Antiviral Strategies and Vaccines against HTLV III/LAV

A. G. DALGLEISH, BSc, MB, MRCP, FRACP, *Consultant Medical Oncologist, Clinical Research Centre, Northwick Park Hospital, Harrow*

The Virus, its Origins and Nomenclature

The causative agent of the acquired immune deficiency syndrome (AIDS) and related conditions was reported in September 1983 by Barré Sinoussi *et al.*[1] working in Montaignier's Laboratory (Pasteur Institute). At that time there was no evidence that this virus had any causal role in AIDS or related conditions. It was Robert Gallo and his colleagues[2] at the National Cancer Institute (NCI) Washington who first reported (May 1984) the successful isolation and production in a permanent cell line of a new retrovirus which induced specific serum antibodies in the majority of AIDS and AIDS at risk persons[3]. The causal nature of this new retrovirus was quickly confirmed by workers in London, who also confirmed that the French and American isolates were serologically identical[4].

The first French isolates were known as LAV (lymphadenopathy virus) or IDAV (immunodeficiency associated virus). Workers in Gallo's laboratory had previously isolated the first human retrovirus now shown to be the causal agent of the adult T cell leukaemia lymphoma syndrome (ATLL) and this was already known as the human T cell leukaemia—lymphoma virus—I (HTLV-I)[5]. A second similar yet different isolate called HTLV-II has yet to be associated with any specific disease[6]. The French group recognised that 'LAV' and 'IDAV' were new human retroviruses and that they had a proclivity for T4 + T lymphocytes. They therefore referred to them as human T cell lymphotrophic viruses. It was not surprising that Gallo referred to his new isolates as HTLV-III, the 'L' standing for lymphotrophic as opposed to leukaemia or lymphoma. Further isolates reported from San Francisco added to the confusion as they were called AIDS retroviruses (ARV)[7]. The confusion has recently been compounded with an international committee declaring the viruses as 'human immunodeficiency viruses' (HIV)[8]. This decision is not unanimous, but in this article I refer to the viruses as HIV if for no other reason than it is shorter than HTLV III/LAV.

The isolation and establishment of a permanent cell line was not an easy feat, as the virus is cytopathic for most host cells. Permanent T4 + leukaemic T cell lines were eventually used to isolate and produce large quantities of the virus which could then be used as antigen for widespread serological screening.

The major 'hold up' in establishing the French isolate as a causal agent was the inability to establish it in a permanent cell line. It was initially isolated in a low producing B cell line and it was not until workers in Prof. Weiss's laboratory in London established 'LAV' in the T4 + leukaemic line—CEM that the causative role could be investigated. The CEM/LAV line was sent back to Montaignier in March 1984[9].

The availability of the virus in a permanent cell culture is a prerequisite for investigating antiviral stratagems *in vitro,* although it is now possible to 'clone' virus isolates from primary culture without the establishment of a permanent cell line.

The Retrovirus Life Cycle

HIV is a retrovirus which means that it is an RNA virus capable of making double DNA copies of itself which may then intercalate into the hosts cell genome or remain within the cytoplasm. Replication is directed by the enzyme reverse transcriptase (RT) which uses the host's cell replicative mechanism for replication (Fig. 1). In order for a virus to enter a cell it must enter through an appropriate receptor. The presence or absence of this receptor will establish the host cell range. The virus must first bind to the appropriate receptor before gaining entry to the cell as part of the virus receptor complex. Most retroviruses enter by the endocytic pathway from whence the virus is 'delivered' to lysosomes which are able to 'uncoat' the virus, thus releasing the infectious core into the cell. Further replication depends on the RT enzyme and post-translational events include glycosylation of the envelope proteins.

Antiviral stratagems may be aimed at any one of these steps, which may be grouped as follows for possible therapeutic manipulation:

1. Binding to the receptor;
2. Endocytic pathway;
3. Reverse transcriptase inhibitors;
4. Post translation modification.

The Receptor

Viruses use specific receptors which may only be present on a very limited number of cells, or they may use a receptor that is present on a broad range of cells sometimes from many species. The host cell range is often established by performing plaque assays and/or infectiv-

