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Viruses and tumours

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1. Introduction

Despite the fact that viruses have been known for nearly 60 years to play a causative role in animal cancer – spontaneous, as well as induced – the final step, isolation of an infective agent, has yet to be taken for any example of the human disease. I want, today, to consider briefly some of the characteristics of virus-cell interaction in the aetiology of animal cancers and the application of such data, if any, to the search for viruses, or virus-like agents, especially for leukaemia and the Burkitt tumour.

2. Virus-cell interaction in the production of animal tumours

2.1. Viruses containing RNA

In general, and we shall consider the exceptions later, the animal tumour viruses which contain RNA multiply in the malignant cells which they induce. This means that they can be visualized in the cells by electron-microscopy, separated from cellular material by a number of methods, and inoculated into test animals, usually newborn, to reproduce the disease. Koch's postulate is thus fulfilled.

Most of these viruses are completed in the cytoplasm of the cell, usually at a membrane, and incorporate host cell antigens into their structure. In addition, the infected cell may manufacture soluble complement-fixing antigens of a non-virion nature and also transplantation-type antigens (non-virion neo-antigens) which can be detected in certain circumstances by graft-rejection phenomena in suitably immunized hosts.

This is a simplified picture and some of the exceptions are of interest for our comparative purposes.

(a) The virus itself may not be the sole determinant of the tumour. The induction of mammary cancer in mice, for example, requires three factors, genetic, hormonal and viral, each of which may determine the outcome.

The murine leukaemia agents may be transmitted vertically from mother to offspring but neonatal thymectomy reduces the incidence of the disease.

There are genetic factors associated with the induction of chicken sarcomas in different strains of fowls by Rous sarcoma agents.

(b) One strain of RSV – RSV(B) – is said to be 'defective' in the sense that, whereas it can transform normal fowl cells into malignant cells, these are unable to achieve synthesis of new virus without the intervention of another virus – RAV – which is normally present as a contaminant virus in laboratory stocks of the former. RAV has structural and biological properties similar to those of the 'defective' RSV(B), but it cannot be excluded that other, unrelated, viruses could act as 'helpers'.

The way in which an RNA virus brings about an apparently genetic change in the transformed cell population is not yet clear. Some observers [1] believe that a complementary DNA is synthesized by the transformed cell and that this becomes incorporated into the genome of the malignant cells.

2.2. Viruses containing DNA

Although DNA-containing tumour viruses have been known since the discoveries of SHOPE in the mid-1930s, it was the discovery of polyoma virus in 1950, and the application of *in vitro* methods to its study that has lead to the very substantial progress in this field.

Whereas, the RNA viruses usually multiply in the transformed cells, the DNA viruses usually do not. Correspondingly they cannot readily be visualized by electron-microscopy in the tumour cells and their presence has to be inferred from other evidence. It is generally believed that the viral DNA is incorporated directly into the cell genome with the consequence that

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the host cell is transformed and new, non-virion antigens appear – soluble, complement-fixing and structural transplantation. *In vitro*, of course, in conditions in which virus multiplies rapidly, virion antigen is also synthesized and it is by no means excluded that, in tumours, occasional cells lyse and liberate very small amounts of virus.

In SV-40 virus-induced tumours of hamsters the capacity of the malignant cells to liberate virus in association *in vitro* with sensitive normal cells has been called "induction by association" [2,3]. A similar phenomenon has been recorded for some rat tumours induced by the Schmidt–Ruppin strain of Rous sarcoma virus [4,5] but the question whether this represents the 'induction' of viral synthesis *de novo* or the 'amplification' of otherwise undetectable amounts of virus produced 'spontaneously' remains to be resolved, This is an important question for the study of human tumours under similar conditions *in vitro.*

The complement-fixing, and transplantation antigens, appear to be specific to the virus, or related groups of viruses responsible for their production. Even after many transplant generations the tumours induced in hamsters by polyoma, or by SV-40 viruses betray their viral origin by reason of these 'new' antigens – complement-fixing or transplantation, or both – which the malignant cells still carry.

It has been comparatively easy, therefore, to screen large numbers of human tumours and sera for complement-fixing antigens (or antibodies) for a variety of viruses – those such as adenoviruses which are known to be oncogenic for animals, and those which, at present, are only under suspicion. The results have invariably been disappointingly negative.

