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The Distribution of Human Coronavirus Strain 229E on the Surface of Human Diploid Cells

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SUMMARY

The distribution of human coronavirus strain 229E (HCV 229E) particles on the surface of human diploid (MRCc) cells was examined. Virus particles showed a totally random distribution on fixed cells and on cells to which virus had been adsorbed in the cold. A marked redistribution of virus particles was observed on warming virus–cell preparations to 33 °C for 20 min, the peripheral areas of the cell becoming relatively devoid of virus particles while the majority of particles were now located some distance from the edge of the cell. Redistribution did not occur in the presence of metabolic inhibitors.

INTRODUCTION

Viropexis is the mode of cellular entry for a number of virus groups (Dales, 1973), including coronaviruses (Patterson & Bingham, 1976). The distribution and point of entry of virus particles on the cell surface has, however, received little attention. During the past decade it has been demonstrated that the binding of bivalent antibody (Taylor *et al.*, 1971), or multivalent ligands (Smith & Hollers, 1970) to plasma membrane receptors can lead to an energy-dependent redistribution of the bound receptors. The principal requirement for such a redistribution is that the ligand be at least divalent and thus allow the formation of a cross-linked two-dimensional matrix. Virus particles have at their surface repeating subunits, the function of which is to enable the virion to bind to the surface of a susceptible cell. Thus, a virus particle may be considered to be a multivalent ligand with the capacity to form a cross-linked matrix with its appropriate cellular receptor molecules. Such binding may result in a redistribution of virus particles on the cell surface by mechanisms similar to those operating in antibody- or lectin-mediated capping. Recent investigations with a picornavirus suggest that such a redistribution can occur (Gschwender & Traub, 1979).

The aims of the present study were to determine by scanning electron microscopy (SEM) the distribution of coronavirus particles on the cell surface and to look for evidence of redistribution. A coronavirus was selected for this work because these viruses are relatively large, 80 to 220 nm in diam. (Tyrrell *et al.*, 1978) and thus easily recognized by SEM. The human coronavirus strain 229E (HCV 229E) was used as it is a typical member of the group. The results suggest that coronavirus particles are initially bound randomly over the cell surface and are subsequently redistributed by an energy-dependent mechanism.

METHODS

Cells and virus. Monolayer cultures of diploid human embryo lung cells of the MRC continuous (MRCc) line, obtained from Dr A. F. Bradburne, were used. HCV 229E was obtained from Dr S. E. Reed and was grown in MRCc cells.

Virus growth and purification. Monolayers of MRCc cells were infected at a multiplicity of 0·1 infectious particles/cell. Following an adsorption period of 1 h at 33 °C, cells were incubated at 33 °C for 32 h in Eagle's BME with 2% newborn calf serum (Macnaughton &

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Madge, 1978). The virus suspension was clarified at 2000 *g* for 30 min at 4 °C and the virus pelleted at 75000 *g* for 1 h. The virus pellet was resuspended in L15 medium to produce a final concentration of 10^{11} to 10^{12} particles/ml as determined by electron microscopy. The particle:infectivity ratio was approx. 10^2:1 using an immunofluorescent foci assay (Macnaughton *et al.*, 1980).

Adsorption of virus to MRCc cells. Monolayers of MRCc cells were preincubated for 20 min at 33 °C in the absence or presence of a combination of 10^{-3} M-sodium azide and 5×10^{-2} M-2-deoxyglucose in L15 medium. They were then cooled on ice and 50 μl virus added and allowed to adsorb for either 30 min or 1 h. Unadsorbed virus was removed by washing three times with cold L15 medium. Inhibitor-treated cells were washed in L15 medium containing inhibitors. The temperature was then raised to 33 °C and the incubation terminated after 20 min by replacing the medium with 3% glutaraldehyde buffered with 0·1 M-cacodylate buffer pH 7·2 containing 5% (w/v) sucrose. In some experiments the last 20 min incubation was carried out on ice and the cells subsequently fixed with ice-cold glutaraldehyde fixative.

In other experiments, cells were prefixed for 1 h at 20 °C with 4% paraformaldehyde buffered with 0·1 M-cacodylate buffer pH 7·2. The fixed cells were then washed in cacodylate buffer containing 0·1 M-lysine to block any free aldehyde groups, and virus added as described above.