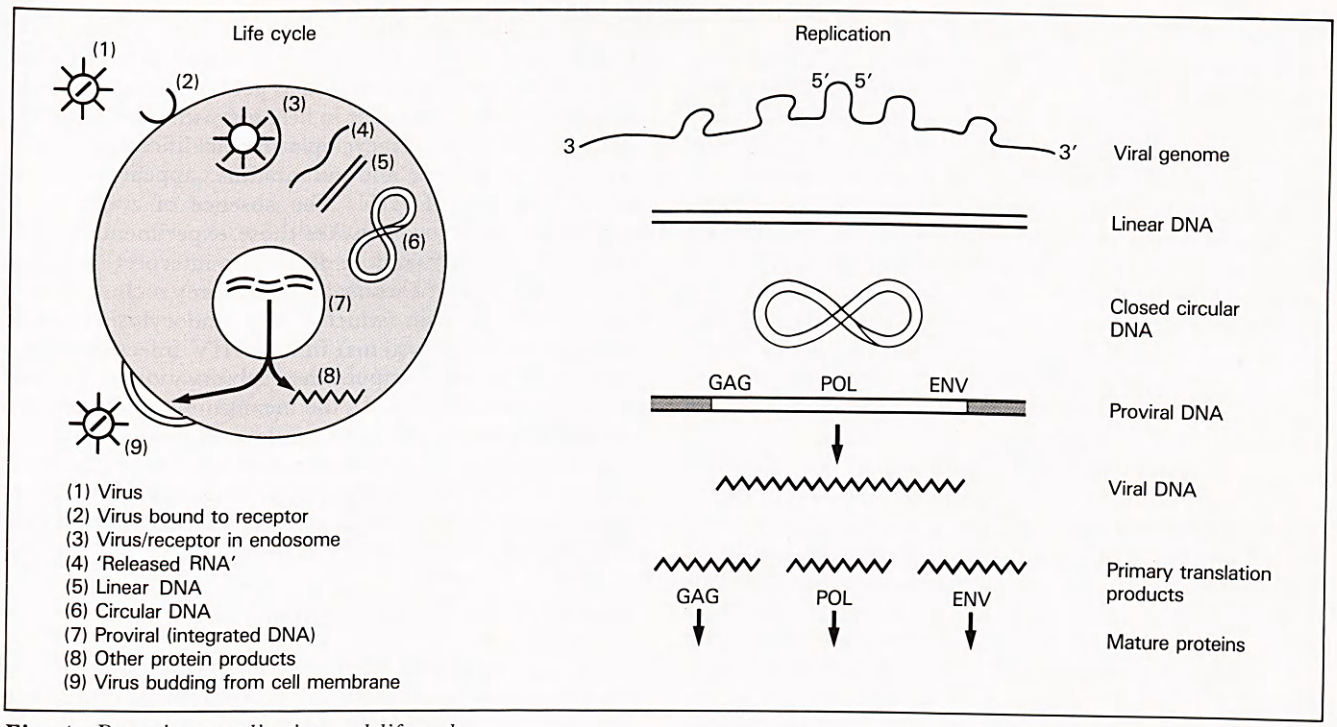


Fig. 1. *Retrovirus: replication and life cycle.*

Table 1. Therapeutic sites of intervention.

1. Plasma ? complement
2. Neutralising antibody
3. Anti receptor/binding, antibody ? idiotype
4. Drugs affecting the endocytic pathway
   - NH_4Cl
   - Amantadine
   - (Chloroquine)
   - (Monensin)
5. Reverse transcriptase inhibitors
   - HPA-23
   - Suramin
   - Phosphonoformate
   - AZT
   - ? others
6. Cytotoxic T cells
7. Anti-sense RNA variants

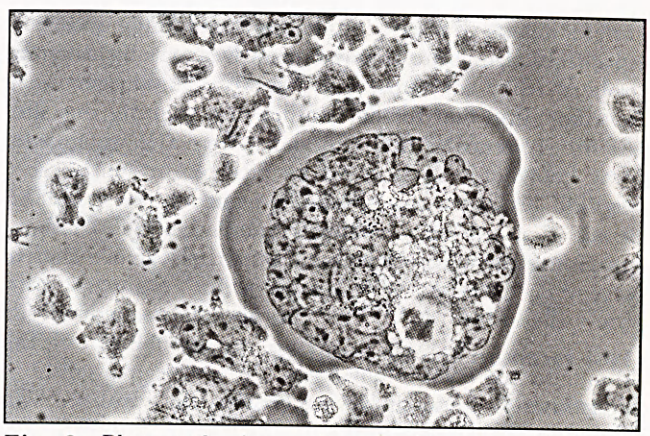


Fig. 2. *Photograph of syncytia or multi-nucleate giant cells formed by the viral 'fusion' of JM T4 + cells.*

