Comparison of Cytoskeletal Organization in Canine Distemper Virus-infected and Uninfected Cells

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SUMMARY

The organization of vimentin filaments, keratin filaments, microtubules and microfilaments was compared in canine distemper virus (CDV)-infected and uninfected cells by indirect immunofluorescence. Infection of tissue culture cells with CDV caused a total reorganization of all the cytoskeletal structures with the most notable changes in the microtubules and intermediate filaments. During virus infection two different patterns of staining were observed for both the intermediate filaments and microtubules, suggesting a step-by-step reorganization of the structures. While the two types of intermediate filaments (vimentin and keratin) had quite different staining patterns, the vimentin (but not keratin) filaments had a distribution pattern similar to the microtubules in both infected and uninfected cells. These results suggest that microtubules and vimentin (but not keratin) filaments may have a close association in CDV-infected cells.

INTRODUCTION

In cultured cells lytically infected with a cytocidal virus such as canine distemper virus (CDV) extensive and rapid changes in cell shape occur which are a part of the cytopathic effect (c.p.e.) The morphological changes are characterized by the formation of large, flat giant cells or polykaryons (Bussell & Karzon, 1962, 1965a, b). However, the c.p.e. will not develop if the cultures are persistently infected (ter Meulen & Martin, 1976; Rima & Martin, 1976). In such a case the cell shape remains the same and virus titres are low, yet the virus remains cell-associated. This suggests that the presence of virus alone does not induce the c.p.e. It is not known how one virus can induce two different states of infection and, furthermore, there is little information on what mechanisms initiate the development of the c.p.e.

Numerous studies have established that in both normal and virus-transformed cells, morphogenesis is one of the several important functions of the cytoskeleton (Hynes & Destree, 1978; Rubin et al., 1978, 1979; Clark & Spudich, 1977; Stephens & Edds, 1976; Goldman et al., 1979). Microfilament reorganization has been used as one indication of viral transformation of cultures (Pollack et al., 1975). More recently, changes in microfilament bundle patterns have been defined as possibly playing a role in the morphological alterations which occur during lytic infections induced by different viruses (Fagraeus et al., 1978; Heeg et al., 1981; Meyer et al., 1981). Moreover, studies on the formation of polykaryons after infection by the parainfluenza virus, SV5, revealed that thick bundles of both microtubules and intermediate filaments reformed into parallel rows during cell fusion (Wang et al., 1979). A recent electron microscopic study (Howard, 1981) demonstrated that intermediate filaments reorganized either into thick bundles or massive aggregates after CDV infection.

Thus, evidence is accumulating that one of the important properties of a lytic virus infection is the reorganization of the cytoskeleton. A complete characterization of the organization of all cytoskeletal elements has not been done in the same virus–host system during a fully developed
lytic infection. Therefore, a collective study was designed to examine and compare microfilaments, microtubules and intermediate filaments in the same host cell–virus system. Hep2 cells were chosen as they readily support CDV growth and further demonstrate typical c.p.e. for this virus. Since this is a continuous epithelial cell line, both vimentin and keratin intermediate filament proteins were examined as it has been shown that both are found in epithelial cells (Franke et al., 1978, 1979). Immunofluorescence techniques were used to define cytoskeletal differences between infected and uninfected cells.

METHODS

**Cells.** Hep2 cells obtained from the American Type Culture Collection (Rockville, Md., U.S.A.) were grown on coverslips in Eagle’s minimal essential medium (MEM) supplemented with Hanks’ balanced salts and 10% calf serum (5% for cell maintenance) at 37 °C.

**Virus.** The Onderstepoort strain of CDV was obtained from Dr S. Krakowka, College of Veterinary Medicine, Ohio State University, U.S.A., and grown in Hep2 cells at a titre of 20 to 50 p.f.u./ml.