Fixation of cells for SEM. After 24 h at 4 °C the glutaraldehyde fixative was removed and the cells washed twice with 0·1 M-cacodylate buffer pH 7·2 containing 5% sucrose, prior to post-fixation for 1 h on ice with 1% osmium tetroxide buffered with 0·1 M-cacodylate buffer pH 7·2. Then, the cells were washed briefly in distilled water and following dehydration in alcohol, were dried at the critical point of liquid CO_2 using a Polaron critical point drying apparatus (Polaron Equipment, Watford, U.K.). Dried coverslips were then mounted on aluminium stubs using 'Electrodag 915' (Acheson Colloids, Plymouth, U.K.) and a thin layer of gold evaporated on to the cells in an E5100 Sputter Coater (Polaron Equipment). Coated specimens were examined in a Philips 500 SEM.

Analysis of results. For each experiment approx. 100 cells/treatment were closely examined by SEM. A series of high-power micrographs were taken of 2 or 3 cells/treatment in order to build montages of single cells at a final print magnification of ×15000. The montages were overlaid with clear acetate paper and the outline of the cell and position of individual virus particles mapped. These tracings were subsequently photographed.

RESULTS

Distribution of HCV 229E on fixed cells and on cells adsorbed with virus in the cold

A typical micrograph showing part of an uninfected MRCc cell is illustrated in Fig. 1 (*a*). A number of microvilli may be observed in this micrograph; the addition of virus or treatment with metabolic inhibitors had no marked effect on their morphology or distribution. Fig. 1 (*b*) shows a typical micrograph of a paraformaldehyde-fixed MRCc cell on which virus had been adsorbed for 1 h at 4 °C. HCV 229E particles were recognized as small spheres with diam. of approx. 80 nm. The particles seemed to be distributed totally at random; there was no area where preferential attachment of virus occurred. A similar distribution of virus particles was observed on the surface of those cells which had been adsorbed with virus for 1 h in the cold.

It is impossible to show an electron micrograph of the whole cell surface clearly showing virus particles, as the particles are too small to resolve at the magnification required for publication. Thus, maps have been drawn from montages of a number of different electron micrographs to show the distribution of virus over the whole cell surface. Fig. 2 is a typical map of a fixed cell, to which virus had been adsorbed for 1 h at 4 °C, showing totally random virus distribution.

