epithelial cells which were shown to contain acetylcholine receptor[19].

The presence of anti-thymus antibodies has been demonstrated in a high percentage of myasthenic patients[7] and we could also detect binding of sera from myasthenics to mouse thymocytes. However, in the spontaneous disease, there is no direct evidence whether the immune response to thymus is a separate independent reaction or whether autoantibodies to AChR cross-react with the thymus. The present study provides direct evidence that anti-AChR antibodies can cross-react with the thymus.

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Epstein–Barr virus-induced cell fusion

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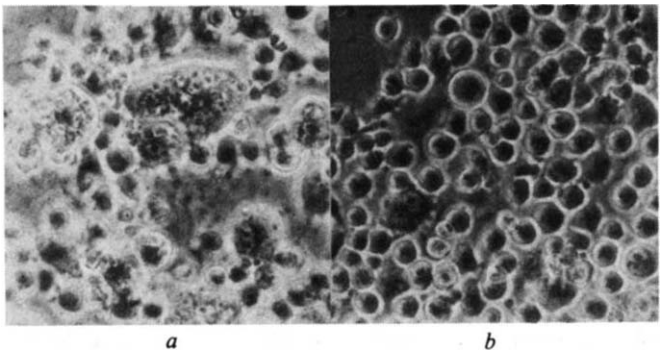
Serological and molecular biological studies[1–3] have shown an association between Epstein–Barr virus (EBV) and nasopharyngeal carcinoma. Although it has been shown that the epithelioid tumour cells carry EBV genomes[4], they are apparently devoid of receptors for EBV (H.W., unpublished observations). Others have suggested that fusion of EBV carrying cells with epithelial cells may be the mode of entry of the virus into cells unable to absorb the virus and that this may be mediated by one of the known syncytium-forming viruses which inhabit the respiratory tract (for example, members of the paramyxovirus group). de Thé and colleagues suggested that intercellular bridges could be seen in NPC tumour material[5]. We have developed a technique which permits the preparation of stable monolayers of viable human lymphoblastoid cell lines[6]. Using this technique we have now demonstrated that EBV can induce fusion between EBV-superinfected lymphoblastoid cells and cells devoid of EBV receptors.



a *b*

Fig. 1 The formation of polykaryocytes in cultures of immobilized superinfected Raji cells. A stock of EBV prepared from P3HR1 cells was used to superinfect Raji cells as previously described[8]. After shaking at room temperature for 1 h, the cells were applied to plastic Petri plates coated with ALG. The infected, immobilized cultures were incubated at 37 °C. *a*, Raji cells superinfected with concentrated virus, 4 h post-infection. *b*, As *a*, but virus diluted 1:100. ×650.

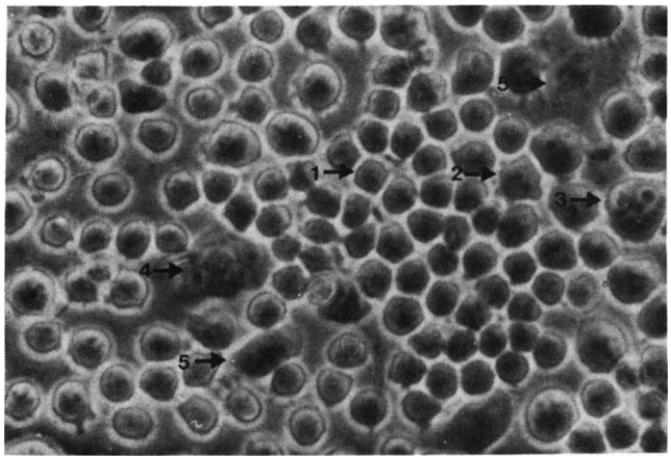


Fig. 2 The formation of polykaryocytes between superinfected and uninfected Raji cells. 10^6 Raji cells were shaken with 0.5 ml of an undiluted EBV stock at room temperature for 1 h. The cells were washed to remove unadsorbed virus and mixed with 9×10^6 Raji cells. The mixture was applied to ALG-coated plates as described in Fig. 1 legend. After 48 h of incubation at 37 °C, the culture was photographed. The various stages of polykaryocyte formation are indicated by arrows: (1) single cells; (2) binucleate cells; (3) cells with up to 10 nuclei; (4) nuclei begin to fuse and disintegrate; and (5) total loss of nuclear identity and disintegration. ×1,400