**Immunoreagents.** (i) Keratin antibody: antibody specific for keratin was prepared in rabbits from calf hoof prekeratin and was characterized by labelling of SDS gels (Eckert & Daley, 1981). The specificity of the antibody has been further demonstrated by labelling of nitrocellulose blots of two-dimensional gels of PtK1 (epithelial) cells (B. S. Eckert, S. E. Caputi & R. Warren, unpublished observations). (ii) Vimentin antibody: vimentin was purified from Triton X-100 residues of 3T3 cells by preparative gel electrophoresis and injected into New Zealand white rabbits. The specificity of the resulting antiserum was demonstrated by immunoradiography of nitrocellulose blots prepared from two-dimensional electrophoretic gels of purified vimentin and human neutrophils. 125I-labelled Protein A was used as the marker. (iii) Myosin and tubulin antibodies: rabbit antibodies to human uterus myosin (Sheetz et al., 1976) and rabbit antibodies to chicken brain tubulin (a gift of Dr M. Simon) were used as specific reagents for staining the myosin and tubulin components respectively, as described previously (Bourguignon & Singer, 1977; Bourguignon & Rozeck, 1980; Bourguignon et al., 1978, 1981).

**Immunofluorescence.** Cells were fixed for 7 min in 3-7% formaldehyde in Dulbecco’s phosphate-buffered saline (PBS) at room temperature, pH 7-2 (calcium and magnesium deleted and 0·1 M-cystine added). The cells were rinsed in PBS and permeated by immersion in 95% ethanol for 10 min followed by rehydration in PBS for 5 min. Cells were incubated with 20 to 30 μl of a specific antibody at 37 °C for 45 min followed by rhodamine-conjugated goat anti-rabbit IgG. The cells were examined on a Zeiss photomicroscope; photographs were taken on Kodak Plus-X film.

RESULTS

**Myosin distribution**

Nearly parallel bundles of myosin stretching from one side of the cell to the other obscuring the nucleus were seen in the uninfected cells (Fig. 1a). In contrast, infection with CDV led to a complete disruption of the bundles and resulted in a diffuse, granular staining (Fig. 1b). This was most clearly evident in the giant cells where, in addition to the diffuse staining, there was a faint outline of the periphery of the cell defining its shape.

**Tubulin distribution**

A pale, wavy network of staining with anti-tubulin was seen in uninfected cells (Fig. 2a). Shorter, more brilliant bundles were concentrated to one side of the nucleus, partially capping it and also associating with a bright focal point nearby. The nucleus itself was dimly stained. The tubulin organization in infected cells was characterized by two patterns. In single-nucleated cells the fine wavy network was still seen but in addition a thicker bundle of microtubules extended from the nucleus deep into the cytoplasm (Fig. 2b). One or more intermediate bright focal points were seen along these bundles. The other pattern, observed only in the polykaryons, showed one or more thick, long bundles crossing near or among the multiple nuclei (Fig. 2c). Occasionally, bright foci were seen.

**Vimentin distribution**

The vimentin pattern in uninfected cells appeared as an interwoven network of fine bundles extending throughout the cytoplasm with a slight outlining of the nucleus (Fig. 3a). A region of brighter staining was also noted occasionally on one side of the nucleus. This pattern was similar
Cytoskeletal changes in CDV-infected cells

Fig. 1. Intracellular localization of myosin by the immunofluorescence technique. (a) Cultured uninfected Hep2 cell; × 900. (b) CDV infection resulting in polykaryon formation and disruption of myosin filaments; × 360.

Fig. 2. Intracellular localization of tubulin by the immunofluorescence technique. (a) Cultured uninfected cells. (b, c) Infection with CDV resulting in formation of long thick bundles of microtubules: (b) Single-nucleated cell; (c) polykaryon. Arrows indicate bundles. All magnifications × 1000.

to that seen in cells stained for tubulin. Two patterns were again observed in the infected cells. The first was nearly identical to that seen in infected cells stained for tubulin (Fig. 3b). The second pattern was seen in the polykaryon where one or more long thick bundles encircled the multiple nuclei (Fig. 3c) or were organized into arrays overlying the nuclear region.
Keratin distribution

The majority of the keratin filaments formed a ring around the nucleus in uninfected cells (Fig. 4a). A very few fine filaments extended out into the cytoplasm. Generally, the filaments did not extend to the edge of the cell. Infected cells were characterized by two different patterns. Frequently, one long thick bundle wrapped around the nucleus and projected into the cytoplasm (Fig. 4b). The other pattern seen in the polykaryons was characterized by a lack of organization. An amorphous mass totally obscured the nuclear area and filled the adjoining cytoplasm. Very fine filament bundles extended from the mass but the periphery of the cytoplasm remained devoid of filaments (Fig. 4c).

