ULTRASTRUCTURAL ANALYSIS OF THE ACTION OF MECLOFENOXATE ON RETROVIRUS-TRANSFORMED CELLS

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Meclofenoxate (MF: USSR name acephen) is an ester of dimethylaminoethyl-p-chlorophenoxyacetic acid, related in structure to the plant growth factors known as auxins [14, 15]. MF has a neuroanabolic and psychoanaleptic action and is widely used clinically in the treatment of asthenic states, neuroses, and varous intellectual and memory disturbances in old age. Treatment of cell cultures with MF leads to reduction of the lipofuscin granules in these cells [3, 4, 10, 12]. It has also been shown that MF considerably potentiated the cytotoxic effect of several anticancer preparations and, in particular, certain alkaloids of plant origin (vincristine, vinblastine), which are inhibitors of mitosis [7, 13]. The authors cited suggested that MF acts on the tumor cell membranes [13]. However, the mechanism of action of MF on cells has not yet been explained.

The aim of this investigation was to study the action of MF on the ultrastructural organization of retrovirus-transformed cells of the lymphoid series (hybridomas).

EXPERIMENTAL METHOD

Hybridomas were obtained by fusing myeloma cells from Sp12 o-Ag14 mice with spleen cells from Balb/c mice, immunized with bacteriophage. The hybridomas were cultured by the method described previously [1]. Seeding was carried out after 3-4 days. MF was dissolved in the culture medium and a fresh portion was added daily from 15 days in a final concentration of $5 \cdot 10^{-4}$ M, after which the cells were taken for electron microscopy. The hybridomas were sedimented by centrifugation (1,000g, 5 min) and fixed for 1-2 h in 2.5% glutaraldehyde, after which they were postfixed for 1 h in 0.5% OsO_4 solution. Both fixatives were made up in 0.1 M Na-cacodylate buffer (pH 7.4). The hybridoma cells were dehydrated in ethanol-acetate and embedded in Epon-Araldite. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in the JEM-100B electron microscope (JEOL, Japan), with an accelerating voltage of 80 kV.

EXPERIMENTAL RESULTS

A characteristic ultrastructural feature of cells of the original myeloma line and of the hybridoma obtained from it is that they contain a relatively large number of retrovirus particles, surrounded by a membrane, both inside and in the extracellular space. An active part in the assembly of retroviruses is played by various cell membranes. The beginning and end of formation of the spherical nucleocapsid (NC) of the virus on the plasma membrane is shown in Fig. 1a. It will be clear that tubulin microtubules (~23 nm in diameter) approach the sites of virion formation. This suggests that the microtubules can take part both in transport of viral ribonucleoprotein (RNP) and in assembly and release of the virion. The cytoskeleton can also facilitate adhesion of RNP to the plasma membrane. The formation of a spherical NC and its adhesions to the plasma membranes with the participation of the cytoskeleton enables the already formed retrovirus to leave the cell by budding.

