1. HOW VIRUSES GET INTO CELLS

R. A. Weiss
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In his presentation, Professor Weiss began by reviewing the major strategies by which viruses can enter cells; he then described in detail the mechanisms of entry of one particular virus, the human immunodeficiency virus (HIV).

The first step in virus infection is binding of the virus to the cell membrane by recognition of specific receptors. Some viruses, such as Sendai virus and herpes simplex virus, have envelopes which fuse directly with the plasma cell membrane, releasing the virus nucleocapsid core into the cytoplasm. Other viruses, with envelopes (influenza virus, vesicular stomatitis virus, Rous sarcoma virus) and naked (reovirus, adenovirus), use a process termed ‘receptor-mediated endocytosis’. Taking influenza as an example, the haemagglutinin glycoprotein anchored into the virus membrane has a cleft which recognises sialic acid residues on the cell membrane. After binding, the virus is endocytosed via a coated pit. As the intravesicular pH falls, the haemagglutinin undergoes a conformational change to expose a hydrophobic domain which allows fusion of the virus envelope with the cell membrane and initiates virus replication.

The mechanism of HIV infection has been studied in great detail since this retroviral infection causes depletion of a particular subset of lymphocytes, the T helper (Th) cells, during the development of AIDS. The HIV molecule has two types of envelope glycoprotein—gp41 embedded in its membrane and gp120 attached to gp41. The gp41 recognises the cell surface receptor and then mediates membrane fusion. The cellular receptor is the CD4 antigen, present on, amongst others, Th cells. Several experiments demonstrate this:

1. Most cells capable of being infected display the CD4 receptor; they include antigen presenting cells in the skin (Langerhans cells), monocytes, macrophages and Th cells. Some cells, however, can be infected in vitro even though they do not possess the CD4 receptor; examples are glioma cells and cells from malignant brain tumours in which low levels of the mRNA for CD4 are expressed.

2. Monoclonal antibodies to CD4 inhibit binding of the virus to the cell.
3. Infected cells that bear the HIV gp120 glycoprotein fuse with CD4 + Th cells to form large syncytia. This process can be blocked with anti-CD4 antibodies.
4. The transfer of the CD4 gene to another cell confers susceptibility to HIV infection. HeLa cells transfected with this gene form syncytia on exposure to the virus.
5. Recombinant gp120 molecules bind to CD4 molecules with high affinity. Assays using colloidal-gold labelled antibodies to CD4 show endosomes within the cell containing the CD4 receptor. However, this receptor-mediated endocytosis is not required for HIV infection since HeLa cells, transfected with CD4 mutants unable to endocytose, show no loss of susceptibility to HIV infection or HIV-induced cell fusion. Therefore fusion at the cell surface appears to be sufficient to allow HIV functional entry into cells.

The CD4 molecule of 433 amino acids has four variable domains. The precise binding site of HIV gp120 has been elucidated by using monoclonal antibodies to several different epitopes of the CD4 molecule. The antibodies Leu 3a and OKT4a, raised against a site on V1, prevent not only the binding of HIV-1, but also the binding of HIV-2 and simian AIDS virus (SIV). Site-directed mutations to the DNA of the region involved in antibody recognition found a 7 amino acid sequence (residues 42–48) on CD4 to be crucial for gp120 recognition.

Various methods can be employed to prevent binding of virus to the cell:

1. Soluble, truncated, CD4 molecules containing the gp120 recognition site will bind to gp120, thus neutralising virus infectivity. Soluble CD4 molecules neutralise all strains of HIV and are therefore being explored therapeutically.
2. Anti-CD4 monoclonal antibodies saturate cellular CD4 receptors.
3. Anti-idiotypic antibodies can be made to the monoclonal antibodies of the CD4 receptor binding site. It was hoped that they would also recognise the specific site of gp120 without competing with the normal functions of the CD4 receptor. However, only weak neutralisation is seen.
4. Some antibodies raised against the viral glycoproteins are highly specific. Anti-gp120 from a particular strain of HIV-I will neutralise HIV-I at a dilution of 1 in 100,000, possibly by preventing virus binding to CD4.

In conclusion, the primary stage of HIV infection, that of the retrovirus binding and fusing with the cell membrane, may be a stage at which intervention can take place and may have a role in antiviral therapy or immunisation.

2. VACCINE STRATEGIES AGAINST INFECTIONS

M. A. Epstein
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In his talk, Professor Epstein gave a concise summary of the types of vaccine and requirements in their development. He also discussed various vaccine strategies, their usefulness and applications in potential future vaccines.