Raji cells, which carry the EBV genome but do not express early or late viral antigens, are susceptible to superinfection with EBV derived from the EBV-producing cell line P3HR1. Superinfected cells enter an abortive or productive cycle of virus replication depending on the multiplicity of infection. Productively infected cells synthesize viral DNA[7] and several virus-specified polypeptides[8] including early antigen (EA)[9] and viral capsid antigen (VCA)[10]. This superinfection system was used as a model to study the interaction of EBV-infected B-lymphoblastoid cells with one another and with unrelated cells. The response of uninfected Raji cells to immobilization on plastic surfaces coated with antilymphocyte globulin (ALG) has been described elsewhere[6]. When superinfected Raji cells were applied to ALG-coated plates in sufficient numbers to yield a confluent monolayer, polykaryocytes were observed within 2 h of immobilization (4 h after addition of the virus to the cells). Figure 1*a* shows a phase contrast photomicrograph of such a culture. Dilution of the virus stock used to superinfect the Raji cells leads to a diminution of the number of polykaryocytes

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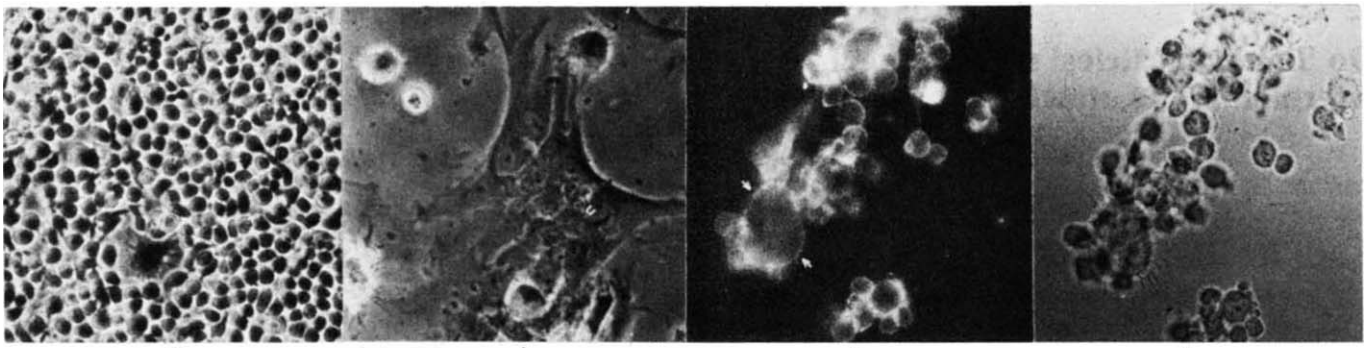

Fig. 3 The formation of polykaryocytes between infected Raji cells and cells devoid of EBV receptors. Raji cells were applied to ALG-coated plates to yield very sparse monolayers (approximately 10% confluent). After the cells had attached, virus was added and 1 h later either Jurkat (T-lymphoblastoid cells; *a*, *c*, *d*) or human embryo fibroblasts (*b*) were added to give a subconfluent monolayer. The cultures were incubated at 37 °C and photomicrographs prepared 20 h after infection. The cultures in *c* and *d* were stained with anti-T-cell serum. *c* Shows a polykaryocyte with T-cell membrane fluorescence. The arrows indicate areas where the polykaryocyte is free of adherent cells (see *d*) and shows clear membrane fluorescence. *d* Is a photograph of the same group of cells taken under normal light. At least three nuclei can be identified within the polykaryocyte. *a*, *b*, ×650; *c*, *d*, ×1,000.