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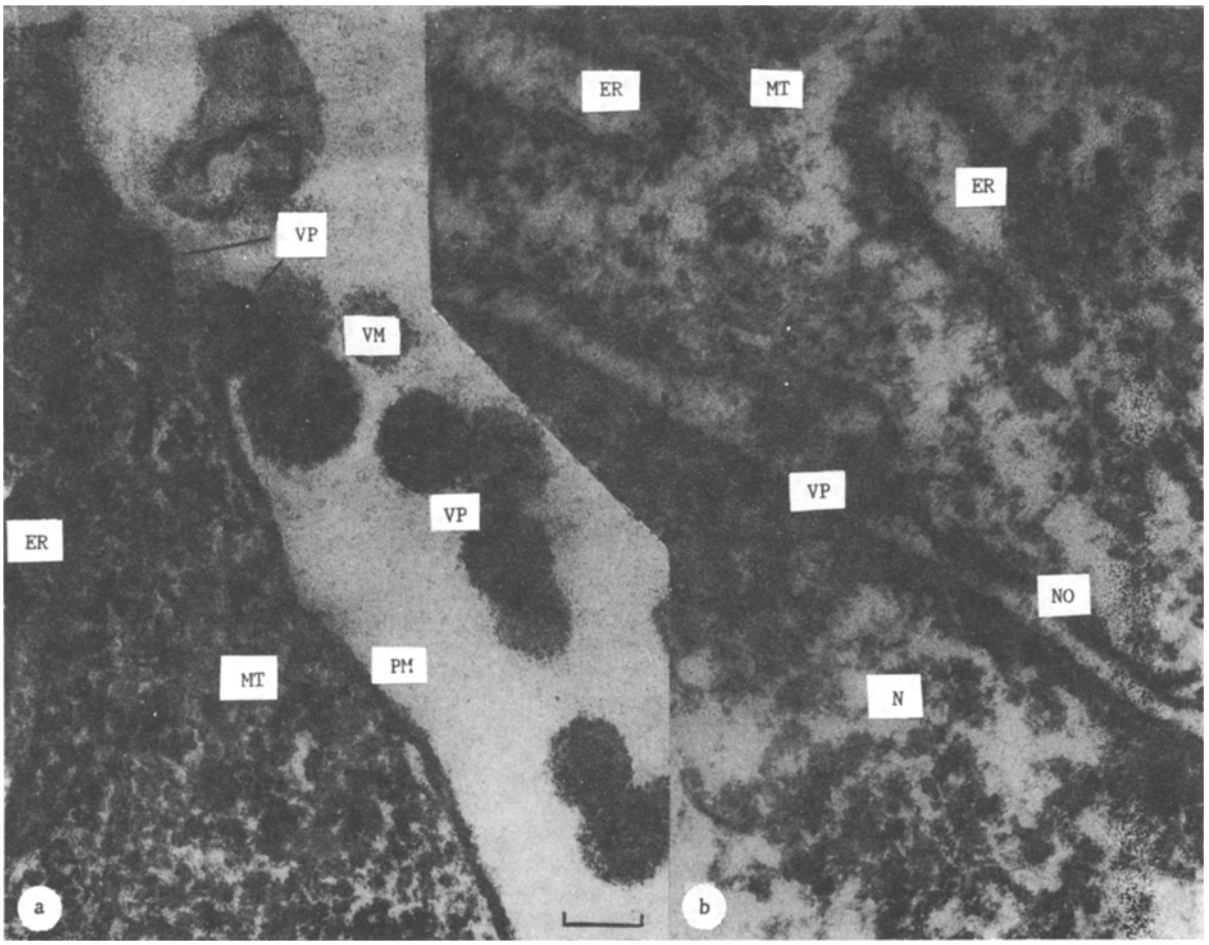

Fig. 1. Morphological features of assembly of C-type retroviruses on the plasma (a) and outer nuclear (b) membrane. Successive stages of formation of two types of virus particles are shown with the formation of spherical nucleocapsids. 115,000×.

Assembly and formation of the spherical NC also take place inside the cell on membranes of the smooth endoplasmic reticulum (ER) and nuclear membranes on the side of the cytoplasm (Fig. 1b, 2). As a result of this process, the virus particles surrounded by a membrane lie within the cisterns of ER and in the lumen of the nuclear membrane. The mechanism of interaction of RNP with the membrane of ER and the outer nuclear membrane differs from the mechanism of binding of RNP with the plasma membrane, evidently because of a difference in the membrane-bound cytoskeleton. As a result of this the retroviruses inside ER and the nuclear membrane are much smaller in size than retroviruses from the extracellular space. This may probably be evidence of different ways of formation of retroviruses in the cell. An alternative suggestion is that interaction of RNP with the intracellular membranes is only one of the stages preceding final maturation of the retroviruses. In some cases retroviruses can be found inside mitochondria (Fig. 2).

A detailed ultrastructural analysis of the formation of retroviruses in hybridomas suggests that RNP of the retrovirus adheres equally probably to the cell membranes mentioned above. The principal way of assembly of the infecting retroviruses in the cell is evidently through interaction of the virus RNP with a site on the cytoplasmic membrane. In this connection it is not yet clear whether retroviruses formed inside the cell, on leaving into the extracellular space, possess infective properties.

During hybridoma culture in the presence of MF, assembly of the retroviruses takes place mainly on membranes of the smooth ER and on the outer nuclear membrane (Fig. 3). Budding of retroviruses from the cell can be detected only in rare cases. Treatment of the cells with MF induces swelling of the mitochondria and also the formation of numerous evaginations of the plasma membrane, with the formation of microvilli, packed with microfilaments (diameter ~5-7 nm), identified as F-actin [2, 11]. An important characteristic of such cells is the absence of contacts between tubulin microtubules and plasmolemma. All

