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# Ultrastructural Studies on Cell Fusion Induced by Epstein-Barr Virus or N-Butyrate and 12-O-Tetradecanoylphorbol-13-Acetate

## Brief Report

By

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With 3 Figures

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## Summary

The morphological changes which occur in Raji cells during EBV induced fusion are described. Of particular interest is the formation of local contacts between cells, at these points the plasmalemmae of the two cells become disorganized and cytoplasmic bridges are formed.

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Our previous studies indicate that superinfection or chemical induction of immobilized Raji cells leads to the formation of polykaryocytes (4, 5, 6). In one of our studies we demonstrated that fusion occured from within and was not caused by a virion component (5). In this report we present the results of electron microscopic studies on Raji cells undergoing fusion in suspension and in monolayers after superinfection or chemical induction.

The gross cytopathological changes characteristic of chemically induced Raji cells observed in this study were identical to those previously described by us (3) and others (1). These consisted of decrease in the number of microvilli, an increase in the number of surface blebs, cytoplasmic vacuoles were occasionally seen and the endoplasmic reticulum was well developed, there was a reduction in the number of chromatin clumps and filamentous structures were observed in the nucleoplasm (data not shown). These alterations were seen in both suspension and monolayer cultures, indicating that immobilization did not change the response of the cells to chemical