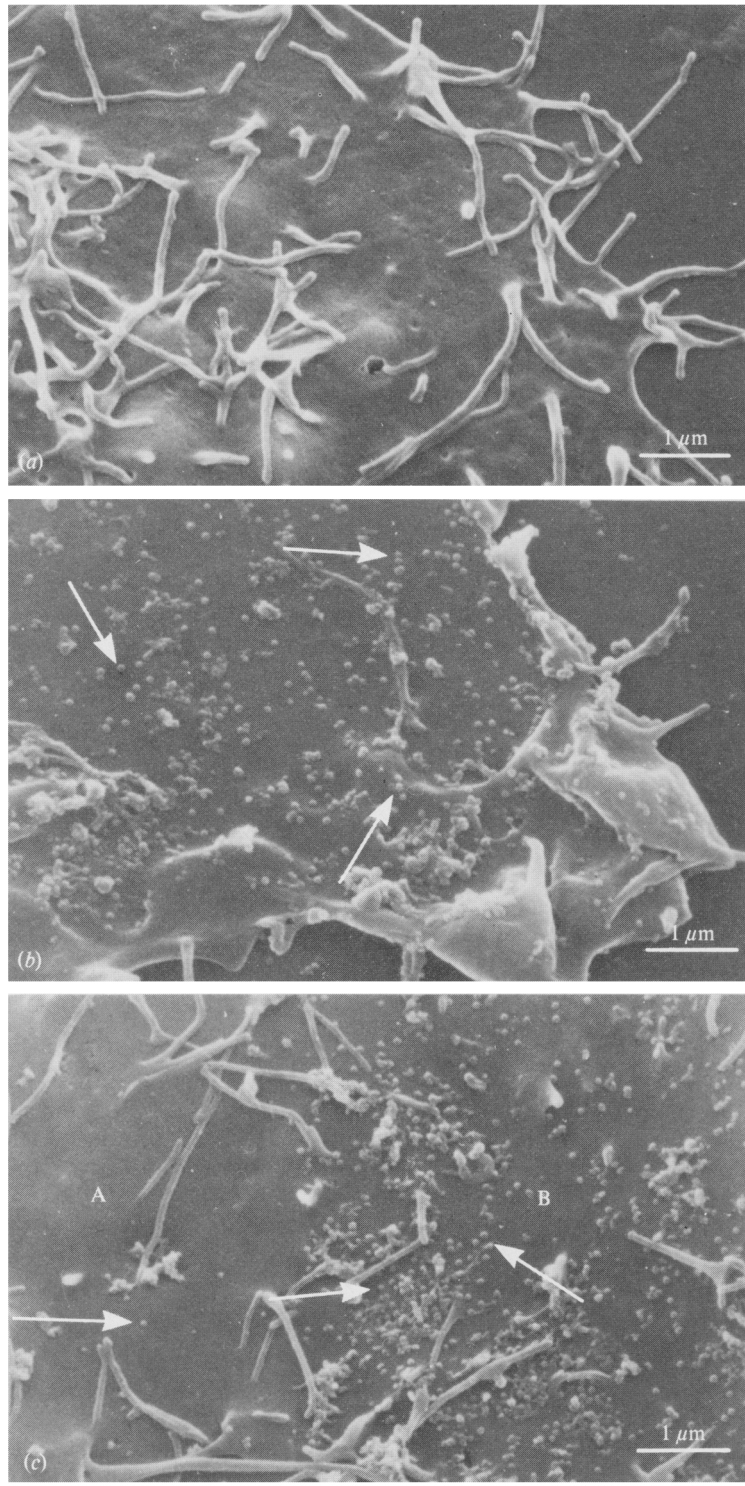

Fig. 1. (*a*) Control uninfected MRCc cell. (*b*) A cell prefixed with paraformaldehyde and then treated with virus for 1 h. Arrows indicate virus particles. Note the random distribution of virus particles over the cell surface. (*c*) A cell adsorbed with virus on ice for 1 h, washed and subsequently incubated for 20 min at 33 °C. Region A is a peripheral area of the cell and is relatively devoid of virus particles whilst in region B there are numerous particles. Arrows indicate virus particles.

