig. 1a). The distribution pattern of the 90 to 110 nm diameter clusters was similar on HeLá and Vero cells. The clusters were randomly distributed on the cell surface and along the processes. No preferential localization of the receptor molecules was found on microvilli or on the bases of cell processes. Labelling of unfixed cells at 4 °C revealed the same arrangement of clusters of gold markers indicating that the binding capacity of the receptor is not influenced by glutaraldehyde fixation (Minor et al., 1984). No specific labelling was seen on BHK21 cells, which are not susceptible to PV infection, or on susceptible cells which were incubated with non-
specific serum or with pAg alone (data not shown). Only a few isolated gold particles were adsorbed in similar numbers to those on the surrounding glass support.

Replicas prepared from the extracellular fracture face after freeze-fracturing of pre-labelled cells (Fig. 1c) revealed a similar patch-like distribution of labelled PVR. The topographical distribution of the intramembranous particles in the labelled regions was not different from that of the surrounding area.

Small groups of two to three gold particles were found attached to the surface of the plasma membrane in ultrathin sections of labelled HeLa or Vero cells (Fig. 1b). The average distance between these clusters corresponded to those seen on replicas. No specific ultrastructures were noticed in these labelled plasma membrane regions.

PV particles were adsorbed to Vero cells at 4 °C for 60 min (10^5 p.f.u./cell corresponding to 10^7 particles/cell) then fixed and labelled with anti-PV antibodies and pAg. The results showed a distribution pattern of labelled PV particles similar to that of the labelled receptor (Fig. 2). Ultrathin sections of these cells demonstrated clearly that the immunogold-labelled 25 to 40 nm structures seen on Pt/C replicas corresponded to adsorbed PV particles. On average, each of the adsorbed viral particles was labelled with one to three gold markers; occasionally, up to three viral particles were attached in one area of the plasma membrane surface. The distances between the adsorbed viral particles in section preparations were nearly the same as those between the clusters of immunogold-labelled PVR.

Further studies aimed at determining the mobility of the receptor–antibody complex within the plasma membrane. When unfixed cells were preincubated with the MAb D171 at 4 °C and subsequently warmed in culture medium for 30 min at 37 °C, the patch-like distribution pattern of gold markers was still visible. Preliminary studies showed that these MAb–receptor complexes were stable for hours during incubation in MEM at 37 °C. The binding of this ligand did not induce ultrastructural alterations within the plasma membrane, which was confirmed by freeze-fracturing as well as ultrathin sectioning. However, the complexes were seen to aggregate into large immune complexes after an additional incubation with rabbit anti-mouse antibodies used as a second ligand (Fig. 3). The topographical distribution of the receptor molecules was remarkably altered by the formation of these complexes.

Our immunocytochemical studies clearly show that the receptor molecules for PV are clustered in small areas of 90 to 110 nm in diameter which may function as multivalent binding sites for PV. However, it was not possible to assess exactly the number of receptor molecules per immunogold-labelled area due to the irregular cell surface topography. Only the number of receptor clusters per square area could be determined on micrographs and this was calculated to be 70 to 100 clusters per 100 μm^2.

The existence of receptor clusters and an average adsorption of only one PV particle to one cluster area as shown here may help to explain the discrepancy between the number of receptor molecules determined by Nobis.
et al. (1985) with $^{125}$I-labelled MAb D171 (approximately $1 \times 10^5$/cell) and that deduced from sections in electron microscopy studies (approximately $1 \times 10^3$ adsorbed viral particles/cell; Dunnebacke et al., 1969). About $5 \times 10^3$ viral binding sites per intact HeLa cell were calculated to exist in virological investigations (Guttman et al., 1977). Further immunocytochemical studies with this MAb may provide additional insights into the features and the possible biological role of this viral receptor. Such studies may also be helpful for following early events taking place at the plasma membrane during the adsorption and penetration of non-enveloped viruses.

The preparation procedures were as follows: coverslip cultures were rinsed with prewarmed serum-free medium and pre-fixed for 20 min at 37 °C with a solution of 1.75% (v/v) freshly prepared paraformaldehyde and 0.025% (v/v) glutaraldehyde. They were rinsed twice with phosphate-buffered saline (PBS) containing 50 mM-glycine (PBG) for 10 min, incubated with the MAb D171 diluted 1:20 in PBG for 20 min, washed again and incubated with rabbit anti-mouse serum (Dako) diluted 1:50 in PBG for another 20 min. After rinsing with PBS the cultures were incubated twice with a suspension of protein A-coated gold particles (12 to 14 nm) for 10 min, washed with PBS and post-fixed with 1.75% (v/v)
glutaraldehyde and 1% (v/v) OsO₄ in sodium cacodylate buffer. The cells were treated for 5 min with 0.05% tannic acid. Pt/C replicas were prepared from the extracellular surface of the cells after dehydration with ethanol and critical point drying with freon. The Pt/C replicas were prepared and cleaned as described previously (Hohenberg et al., 1985; Hohenberg, 1989). For replication of the extracellular fracture face, the cultures were incubated with 20% (v/v) glycerol after glutaraldehyde fixation and freeze-fractured in situ according to techniques already described (Hohenberg et al., 1985; Hohenberg, 1989). Ultrathin sections prepared from flat embedded cultures in ERL were counterstained with uranyl acetate and lead citrate.

The authors gratefully acknowledge expert technical assistance by Mss I. Andresen, C. Burbank and U. Neumayer. This work was supported by Gemeinnützige Hertie-Stiftung, Frankfurt am Main. The highly purified PV and the anti-PV immune serum were kindly provided by G. Koch and R. Dernick. The Heinrich-Pette-Institut is financially supported by Freie und Hansestadt Hamburg and Bundesministerium für Jugend, Familie, Frauen und Gesundheit, Bonn.

References


(Received 3 January 1990; Accepted 24 July 1990)