Electron microscopic evidence of herpesvirus in association with oesophageal carcinoma

ISOBEL M. SPENCE

Summary

It is now accepted that the herpesvirus group is an aetiological agent in oesophagitis, and it is known to play a role in various neoplasias. This is the first report of the finding by electron microscopy of herpesvirus in biopsy material from a clinically diagnosed case of oesophageal carcinoma.

No definitive answers on the aetiology of carcinoma of the oesophagus, so common among our black and coloured populations, have as yet been advanced, the consensus being that it is multifactorial. Oesophagitis, which in Iran may be a predisposing factor for oesophageal cancer, is also well recorded in patients with other neoplasias or severely debilitating disease. Strains of herpes simplex virus type 1 (HSV-1) and cytomegalovirus have been isolated from patients with oesophagitis, and herpetic inclusions have been found in histological preparations or by electron microscopy. This report records the first demonstration of herpesvirus in association with oesophageal cancer.

Material and methods

A small portion of tissue obtained at biopsy from a black woman with a clinical diagnosis of oesophageal carcinoma was fixed in 5% glutaraldehyde in cacodylate buffer, post-fixed in 1% osmium tetroxide in the same buffer, dehydrated with acetone and embedded in Araldite. Thin sections, post-stained with uranyl acetate and lead citrate, were examined in an Hitachi HU 11B electron microscope at 75 kV.

Results

Histological examination showed fibrofatty tissue with a small island of atypical squamous epithelial cells and some lymphoid tissue which was normal in appearance. There was no evidence of either an inflammatory reaction or a viral infection, but the sample was reported as being too small for adequate study. Only epithelial cells were present in the material examined in the electron microscope. These could be classified as: (i) apparently normal oesophageal tissue identified by the narrow intercellular spaces, well-developed and numerous intercellular desmosomes and a network of cytoplasmic microfilaments (Figs 1 and 2); (ii) quasinormal oesophageal tissue which resembled the normal counterpart in all respects except for the presence of occasional herpesvirus particles (Figs 2 and 3), thick-walled vesicles (Fig. 2), intranuclear fibres (Fig. 1) and coronavirus-like particles within cisternae of the endoplasmic reticulum (Fig. 4); and (iii) neoplastic cells, which were usually separated by wide intercellular spaces, had fewer and less-well-developed intercellular desmosomes but contained many intracytoplasmic desmosomes (Fig. 5), numerous fibrogranular mitochondria with vestigial cristae (Fig. 5) and some mycoplasma-like elements (Fig. 6). No virus particles were present within these cells, nor was there any thickening of the well-defined trilaminar plasma membrane. Coated vesicles and numerous microfilaments, dispersed or in dense bundles (Fig. 5) and frequently in a paranuclear position, were found. Non-membrane-bound lipid globules were present in all three

Fig. 1. Cell showing nucleus (N) with large nucleolus and intranuclear fibres (white arrows), desmosomes (d) between adjacent cells and microfilaments within the cytoplasm of both cells (black arrows) (x 23 700).

Fig. 2. The cytoplasm of adjacent cells showing the thickened plasma membranes (m), well-defined desmosomes (d), thick-walled vesicle (thick arrow) microfilaments (thin arrows) and an unenveloped herpesvirus particle (vp) (x 80 000).
types, but appeared to be more densely osmiophilic in the cancer cells. The nuclei of the latter had irregular outlines, but in all cells the nucleoli were prominent and the chromatin clumped and marginalized (Fig. 1).

Discussion

The ultrastructural features of the apparently normal oesophageal cells were no different from those described by other authors, but it is recognized that these normal-appearing sections could equally well be regions of quasinormal cells which do not contain the dense-walled vesicles or intranuclear fibres considered by Johannessen to be characteristic of epithelial pearls. The presence of herpesvirus particles within these cells raises the possibility that only the cells of the epithelial pearl were vulnerable to infection, which seems unlikely since a florid infection may be ruled out owing to the paucity of the particles. The more likely explanation is that the occasional production of viral progeny arises from a latent infection — a known characteristic of the herpesvirus group. The association of coronavirus-like particles may represent an opportunistic infection of pre-stressed cells similar to the *Candida albicans* association in herpesvirus-induced oesophagitis (where electron microscopic examination also confirmed the presence of yeast and herpesvirus within the material). However, coronaviruses have been identified in cases of Balkan nephropathy in which urinary tract tumours do occur, in lympho-epitheliomas of the nasopharynx and tonsil, lymphomas of the tonsil and a case of transitional cell carcinoma of the bladder. The role of the herpesvirus group in oesophagitis is well documented; among the many publications are records of tissue culture isolations of HSV-1, and cytomegalovirus, herpesvirus-like and cytomegalic inclusions have been found in histological sections, and varicella zoster virus has also been implicated. The fibrillar structures in the nucleus are similar to those described by Luetzeller and Heine within the nucleoplasm of herpes simplex virus-infected WI-38 cells 24 hours or later after infection. In the specimen from my case no lymphocytic, polymorphonuclear or parakeratotic cells were noted, neither were membrane whorls nor oedematous changes seen, all these being ultrastructural features reported by Hopwood et al. in 20 patients with oesophagitis. Underlying malignant or debilitating disease is often associated with oesophagitis, which in Iran is considered to be a predetermining feature of oesophageal carcinoma.