ity assays on various cells. As retroviruses do not readily lend themselves to plaque assay systems[10], alternative assays have been devised. The simplest is the syncytial induction assay (Fig. 2) which utilises the ability of viruses to fuse receptive cells so that giant or multi-nucleate cells are seen by light microscopy[11]. Replication within a cell can be assessed by measuring the reverse transcriptase levels. Cells may 'fuse' and form syncytia in the presence of various physical or chemical conditions other than a virus, and conversely not all cells infected with retroviruses necessarily form syncytia. In order to investigate further the viral membrane antigens and the host cell receptors the ability of viruses to combine randomly has been usurped in the form of the pseudotype assay (PT)[12,13]. Briefly, a virus which can readily be used in a quantifiable plaque assay (VSV) is grown through cells producing the retrovirus under investigation. Some of these viruses will form hybrids, being VSV cores with retroviral membrane antigens. Non-hybrid viruses can be 'neutralised' using anti-VSV monoclonal antibody. Such pseudotypes can then be titrated onto various cells, and provided they have receptors for the retroviral antigens they will allow the hybrid virus to enter the cell, thus enabling the VSV core to undergo replication which can then be measured quantitatively by applying an overlay of cells suitable for a VSV plaque assay. Using these assays we were able to show that most HIV infectable cells were T4 + leukaemic cell lines[13], and that those that were not expressed the T4 antigen. Furthermore, using a range of monoclonal antibodies made against various leukocyte surface antigens

obtained from Peter Beverely (ICRF) we were able to show that all anti-T4 (CD4) monoclonal antibodies were able to block both the S1 and PT assays[13]. We concluded that the T4/CD4 antigen was an essential component of the HIV receptor. The possibility that T4 is the only receptor is exciting as this may lead to a therapeutic handle against viral replication. Unfortunately, studies on animal retroviruses have shown us that retroviruses may learn to use more than one receptor[10]. Our further studies showed that no other T cell antigen acts as a component of the receptor (Dalgleish *et al.*, unpublished observations) and that not all epitopes of T4 block infection, i.e. OKT4 as well as some other antibodies to epitopes of T4 in the OKT range do not block infection (Sattentau *et al.*, submitted).

The definitive experiment to show that T4 is the HIV receptor would involve the transfection of the *c*DNA T4 clone into various cell lines that do not normally express the T4 antigen. The recent cloning of the *c*DNA of T4 by Maddon *et al.*[14] has allowed the construction of a wide range of cells expressing human T4 antigens. Results of these studies are in press[15] and show that human cells expressing T4 + antigen are infectable with HIV. Furthermore it has been shown that T4 and the gp110–120 envelope antigen of HIV not only bind but also specifically immunoprecipitate together[16]. What is important is that no cellular antigen other than T4 is brought down. These results are exciting in that they suggest that a specific binding site may be preserved among different isolates (they all appear to use the T4 antigen as a receptor) and that further studies may identify this site. As the sequence of both T4 and many HIV isolates are now known[14,17] it could be possible to synthesise a peptide and raise an antibody against this site. This would clearly be a very important therapeutic strategy, providing the virus did not learn to use an alternative receptor.