Long, thick bundles of intermediate filaments were seen adjacent to the nucleus when CDV-infected cells were examined by electron microscopy (Fig. 5b), and often the filaments were parallel to the nucleus with randomly scattered microtubules in the vicinity. Very few filaments or tubules were seen in the cytoplasm of uninfected cells (Fig. 5a).
DISCUSSION

We have demonstrated by indirect immunofluorescence staining techniques that CDV disrupts the normal cytoskeletal patterns of all four elements examined. The distribution of myosin in the infected cells correlated well with previous reports on the actin–myosin complex in both transformed (Pollack et al., 1975; Weber & Grolscher-Stewart, 1974; Bourguignon & Rozek, 1978) and lytically infected cell cultures (Fagraeus et al., 1978; Meyer et al., 1981; Heeg et al., 1981; Rutter & Mannweiler, 1977). In the case of tubulin, vimentin and keratin, at least two different organization patterns were noted in the infected cells after c.p.e. was well established. Finally, the redistribution of vimentin filaments and microtubules (but not keratin filaments) was nearly identical in infected cells.

The multiple organization patterns for these cytoskeletal elements (tubulin, vimentin and keratin) have not been previously reported for cells infected with a lytic virus. Although a timed sequential study of the changes has not been carried out, it is possible that the patterns seen in the single-nucleated cells represent an intermediate stage of cytoskeletal disruption (see Fig. 2b, 3b and 4b). Depending on the infective dose, advanced stages of infection are generally characterized by well-developed large polykaryons (see Fig. 2c, 3c and 4c). Therefore, the cytoskeletal patterns of these cells would be a feature of late infection (Fig. 2c, 3c and 4c). However, if examination of cultures early in infection, preceding polykaryon formation, reveal
the same pattern as that in the single-nucleated infected cells then these cytoskeletal changes could be used to predict infections before full c.p.e. develops. This would be a useful tool to define infection when manipulations were used to disrupt early virus–host interactions.

Our immunofluorescence staining patterns for intermediate filaments and microtubules agree nicely with those described previously in transformed (Hynes & Destree, 1978), virus-infected (Wang et al., 1979) and uninfected cells (Geiger & Singer, 1980). Apparently, in a lytic infection, vimentin filaments and microtubules showed very similar reorganization patterns (Fig. 2 and 3). In contrast, the keratin filaments displayed an entirely different staining pattern (Fig. 4) from that of vimentin and microtubules (Fig. 2 and 3). In particular, the capped appearance of keratin in infected cells (Fig. 4b, c) may be indicative of a so-called ‘intermediate filament organizing centre’ (IFOC). It has been suggested (Eckert et al., 1982) that an IFOC may be closely involved in capping of keratin filaments and in controlling their distribution. Formation of a keratin cap may be a stage in reorganization of these filaments in virus-infected cells.

A recent report has indicated that the disruption of vimentin filaments by reoviruses does not affect the microtubule organization (Sharpe et al., 1982). The differences between these results and ours are most likely due to the characteristics of the individual viruses. The reoviruses, unlike CDV, do not have an envelope and do not induce polykaryon formation. Thus, the interactions of the virus with the cytoskeleton are different depending on the virus used. The morphological changes defined as the c.p.e. in lytically infected cells are in part due to the redistribution of all the cytoskeletal elements. Having characterized the cytoskeleton of the lytically infected cells, it is now of interest to investigate the cytoskeleton of the persistently infected cells. If one or more of the cytoskeletal elements are even minimally rearranged in the persistent infections, this could be used as a probe to define the infection. Furthermore, it would be of interest to establish when these changes start to occur and whether prevention of the reorganization would disturb the formation of the virus.

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REFERENCES


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