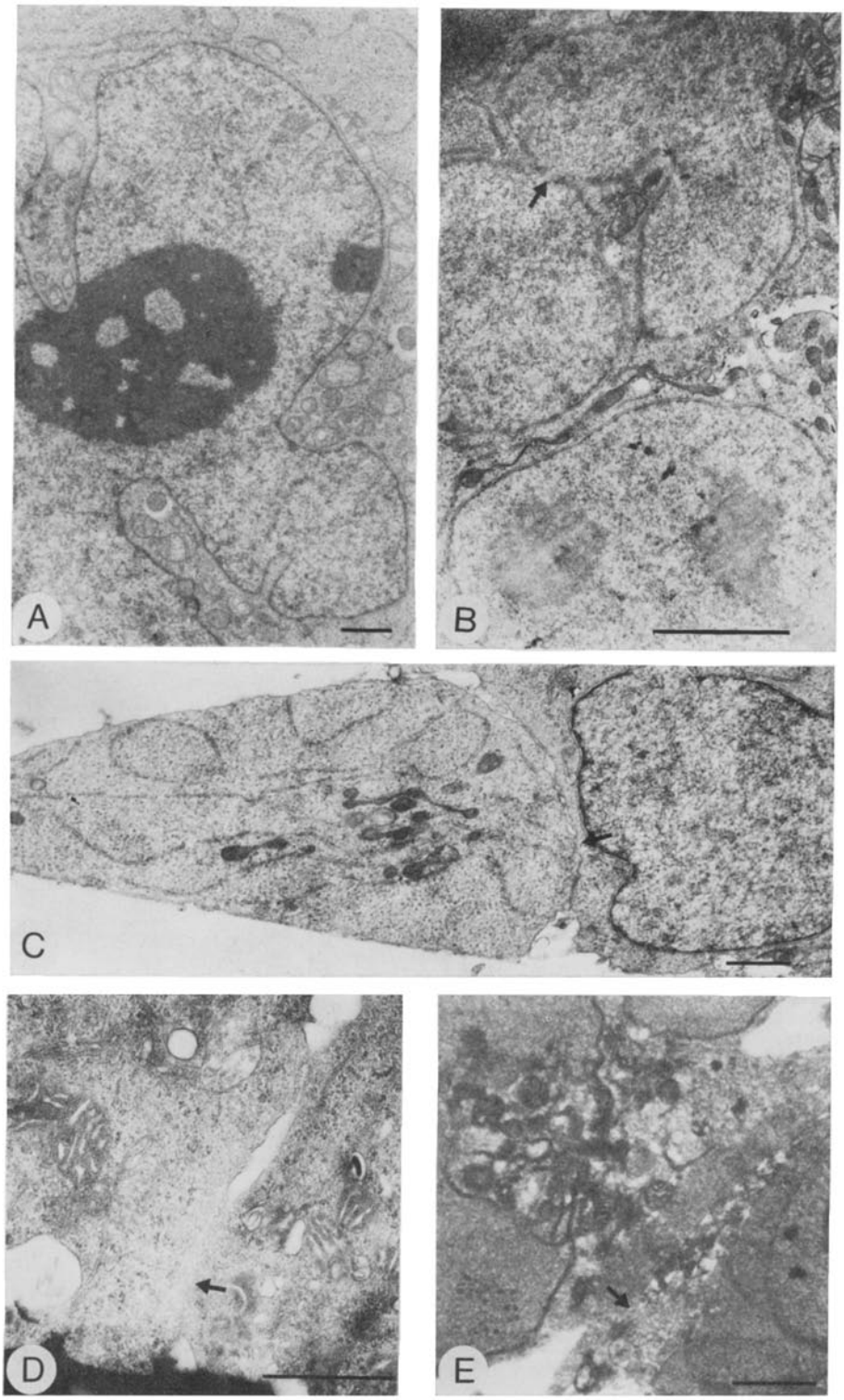


Fig. 1

induction. No polykaryocytes were observed in the suspension cultures, however, after immobilization on plastic films coated with anti-lymphocyte globulin, ALG (6, 12) giant multinucleate cells were observed. Occasionally a multilobulate nucleus was observed indicating that the nuclei of the individual fusion partners had coalesced (Fig. 1A, B). The cells in the monolayers are in close contact with one another at irregular sites on the cytoplasmic membrane (Fig. 1C), and at these points of contact the membranes become disorganised (Fig. 1D, E).

After superinfection Raji cells were also found to undergo cytopathological changes similar to those previously described, these included the appearance of filamentous structures in the cytoplasm (9), replacement of the mitochondrial christae with a beaded electron dense material and reduplication of the nuclear membrane (13) in addition to the changes seen in the induced cells (data not shown). The suspension cultures contained large clumps of cells, the cells within these clumps were in close contact at discrete sites along their surfaces, and at certain points the membranes of two cells fused forming cytoplasmic bridges (Fig. 2A, B). Fusion of the virion membrane to the cytoplasmic membrane was observed, however, we never observed a simultaneous fusion of a virus particle to two cells. Sixteen hours after infection polykaryocytes were observed at low frequency in suspension cultures (Fig. 2C), indicating that cell fusion was not an artifact caused by forcing the cells to form monolayers by immunobilization on ALG coated surfaces. The rare fusion events always appeared to occur in the cell clumps (Fig. 3A), the microvilli of the clumped cells appeared to congregate at the points of contact between cells and were not evenly distributed over the cell surface as is the case with uninfected cells (Fig. 3B, C, D). Polykaryocytes were observed with greater frequency in immobilized, superinfected Raji cells. An example of such a polykaryocyte is seen in Fig. 2C and D (note the remnants of the plasmalemmae of the fusion partners within the cytoplasm). As with the suspension cultures sites of intimate cell to cell contact were observed as were cytoplasmic bridges (Fig. 2A).

The formation of multinucleate cells by cell fusion requires that two cells come into intimate contact with one another, however, it has been shown that close proximation of 2 cell surfaces is normally hindered by a

Fig. 1. Transmission electron micrographs of immobilized, chemically-induced Raji cells. Raji cells were induced to synthesize EBV early antigens by incorporating sodium butyrate and tetra phorbol acetate into the tissue culture fluid (final concentrations 3 mM and 40 ng/ml respectively) (3). The induced cells were immobilized on ALG coated plastic films and incubated for 44 hours *(C)*, 43 hours *(A, B)*, or 38 hours *(D, E)*. The cells were then processed for TEM as previously described (2). All bars = 1 μm. *A* Giant lobulate nuclei are present in some fused cells. *B* Partial fusion of individual nuclei (arrow). *C* Close cell to cell contact at irregular intervals between cells and small areas of disorganized membrane (arrow). *D, E* Cellular membranes lose integrity at points of contact (arrows)