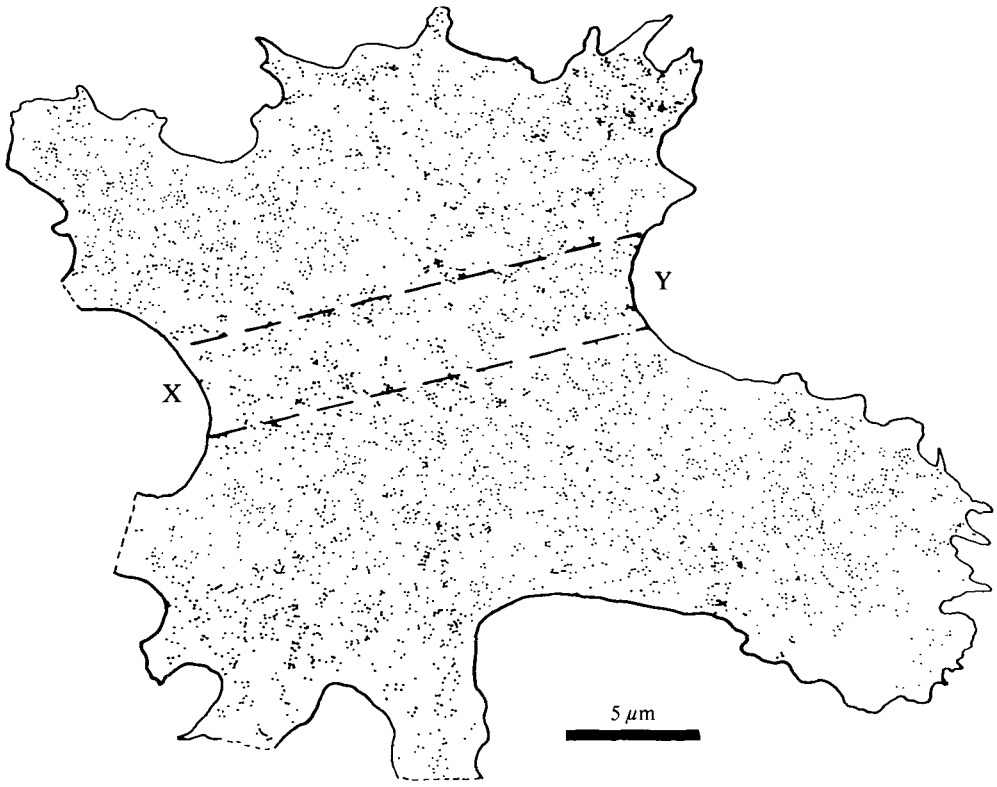

Fig. 2. Distribution of virus particles on the surface of a cell prefixed with paraformaldehyde and then treated with virus for 1 h. The distribution of virus particles across the cell from X to Y is shown in Fig. 4 (*a*).

Effect of warming on the distribution of HCV 229E particles

Fig. 1 (*c*) shows a typical example of part of an infected MRCc cell on which virus had been adsorbed in the cold for 1 h and which was then warmed to 33 °C for 20 min. Virus was redistributed and this is shown clearly on the map of a similar cell (Fig. 3). Redistribution was particularly marked on the surface of large lamellae extending from the main cell body. In all cells the number of particles at the periphery was reduced and the majority were located some distance from the edge of the cells.

Transects across montages of infected cells were drawn and the distribution of particles across the transects plotted as histograms. Typical histograms are shown in Fig. 4. Fig. 4 (*a*) shows the number of virus particles from X to Y across the cell shown in Fig. 2, that had been prefixed with paraformaldehyde before infection. A random distribution of virus was observed. However, there was a marked redistribution of virus particles from X to Y across the cell shown in Fig. 3, where, after adsorption with virus at 4 °C, the cell was incubated for 20 min at 33 °C. The periphery of the cell was relatively devoid of virus particles whilst approx. 4 μm from the edge of the cell there was a sharp increase in the number of particles. Moving further across the cell, the concentration of virus particles fell to a level similar to that found on the surface of the prefixed cell (Fig. 4 *b*). Quantitative analyses carried out on a number of cells showed similar distribution patterns. Transects passing through only the peripheral areas of the cell were not drawn since these would fail to show the redistribution phenomenon.

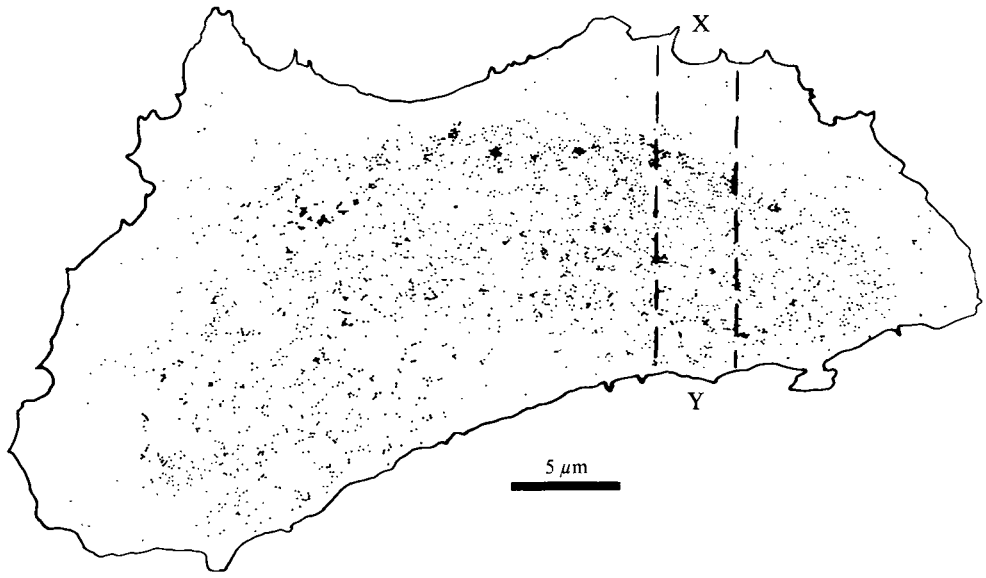

Fig. 3. Distribution of virus particles on the surface of cells adsorbed with virus on ice for 1 h, washed and then incubated for 20 min at 33 °C. The distribution of virus particles across the cell from X to Y is shown in Fig. 4 (*b*).