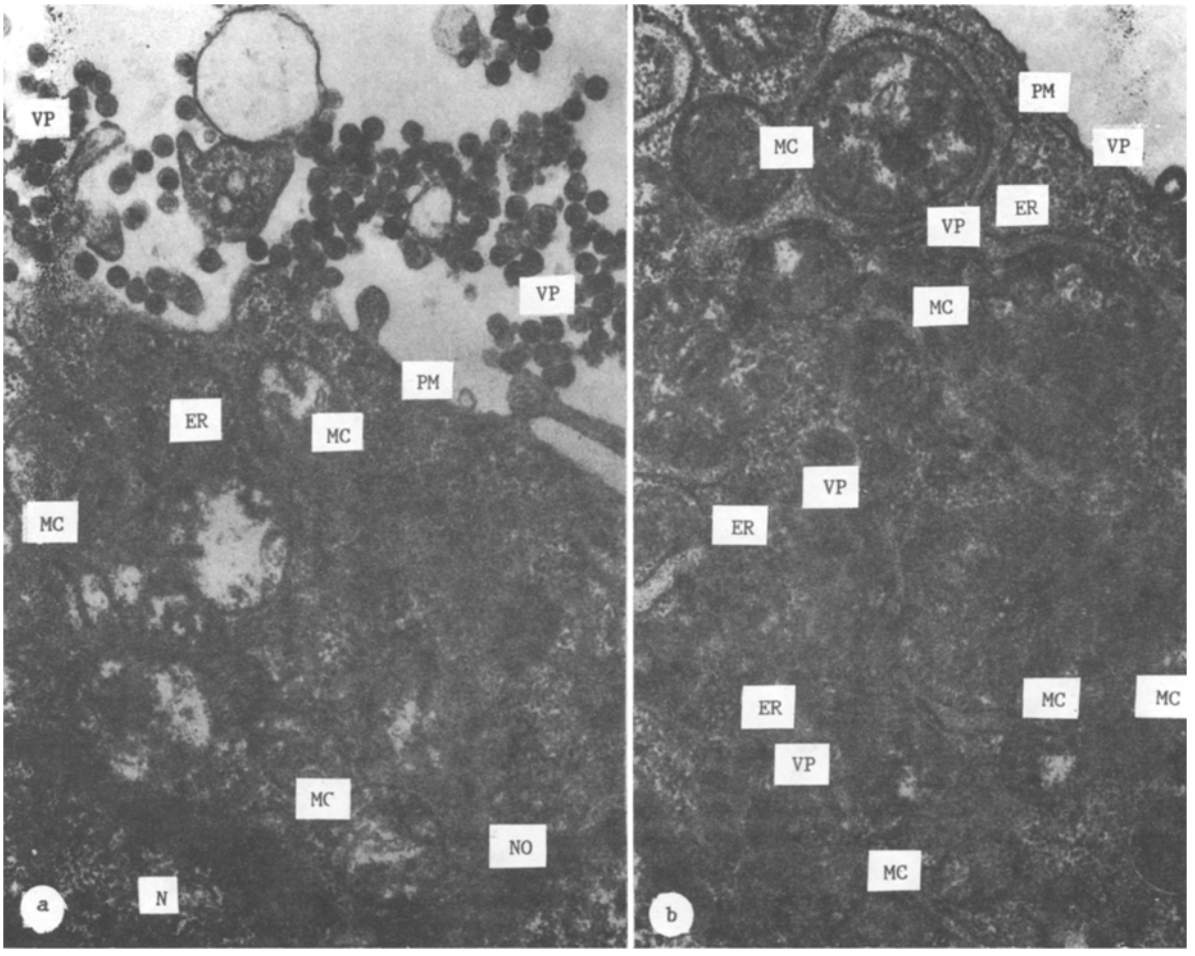

Fig. 2. Accumulation of retroviruses in the extracellular space (a) and intracellular compartment (b): in the cisterns of ER and the mitochondrial matrix. Here and in Figs. 1 and 3: GA) Golgi apparatus, VM) virus membrane, VP) virus particle (fully formed retrovirus), MV) microvilli, MT) tubulin microtubules, MC) mitochondria, MF) microfilaments, PM) plasma membrane, ER) endoplasmic reticulum, N) nucleus, NO) nucleolus. 23,000×.

this indicates the effect of MF on the plasmatic membrane and on the cytoskeleton cells connected to it (at least, on such components as tubulin and F-actin).

It will be noted that the retroviruses remained inside ER during cell division. We found that MF ($5 \cdot 10^{-4}$ M) had no appreciable effect on proliferation of hybridomas or on production of specific antibodies to phase λ by them.