The aim of vaccination is to induce protective immunity against a noxious agent. The toxins may be altered to form a toxoid, such as the formaldehyde inactivated tetanus toxin which can be safely used in immunisation, and the formaldehyde inactivated poliovirus used in the Salk vaccine. Alternatively, infectious agents may be attenuated by continuous passage in culture to lose virulence yet maintain antigenicity, as in the Sabin poliovirus vaccine. In smallpox vaccine a similar strain of poxvirus, vaccinia, is used which is not as pathogenic but stimulates antibodies to provide immunity against the virulent agent. Subunit vaccines are also used: the surface antigen of hepatitis B virus is the major determinant of pathogenicity and is present in all hepatitis B carriers; vaccination with the purified antigen can give protection against whole virus infection.

In developing a vaccine certain requirements are imposed: (1) a susceptible test animal must be available on which to test the vaccine by exposing it to virulent organisms when protected with the immunogen; (2) an assay to monitor antigen; (3) an efficient method for antigen preparation; (4) the final product must be potently immunogenic; (5) quantitative tests for antibodies and specific cell responses to challenge with the infectious agent.

In recent years a number of alternative vaccine strategies have been developed. Specific immunogenic agents may be cloned into a vector which may be bacterial, viral, yeast or insect cells. This method has been particularly important in the second generation of hepatitis B vaccines where the HBSAg used in immunisation is expressed in yeast and harvested from bulk culture. Vectors have also been introduced into caterpillars and the gene product harvested from the bulk mass of the insect. Recombinant viral vectors are made by cloning the specific gene into a non-pathogenic virus which, on vaccination, will induce an immune response to both the virus and the foreign antigen. Professor Epstein and Dr Mackett have carried out research using the poxvirus strain WR, into which they inserted the gene encoding EBV MA gp340, the surface glycoprotein and major antigenic component of Epstein Barr virus. This glycoprotein elicits both neutralising antibodies and MHC restricted cytotoxic T cell production. When tamarins are vaccinated with the recombinant virus and subsequently challenged with a 100% lymphogenic dose of EBV, 3 out of 4 animals overcame the infection within 25 days. The unvaccinated animals grew tumours 10–14 days post-infection and died within a further week. This work used the virulent WR strain of virus which could never be used for vaccination in man. The avirulent vaccine strain with an inserted gp340 gene gave no protection owing to the low levels of gene expression. Genes have also been inserted into varicella zoster virus which, as a herpes virus, may give life-long immunity, but an animal model is not yet available on which to test the vaccine.

Other methods of vaccine production in early stages of development involve (a) preparation of synthetic subunits (this can prove difficult owing to large and complicated molecules), (b) genetic manipulation of organisms, deleting genes responsible for virulence, and (c) manipulation of vaccines to insert novel antigenic determinants into an already safe live virus vaccine, eg poliovirus Sabin vaccine.

The most promising vaccine development in Professor Epstein’s laboratory involves the large-scale purification of antigens and vaccinating animals with adjuvants such as immunostimulating compounds (ISCOMs) and muramyl dipeptide analogues. ISCOMs act as cage-like structures around the antigen and act as adjuvants when injected at the same time as the antigen. Complete protection from tumour induction is attained in tamarins injected with ISCOMs plus gp340 and subsequently challenged with EBV.

Muramyl dipeptides are powerful immunogens but MDP toxicity occurs with symptoms varying in severity from pyrexia to CNS neuropathy. An MDP-threonyl derivative, when co-injected with purified gp340, produced neutralising antibodies and specific cytotoxic T cells, and conferred protection from tumour formation when challenged with EBV. No toxicity has been shown in any animal tested. A French group has begun clinical trials with the haemagglutinin glycoprotein of influenza virus, and Professor Epstein eagerly awaits the commencement of clinical trials using the gp340 of Epstein Barr virus. This research is now at an exciting stage.

3. ONCOGENES AND CANCER TREATMENT

K. Sikora
Royal Postgraduate Medical School, London

Professor Sikora discussed the role of oncogenes and anti-oncogenes in the development of cancer. One in three people will get cancer; one in four people will
die of it. The frequency of some cancers has increased owing to changes in lifestyle; others have stayed fairly constant over the years. The clues to the treatment of cancer lie in understanding the molecular biology of the cell, and how this relates to the control of cell growth.