observed (Fig. 1*b*; virus diluted 1 : 100). Immobilization of Raji cells without addition of exogenous EBV does not induce polykaryocyte formation. With minor modifications this technique can be used to titrate stocks of EBV. A similar series of experiments, in which infected cells were treated with sodium azide or cycloheximide, or in which cells were infected with UV-ray inactivated virus or virus treated with neutralizing antiserum, indicated that at least partial expression of EBV is required to induce cell fusion.

The high multiplicities of infection necessary to induce a full or partial virus reproductive cycle prevents observation of the interaction between infected and uninfected cells. To obviate this problem, superinfected Raji cells were washed to remove unadsorbed virus and mixed with uninfected Raji cells in a ratio of 1 : 10. The mixture of cells was applied to ALG-coated plates and the cells were allowed to attach as described above. After 48 h, numerous multinucleate cells could be observed and in some of the syncytia the nuclei had begun to disintegrate or coalesce. Figure 2 illustrates the various stages of syncytium formation.

These experiments show that EBV can induce fusion between cells having receptors for EBV, but not whether EBV can induce fusion between receptor-positive and -negative cells. We studied the interaction of superinfected Raji cells with human T-lymphoblastoid cells (Jurkat) or human embryo fibroblasts (HEF). Raji cells were applied to ALG-coated plates to give very sparse monolayers with a cell density of less than 10% of confluency. These cells were infected *in situ* by adding a stock of EBV; after the virus had been adsorbed by the Raji cells, Jurkat or HEF cells were added. After incubation at 37 °C for 20 h, the cultures were photographed (Fig. 3*a*, *b*). From these photographs it is apparent that the cells had fused giving rise to polykaryocytes. Fluorescent antibody staining of the cultures showed that the polykaryocytes contained EBV antigens (EA and VCA) and that the polykaryocytes formed between superinfected Raji cells and Jurkat cells had T-cell membrane determinants on their surface (Fig. 3*c*, *d*).

Our observations suggest that close cell-to-cell contact, as it occurs in closely packed monolayers, is necessary for the development of multinucleate cells. A close contact between epithelial cells and lymphocytes (which may carry EBV genomes) has been observed in the lymphoepithelium of Waldeyer's ring of the throat[11]. The exceptionally close contact between the lymphocytes and epithelial cells in this unique tissue may provide the necessary conditions for EBV-induced fusion between the two cell types. For further considerations of this aspect, and the relationship of EBV to other lymphoepithelial and undifferentiated carcinomas of the Waldeyer's ring see ref. 12.

In situ hybridization studies[13] with NPC tumour material indicate that the cancerous cells carry a heavy load of viral genomes. This could be achieved in one of two ways: either the virus is activated, enters into a lytic cycle and replicates independently of the host cell using virus-specified DNA polymerase[14] or, as in cell lines which carry the genome but cannot enter a lytic cycle, the viral DNA can be replicated using host replicative machinery (for example, see ref. 15). Lymphoblastoid cells in a lytic cycle which contain several thousand copies of the viral genome[16] could fuse with epithelial cells transferring its entire viral load. Tumour cells contain large quantities of viral DNA, the near diploid or near triploid karyotype and chromosomal abnormalities[17] fusion of EBV-carrying lymphocytes with epithelial cells could generate such abnormal cells. In addition the hybrid cell could then eliminate most of the genetic material originating from the lymphoblastoid cell and regain a near normal chromosome number. The efficiency with which EBV can induce cell fusion in tissue culture supports the hypothesis that EBV-induced cell fusion may be important in the infection of epithelial cells, which are normally refractory to infection by this virus, provided that certain conditions of virus activation and close cell contact are met.

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