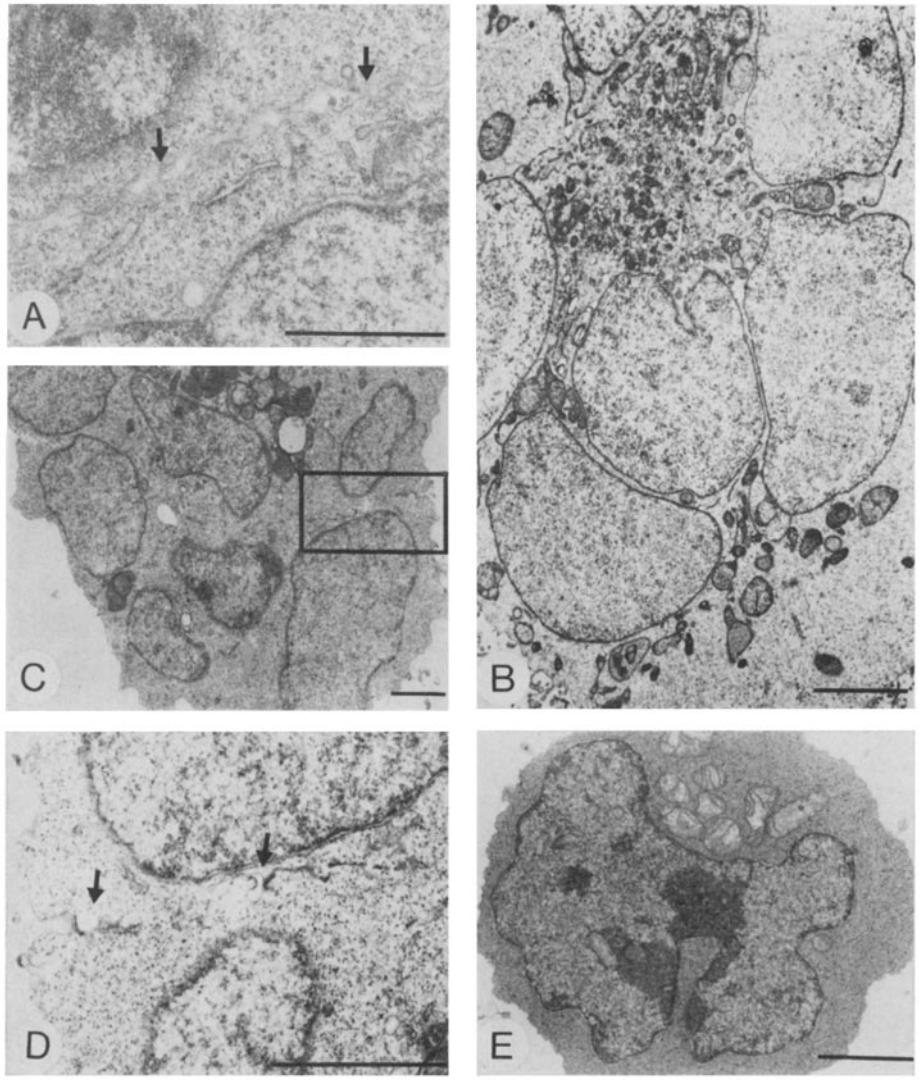


Fig. 2. Transmission electron micrographs of superinfected Raji cells from suspension cultures *(A, B)* and immobilized, superinfected Raji cells *(C, D, E)*. Raji cells were superinfected with EBV derived from P 3 HR-1 cells (4), incubated at room temperature on a shaker for 1 hour and then returned to 37° C *(A, B)* or immobilized on ALG coated plates before further incubation. All bars = 1 μm. *A* Superinfected Raji cells 30 minutes after addition of the virus showing cytoplasmic bridges (arrows). *B* A polykaryocyte from superinfected Raji cells in suspension 16 hours after infection, 5 nuclei can be seen. *C* A polykaryocyte observed in superinfected Raji cells 20 hours after immobilization (7 nuclei). *D* A higher magnification of the boxed area in panel C, the arrows indicate remnants of the plasmamembranes of the fusion partners in the cytoplasm of the polykaryocyte. *E* An uninfected, immobilized Raji cell from a control culture showing the typical morphology of a lymphoblastoid cell

considerable barrier of repulsive energy (11). The experiments presented in this paper show that this barrier can be overcome by 2 different mechanisms. Superinfection of Raji cells in suspension clearly obviates this problem, the superinfected cells form aggregates, and indeed points