The relationship between the herpesvirus group and neoplasia is well known in Burkitt's lymphoma, and roles for HSV-1 in carcinoma at the site of cold sores, herpes simplex virus type 2 (HSV-2) in carcinoma of the uterine cervix and cytomegalovirus in Kaposi's sarcoma have been advanced. Furthermore, a 'hit-and-run' theory is under consideration for some viruses which suggests that the continued presence of viral DNA is not required to maintain the transformed phenotype; experimental evidence for this has come from studies with the Abelson murine leukaemia virus and HSV-1 and HSV-2. The absence of herpesvirus particles in the neoplastic type 3
cell described here is not surprising, since even in Burkitt's lymphoma no virus has yet been found in vivo, although its DNA can be identified within the host cell genome in approximately 87% of cases; viral progeny can nevertheless be induced in vitro under special conditions. Mycoplasmas are often found as contaminants of neoplastic tissues, and although no proven explanation for their presence has been advanced they are not known to be oncogenic. Intracytoplasmic desmosomes are occasionally found in the normal oesophagus but occur very frequently in oesophageal cancer. Gottrann's carcinoid papillomatosis, spinocellular carcinoma, kerato-acanthoma and Bowen's disease and also in human amion tissue cultures infected with varicella-zoster virus. The association of herpes-viruses and oesophagitis, the presumptive relationship between oesophagitis and oesophageal cancer (at least in Iran) and the known role of these viruses in neoplasia suggests that a herpesvirus could initiate malignant change in the oesophagus. However, the findings in this one case cannot be construed as evidence for or against such a supposition.

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REFERENCES

Suicide in Scandinavia
There is a popular tradition in the Western World that Scandinavians are more prone to commit suicide than other Europeans. However, one has always wondered whether the truth of the matter was not that Scandinavians are more honest in reporting their suicides. Suicide statistics are notoriously unreliable, especially in cases of death by poisoning or drowning, but two articles in the Tidsskrift for den Norske Lægeforening suggest that suicide statistics in Norway are indeed relatively reliable and that, as in many other Western countries, there is a definite increase in the number of suicides among younger adults and adolescents.

A physician, a psychiatrist and a toxicologist (Ekeberg et al., Tidsskr Lægeforen 1985; 105: 123) made an independent study of 210 records of medicolegal autopsies and were asked to classify them as 'certain suicides', 'possible suicides', or 'very unlikely suicides'. All but 3 of these deaths were caused either by poisoning (141), by carbon monoxide inhalation (25) or by drowning (41). The evaluation was compared with the official cause of death recorded in the Central Bureau of Statistics. This comparison showed that at the most there was an under-reporting rate of about 10%. Since poisoning and drowning comprise only 40% of all suicides and it is likely that other methods such as shooting or hanging are fairly accurately diagnosed, the total error in the estimates must be well below 10%.

The other study by Tetterstad et al. (Tidsskr Nor Lægeforen 1985; 105: 119) examined all the recorded suicides in Oslo in the age group 15-29 years over the 7-year period 1975-1981. There were a total of 148, 110 men and 38 women, and in both sexes the commonest method of suicide was poisoning. Two main conclusions arise from this study. The first is that suicide among adolescents and young adults in Norway is on the increase to a greater extent than in Denmark, Sweden or Finland, although the Norwegian suicide rate is well below that in the other three countries. The second conclusion is that many of these young people had been at risk: 42 had previously been admitted to a psychiatric institution, 20 with a diagnosis of psychosis and 22 with an alcohol or drug problem. In another 52 people it was known that an alcohol or drug-abuse problem existed, while in a total of 73 of the suicides either alcohol or a drug of abuse was found in the blood at autopsy.

In an accompanying editorial Lavik (Tidsskr Nor Lægeforen 1985; 105: 111) points out the vulnerability of both males and females in this age group. It is a time when important decisions have to be made about further education, occupation, sexual relationships and ideological responsibilities. If the choices are difficult or obstructed, the result may be self-destruction. This is both a tragedy for the individual and a loss of a valuable resource for the community.