The first indication that 'cancer-producing' genes existed was shown by Rous in the early 20th century. He found that injection of a chicken sarcoma into a chicken caused a tumour; the tumourigenic agent was also present in a cell-free extract. The agent was called Rous sarcoma virus and could transform monolayers of cells in vitro, causing loss of contact inhibition and focus formation. This retrovirus, a single-stranded RNA virus, replicating by using reverse transcriptase, had an extra gene named src. v-src mutants were defective in transformation, and v-src was named an oncogene.

Many other animal cancers can be caused by viral oncogenes: v-erbB causes chicken erythroblastosis, v-myb causes chicken myeloblastosis and v-sis causes monkey sarcoma. This is not true for human cancers, but research into viral oncogenes revealed areas of human DNA with considerable homology; they are called c-ons or proto-oncogenes as opposed to the v-ons found in retroviruses. The proto-oncogenes are highly conserved throughout the animal kingdom, the human c-myc gene showing 80% homology with the mouse c-myc gene. The retrovirus has acquired all or part of the proto-oncogene during the dis-integration stage of replication in the nucleus of the cell.

Although many c-ons have been identified, cloned and sequenced within the human genome, their function is not always clear. Many are tyrosine kinases, others growth factors or their receptors; c-sis is part of platelet-derived growth factor (PDGF); erbB-1 is part of epidermal growth factor receptor; c-ras is a GTP-binding protein; c-myc and c-myb are nuclear proteins; c-jun is a transcription factor related to an elongation factor of protein synthesis. The presence of these genes in the cell is not enough to cause cancer, rather they are involved in the regulation of cellular growth and homeostasis. Increased or abnormal expression of the c-ons can cause aberrant growth leading to cancer.

In some tumours there is a constant genomic aberration at the site of an oncogene, seen as a breakpoint, translocation or deletion in the metaphase chromosomes. For example, in chronic myeloid leukaemia, c-abl is translocated from the long arm of chromosome 9 to the long arm of chromosome 22 to form the characteristic Philadelphia chromosome. Similarly, in Burkitt's lymphoma the 8q : 14q translocation involves the c-myc oncogene.

Amplification and mutation of oncogenes can correlate with the progression and prognosis of a tumour. In the past 3 years experiments have been carried out using cloned oncogene probes to score the amplification and genomic rearrangement of oncogenes in particular cancers compared with tumour progression.

Amplification signals tumour aggressiveness and a poor prognosis. Tumours can thus be classified in terms of oncogene expression and patient survival. For instance, neuroblastoma can be graded I to IV, where stage I has 100% survival and stage IV 50% survival; when more than 10 copies of the nuclear oncoprotein n-myc are present there is a 70% survival rate.

These oncogenes and their gene products have potential clinical application in (a) tumour markers, (b) immunohistology, to localise the specific proteins in the tumours, (c) tumour localisation (eg monoclonal antibodies to erbB, when labelled with 125I, will show up a small cell lung carcinoma on a mediastinal X-ray), (d) prognosis (the expression of c-myc in invasive cervical carcinoma in high or low amounts correlates with a poor or good prognosis), (e) cancer risk prediction.

In conclusion, Professor Sikora briefly mentioned the role of anti-oncogenes in tumour development. These are recessive genes whose absence of expression predisposes the individual to a malignant state, eg retinoblastoma and Wilms' tumour. Retinoblastoma is associated with specific chromosomal deletion at 13q14. The cancer can be sporadic in nature as the result of a random deletion on both chromosomes (by, for instance, mitotic non-disjunction). Alternatively, in familial retinoblastoma, one deleted gene is inherited and a second deleting event occurs in childhood before the retinoblastoma develops. Wilms' tumour involves a double recessive deletion at 11p13. Similarly, in the chromosomes of patients with familial adenomatous polyposis coli, a deletion is seen on 5q21-22. This gene needs to be present to maintain normal colonic epithelia, and its deletion can lead to colonic carcinoma.

The above summaries are from papers delivered at the Science and Medicine Conference held at the Royal College of Physicians in November 1988.

| Oncogene | Tumour    | Amplification | Rearrangement |
|----------|-----------|---------------|---------------|
| c-myc    | Breast    | +             | -             |
|          | Burkitt's | -             | +             |
|          | lymphoma  |               |               |
|          | Stomach   | +             | -             |
| n-myc    | Neuroblastoma | +   | -             |
|          | Retinoblastoma | +  |               |
|          | Breast    | +             | -             |
|          | Lung      | +             | -             |
| c-erbB   | Breast    | +             | -             |
|          | Glioma    | +             | -             |
| c-erbB-2 | Breast    | +             | -             |

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