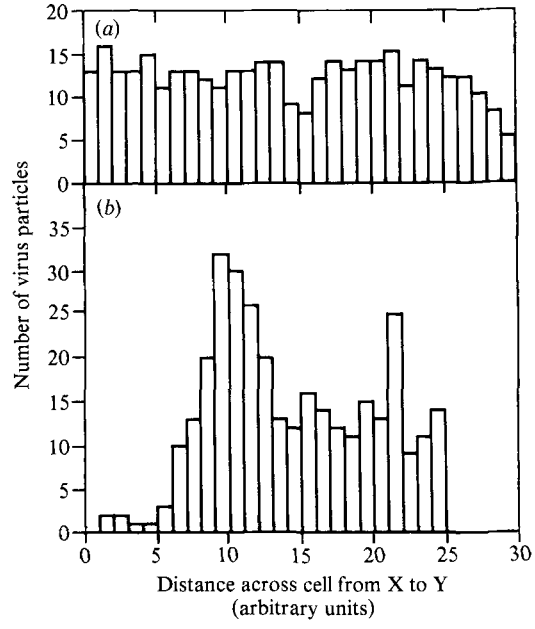

Fig. 4. Histograms of the number of virus particles in equal segments across infected MRCc cells. (*a*) Cell prefixed with paraformaldehyde and then infected with virus in the cold (Fig. 2). (*b*) Cell adsorbed with virus at 4 °C, washed and then incubated for 20 min at 33 °C (Fig. 3).

Effect of inhibitors on the distribution of HCV 229E particles

Some MRCc monolayers were incubated with a combination of 10^{-3} M-sodium azide and 5×10^{-2} M-2-deoxyglucose before adsorption of virus. When these cells were warmed, no

redistribution occurred. The distribution of virus particles was similar to that observed in fixed (Fig. 2) and unfixed cells maintained at 4 °C.

DISCUSSION

The Singer–Nicolson fluid mosaic model is currently the most widely accepted model of membrane structure (Singer & Nicolson, 1972). This model suggests that the plasma membrane is composed of a two-dimensional lipid fluid into which proteins may be inserted and are free to diffuse laterally. Fixation with paraformaldehyde, as in the present experiments, should result in the immobilization of membrane receptors. HCV 229E was found to bind to fixed MRCc cells. This suggests that there is no requirement for receptor movement for the attachment of HCV 229E. In addition, the random distribution of virus particles over the cell surface suggests a relatively even distribution of receptor molecules in the plasma membrane.

The distribution of virus particles observed after warming the cells could be accounted for by preferential loss or internalization at the periphery of the cell or by transport of virus across the cell surface after attachment. The greater concentration of virus particles found approx. 4 μm from the edge of the cell (see Fig. 4*b*), compared with that found on the more interior surfaces, could be accounted for by movement from the periphery, and thus supports the latter hypothesis. Redistribution of virus particles was not observed in the cold or in warmed cells in the simultaneous presence of the oxidative phosphorylation inhibitor, sodium azide, and the glycolytic inhibitor, 2-deoxyglucose. These findings suggest that an energy-dependent mechanism is responsible for the redistribution. As mentioned previously, the principal property required by a ligand to mediate energy-dependent receptor capping is that it is at least divalent. Virus particles, with their repeating receptor-binding units, fulfil this requirement. Although there are similarities between capping and the energy-dependent redistribution of HCV 229E particles observed in our experiments, other mechanisms of redistribution cannot be totally excluded. Particles of carbon, colloidal gold and ion-exchange resins have been shown to be transported from the cell margin to an area of membrane lying over the nucleus. It has been proposed that this is mediated by membrane flow (Harris, 1973). Such membrane flow could play a role in the redistribution of virus particles on the cell surface. Allison (1973), however, has proposed that particle transport and antibody-mediated capping are essentially similar events and suggests that particles binding to the cell surface cross-link membrane proteins in a manner similar to antibody.

Redistribution of virus on the cell surface may lead to preferential sites for internalization. Whether this would confer any biological advantage on the infecting virus particle or on the resistance of the cell to infection is at present unknown.

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