Post-receptor: the Endocytic Pathway

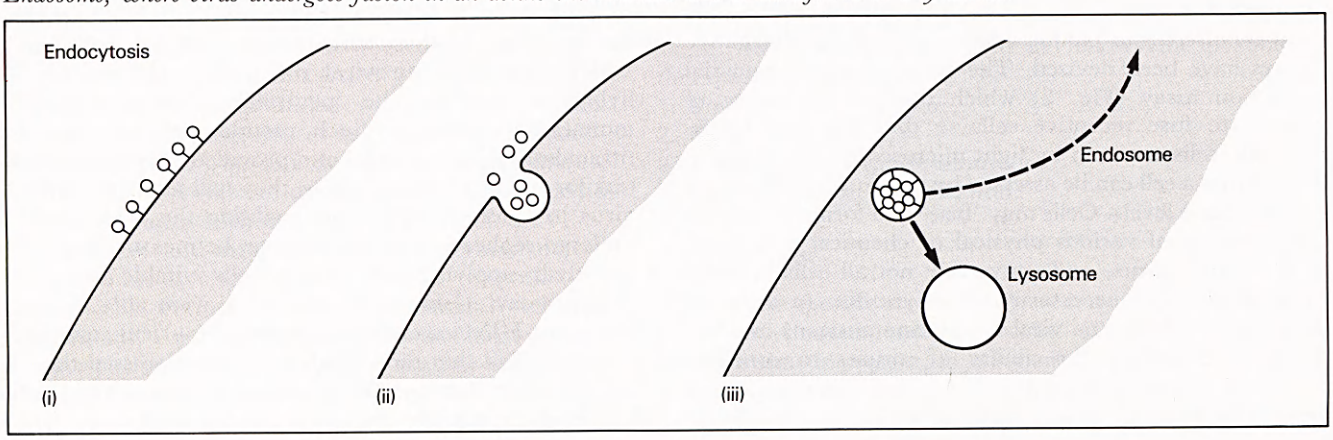
Following binding to the receptor the virus/receptor complex has to enter the cell if infection is to take place. In the case of many viruses this is achieved via the endocytic pathway[18] (Fig. 3). Fusion of the virus requires an acidic environment, without which the virus does not reach the lysosomes and get 'decoated'. Preliminary data have shown that in keeping with other retroviruses, inhibitors of intracycloplasmic acidification such as ammonium chloride and amantadine, appear to reduce the infectability of HIV. The absence of a one viral-replication cycle assay makes those experiments technically difficult and therefore difficult to interpret. We have yet to confirm that carboxylic ionophores such as chloroquine and monensin (which inhibit endocytosis in some animal retrovirus systems) inhibit HIV infection (Dalgleish and Marsh, unpublished observations). Further studies are in progress. In the meantime it may be worth considering amantadine, or similar compound, in a pilot therapeutic trial, as it is readily available. A combination of amantadine with another drug which acts at a different stage of replication, for example reverse transcriptase (RT) inhibitors, may be even better.

Reverse Transcriptase Inhibitors

Reverse transcriptase inhibitors may act at different sites in the enzymatic reaction, as enzyme-binding compounds, template-binding compounds, substrate or product analogues, divalent cation binding agents and miscellaneous compounds[19]. A large number of compounds are known to be inhibitory against the RT of many animal retroviruses[20]. These include (1) suramin that binds directly to the enzyme and other diasylimidazoline derivatives which bind to the template such as Evans blue and direct yellow 50, adenosine analogues such as Ara-A, ribavirin, antimoniotungstate, (HPA-23), pyrazofurin and 5-iodo-2′-deoxy cytidine, thiosemicarbazone R (a cation binding agent) and phosphonoformic acid (PFA).

Following the report that suramin was active against HTLV-III *in vitro*[21], we screened a number of suramin analogues which were found devoid of anti-HIV activity. We then tested PFA *in vitro* and found it to be an extremely active inhibitor of reverse transcriptase at doses of 100–200 μg/ml which are readily attainable *in vivo* (Fig. 4) (Dalgleish *et al.*, unpublished observations). The *in*

Fig. 3. *Endocytosis. 1. virus attached to receptor; 2. virus receptor complex crowd together ('cap') and undergo invagination; 3. Endosome, where virus undergoes fusion in an acidic environment and is transferred to a lysosome.*



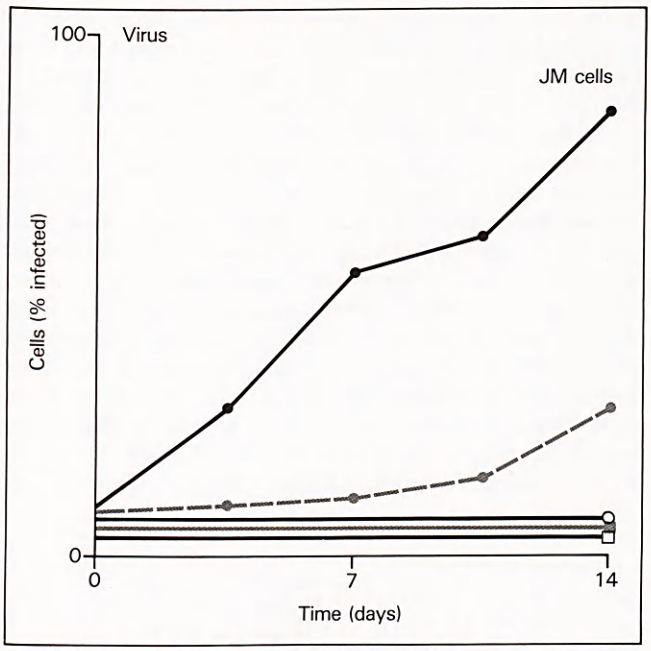


Fig. 4. *The effect of varying doses of PFA on an* in vitro *infectivity assay using high virus producing cells (H93) and very susceptible T4 + cells* (JM). *These results show that PFA at a dose of 100 μm/litre and above inhibit viral replication.*