2.3. Difficulties in interpretation

It might not be inappropriate at this point to consider some of the difficulties in the interpretation of results obtained in these ways before considering leukaemia and the Burkitt tumour in more detail.

First, considerable cross-reaction in complement-fixation tests for 'specific tumour antigens' have been encountered in studies of SV-40, polyoma and non-viral (F.Sa3) hamster tumours [6] and the greatest care is therefore required in the conduct of the tests.

Second, SV-40 and human wart virus appear to induce cross-reacting transplantation antigens *in vitro* [7].

Third, viral hybridization may complicate the whole picture. In brief, adenovirus 7 (which had been passaged twenty-two times in monkey cells containing SV-40 virus but had been freed from SV-40 subsequently) produces the virion antigens of adenovirus 7 but the non-virion neo-antigen of SV-40 [8]. Type 3 adenovirus may also be hybridized but it remains to be seen to what extent, e.g. 'non-oncogenic' (for animals) DNA virus can 'rescue' oncogenic DNA from (malignant) cells and become oncogenic hybrids. If the hybrids can transform cells without being further reproduced their detection may be extremely difficult. In this connection it has been reported that SV-40 virus DNA can transform cells *in vitro* but these cells may show neither specific complement-fixing nor transplantation antigen [9].

Fourth, the relationship of the two types of non-virion neo-antigens needs to be clarified. Most observers agree that they are different and one possibility is that the soluble antigens represent new enzyme moieties induced in the transformed cell for the production of viral DNA.

Fifth, the superinfection of transformed cells by viruses or other organisms may again lead to difficulties in the interpretation both of electron-microscope and of immunological data. It must surely be obligatory for those who are looking for aetiological agents for malignancies in man to specify the steps they have taken to exclude the contribution of contaminating, or super-infecting, organisms to their results. This is especially true for those who purport to have found "tumour specific antigens" in such malignant cells. Resistance of tumour cells to viral superinfection is not a criterion for their own viral transformation, and one suspects that many of the cells, or cell lines, or even culture media used for association *in vitro* with cancerous tissues (or extracts of them) of man harbour more than one active or latent, inducible virus or virus-like agent with which to surprise the investigator, whether he uses electron microscopy, density-gradient centrifugation, or animal inoculation – or all three.

Sixth, and last, we know that virus-induced animal tumours, transplanted in normal hosts, may lose, or appear to lose, their induced transplantation antigens [5,10,11]. Whether this is loss, or masking, remains to be seen but the essential point to grasp is that most types of cancer in man have many years in which to 'progress' and, by the time they have become clinically apparent, the cells may bear slight relationship in biological properties to those with which the tumour began.

2.4. Viruses and leukaemia in man

I cannot repeat each time the deciderata which I have given above but I would ask you to bear them in mind while I briefly review progress in two related fields – leukaemia and the Burkitt lymphoma.

Leukaemia – Great interest was aroused by the discovery of so-called "clusters" of leukaemia in children and the subsequent suggestion that those of Niles, Ill. and Orange, Texas, were associated with congenital heart disease [12]. Some 15% of very young children with such heart malformations showed cyto-pathic agents in their kidneys [13].

Electron-microscopists have seen a number of different agents associated with leukaemia – (i) Myxovirus-like and 178 μm in diameter in the sera of acute leukaemic children (138 μm for those with infectious mononucleosis) [14]. Some 3% of normal children (but not normal adults) showed similar particles [15] and it has been suggested that these are platelet breakdown products. (ii) Eight out of 56 acute leukaemics showed 80–90 μm particles (with 1 or 2 membranes) in their blood. These particles had tails when prepared in PTA [16]. (iii) Nuclear (or cytoplasmic) particles (45–70 μm) were found in eight out of thirty patients with acute granulocytic leukaemia [17] and (iv) virus-like particles (similar to murine leukaemia viruses) have been "seen" in bovine and human milk [18].

Attempts to show that leukaemic cells induced antibodies in the same patient were unsuccessful in one series [19] but, in a different approach [20] fluorescein-labelled rabbit anti-sera to leukaemic human plasma reacted with the bone-marrow and blood of 41 out of 72 patients with leukaemia – but not with those of 25 controls. The same antiserum reacted with 50% of the cells cultured by Epstein from a Burkitt lymphoma. Labelled rabbit antiserum against Rauscher's leukaemia virus behaved in the same way in both tests.