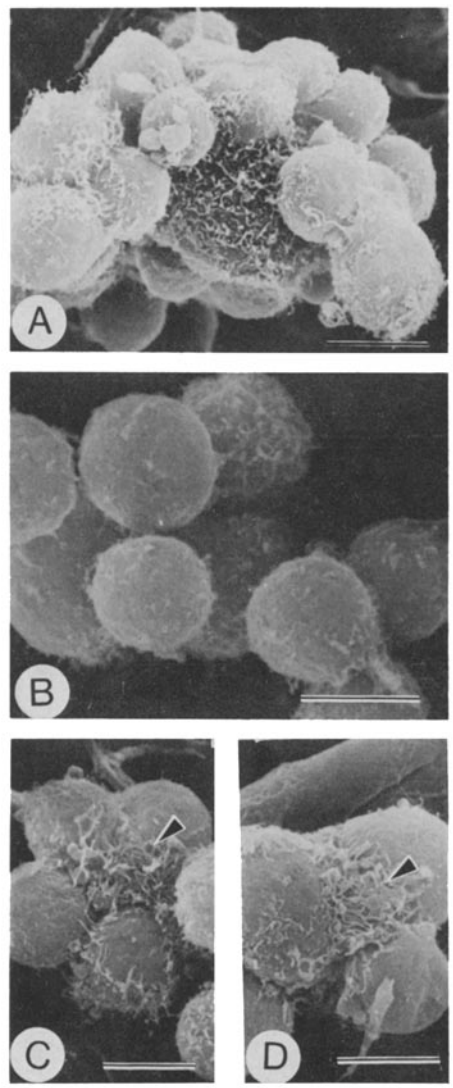


Fig. 3. Scanning electron micrographs showing the morphology of *A*, *C*, *D* superinfected Raji cells and *B* uninfected Raji cells as control. All cells were prepared from suspension cultures. All bars = 10 $\mu$m. *A* Superinfected Raji cells 16 hours after infection showing a polykaryocyte surrounded by several single cells. *B* A control Raji cell showing an even distribution of the surface microvilli. *C*, *D* Raji cells 30 minutes and 60 minutes post infection note the accumulation of the microvilli at the site of cell to cell contact

of contact and even cytoplasmic bridges can be observed shortly after superinfection (Fig. 2A, B). Perhaps the incorporation of virion membrane components into the cell membrane modifies the surface in a way that allows cells to adhere to one another at discrete sites. Fig. 2A shows that indeed cell contact is at discrete sites and not continuous. EBV penetrates Raji cells by fusing with the cell surface, the virus envelope remains integrated with the cell membrane and the naked nucleocapsids enter the cytoplasm and does not enter the cell by an endocytotic process as many other viruses, this observation is in agreement with previously published observations (10, 13). Previous studies demonstrated that virion components were not necessary for cell fusion (4, 5) and although we carefully examined numerous sections of superinfected Raji cells, we were never able to observe the simultaneous fusion of a virion to 2 adjacent cells, or even fusion of areas of the cytoplasmic membranes immediately above the naked nucleocapsids after they had penetrated the cytoplasm. These results suggest that the fusion induced by EBV differes from that caused by UV inactivated Sendai virus which can directly fuse cells without the expression of the viral genome (7, 8). The fact that chemically induced Raji cells, when cultured in suspension, do not undergo fusion, supports the hypothesis that modification of the cell surface by a virion component is essential for cell aggregation and fusion in suspension, early EBV induced membrane changes (e.g. the incorporation of early EBV membrane proteins) do not appear to overcome the mutual repulsion of two cells.

The second process which allows the close contact of lymphoblastoid cells is the preparation of monolayers by immobilization of cells on surfaces coated with ALG. As indicated in Fig. 1 (C, D, E) the areas of contact between chemically induced Raji cells are discrete as is the case in the suspension cultures of superinfected cells. How immobilization overcomes the intercellular repulsion is not clear. It may either be due to the localized modification of the cellular membrane upon immobilization, or simply immobilization forces the cells into close contact by nonspecific mechanisms. Once this barrier to aggregation is overcome then the presence of EA within the induced cells is sufficient to induce cell fusion.

We have previously speculated (4, 5) that EBV induced fusion might be a mechanism by which EBV can penetrate cells which lack receptors for the virus, thus giving rise to the progenitor cells of nasopharyngeal carcinoma. One of the arguments against this hypothesis was that most of the polykaryocytes shown by us were extremely large and eventually degenerated, whilst it is possible to present arguments against this objection the results of this paper show that EBV can induce cytoplasmic bridges or microfusions which might allow the transfer of viral genomes to the fusion partner, then before a full fusion can occur the process is interrupted, producing a cell containing EBV genomes, which is normally refractory to EBV infection.

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