It was suggested previously that MF acts on the plasma membranes of several cancer cells in culture [13]. In these investigations the greatest effect of MF was noted on transformed lymphoid cells, evidently retroviral in nature. Among the anticancer preparations which intensify their cytotoxic properties in the presence of MF, the cytostatics vincristine and vinblastine were used; their action is interlinked with elements of the cytoskeleton such as tubulin and actin. In addition, a similar action of MF on cells (the formation of microvilli) has been demonstrated for antipsychotics of the chloropromazine series, which also interact with the cytoskeleton [8]. The principal effect of these preparations is inhibition of the Ca-calmodulin complex. Since MF and compounds of the chlorpromazine series are very similar in their chemical structure it can be tentatively suggested that MF can interact similarly with the Ca-calmodulin system, bound with the cytoskeleton. We know that at neutral pH MF is quickly hydrolyzed (half-decomposition time ~1 h) into p-chlorophenoxyacetic acid and dimethylaminoethanol, and that these products themselves have no such effect on cells [5]. A fresh portion of this preparation was therefore administered daily both in vivo and in vitro.

Culture of hybridoma cells in the presence of MF ($5 \cdot 10^{-4}$ M) thus leads to reduction of exocytosis of retroviruses, probably as a result of its action on elements of the membrane-

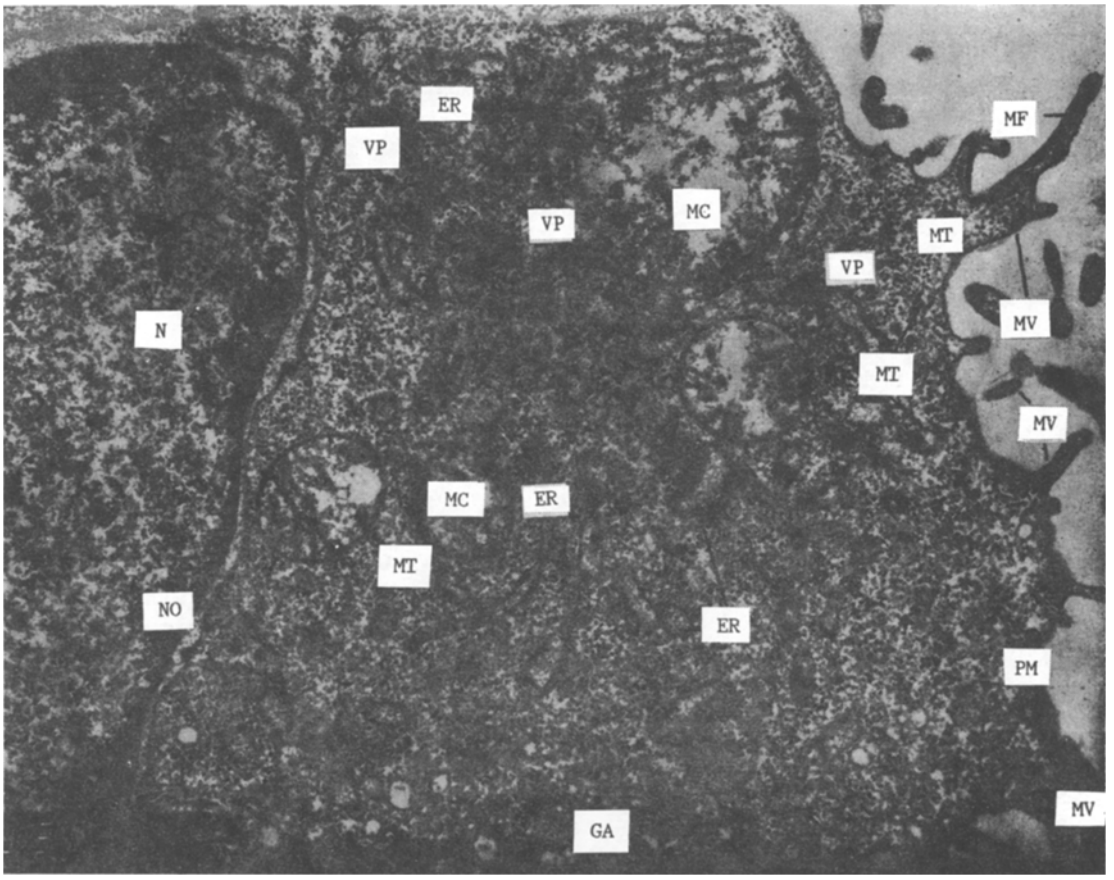

Fig. 3. Ultrastructure of hybridoma cell on 10th day of culture in the presence of MF ($5 \cdot 10^{-4}$ M). The principal intracellular compartments accumulating retroviruses are the cisterns of the endoplasmic reticulum and the nuclear membrane. Swelling of mitochondria and formation of numerous outgrowths of the plasma membrane, in the form of microvilli, can be seen. 23,000×.

bound cytoskeleton. An important feature of MF is the absence of cytotoxicity, when administered chronically to animals [6, 9] and during continuous culture of hybridomas in vitro, for 15 days at least.

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