Isolation experiments from leukaemic bone-marrow or sera were originally believed to have demonstrated a virus, cytopathic for human embryo kidney cells in culture [21,22]. Subsequent work has demonstrated that this is most probably a cytopathic mycoplasma (PPLO) [23,24] unrelated serologically to those hither to described in man. Some observers believe that a virus may also play a role in this complex [25] but mycoplasma have now been isolated directly in agar culture from leukaemia bone-marrow [26] or visualized with the electron-microscope [27].

Whether these agents are 'drivers' or merely 'passengers' remains to be determined, but it is of some interest in this connection that eighteen out of twenty-two mycoplasma strains tested by Schmidt *et al.* [28] blocked or destroyed the I receptors of normal red blood cells *in vitro* and that, whereas less than 0.1% of normal individuals are I-negative, thirty-eight out of 124 leukaemic individuals were found to be so.

Burkitt lymphoma – I shall assume, for reasons of space and time, that you all know the background to this story and the reasons why it appeared to be worthwhile to conduct a search for a transmissible (probably insect-transmissible) agent. The epidemiological situation has now changed and I must comment on this before I describe the most recent attempts at virus isolation. Although the geographical distribution of the disease within Africa remains much as Burkitt has postulated – and it has also been seen in other rain-forest areas such as New Guinea – exceptions have been described both in the age distribution and the finding of cases in children living above the 5000 ft. contour [29].

O'Conor [30] has stressed that there are only two differences between African (Burkitt) and European or American lymphomas – the unusally high frequency in Africa and the predilection for the bones of the jaw and face, and both he [31] and Dorfman [32] have recently described cases in the files, respectively, of the Armed Forces Institute of Pathology in Washington and the Department of Pathology, Washington University School of Medicine, St. Louis, Miss., USA.

Direct virus isolation studies from the tumours have revealed – (i) Herpes simplex virus [33,34] – but the normal population secrete this virus, too; (ii) reovirus III [35] – again a virus with a very wide distribution; and (iii) a number of unidentified viruses [36] – which may, indeed, be mycoplasmas.

Cultures of lymphoblasts from Burkitt tumours have revealed herpes-like viruses in electron-microscopic examination of the cells [37,38]. This virus was not Herpes simplex but cell cultures carrying it appeared to show viral interference [39]. The probable identity of this as cytomegalic inclusion disease virus has been suggested by Huebner [40]. This is a member of the herpes virus group, has similar size and biological properties, is widespread (especially where socio-economic conditions are poor) and has a particular affinity for salivary glands [41].

There has also been a claim that the disease could be transmitted to young African green monkeys by direct inoculation of biopsy material [42]. However, two separate groups of investigators [43,44] claim that the lesions seen in these monkeys have also been seen in untreated 'normal' African green monkeys kept in captivity and the relationship to the inoculum is therefore questioned.

Dalldorf [45] believes that the significant fact about the Burkitt lymphoma is its geographical association with malaria and the role of any, or all, of the agents so far described remain to be determined. They might all be passengers, one or other could be the aetiological agent but, even so, might not act alone but in association with other factors which *per se* might be geographically determined.

Finally, a few comments on aspects of the search for viruses as a cause of human tumours.

I am impressed by the absence of tumours in hamsters which have received inoculation of very many different viruses of human origin [46]. Only the adenovirus group provided oncogenic strains.

The herpes virus group is, however, of equal interest – and must be more closely investigated. The association with the Burkitt lymphoma may not be just coincidence. We should recall first that herpes simplex has been implicated in carcinoma of the lip [47]; second, that these viruses can cause chromosome breakage in infected cells

in vitro – herpes zoster also acts like colchicine on human embryo lung [48] – and third, that they (and other viruses [49]) can act synergistically with chemical carcinogens in the induction of papillomas in mice [50].

The possible intervention of adenoviruses in the aetiology of cancer in man is being throughly investigated especially with reference to cancer of the respiratory tract. It would be a mistake to exclude the myxoviruses from this consideration. Kotin and Wiseley [51] employed influenza viruses combined with a synthetic Los Angeles 'smog' to produce adeno-carcinomas of the lung in mice. PR8 virus transforms mouse kidney cells in culture [52] and Harris and Negroni [53] have obtained malignant lung cancers in mice exposed to aerosols of influenza viruses alone during our investigations of a possible synergism between cigarette smoke and influenza virus in the aetiology of lung cancer in man.

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