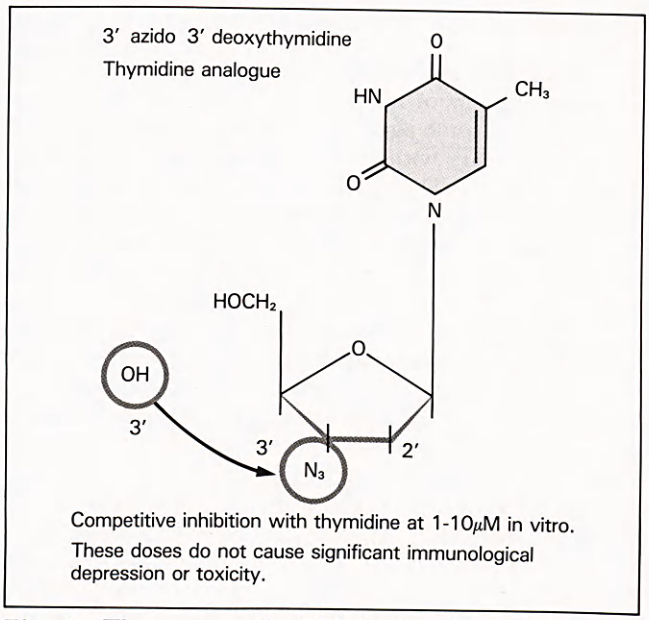


Fig. 5. *The structure of AZT showing the similarity with thymidine with only N_3 replacing OH at the 3′ position.*

vitro findings that PFA inhibited HIV have since been confirmed by other workers[22,23]. As PFA is licensed for use against cytomegalovirus (CMV) and appeared to improve the overall condition of some AIDS patients to whom it was given for CMV infection, we undertook a pilot study of PFA in patients with AIDS and AIDS related conditions, the results of which will be published shortly (Farthing *et al.*).

In vivo studies with suramin[24], HPA-23 [25] and AZT[26] have now been reported. HPA-23 (antimoniotungstate) has been given to three patients with AIDS and one with prodromal symptoms. Although considerable clinical improvement was reported after a short follow-up period, the ability to isolate the virus was reduced during therapy but returned following cessation. A large trial is at present in progress, although considerable toxicity (to marrow) and the inability to cross the blood/brain barrier will limit its further usefulness. Similarly, suramin has been used on 10 patients with AIDS and ARC. Toxicity is also a major limitation, although there is some evidence that this drug is much better tolerated in African patients, and, again, it is not able to cross the gut or blood/brain barrier. Viral isolation studies concur with those for HPA-23. More recently, a new drug known as azido deoxythymidine (AZT)[26] an analogue of thymidine (Fig. 5) has been used *in vivo*. The advantages of a high therapeutic index, oral administration and easy access across the blood/brain barrier make this an attractive therapeutic candidate for long-term administration. A multi-centre pilot study[26] reports significant improvement in clinical parameters, T4 positive lymphocyte counts and delayed-type hypersensitivity skin reactions, as well as a failure to isolate virus from patients given a high dose of parenteral and oral AZT for six weeks. The reported short term toxicity was limited to headaches and leukopenia. Only 19 patients were investigated in this study which needs to be extended to more patients with longer treatment and follow-up periods.

Other Drugs

What is exciting about the results seen with AZT is that similar substitutions in either purines (adenosine, guanosine inosine) or pyrimidines (cytidine, thymidine) could also prevent RT transcription. Therefore a wide variety of possible compounds may lead to an ideal *in vivo,* anti-HIV formulation.

Other drugs have been reported to have potential anti-HIV activity. Ribavirin, an analogue of guanosine, is able to inhibit HIV replication *in vitro*[27] but is unable to protect against cytopathic effects *in vitro* and appears to have no obvious clinical benefit[28].

Dithiocarb (sodium diethyldithiocarbamate) and inosine pranobex (Isoprinosine) also inhibit HIV expression in infected cells[29]. Both drugs are said to increase the number of T4 lymphocytes and are used as 'immunomodulators'. The antiviral effect is weak and is thought to occur at the stage of virus transduction through the membrane and/or DNA integration in the nucleus. A novel lipid compound AL 721 has also been claimed to inhibit HIV replication *in vivo* by altering the cell membrane cholesterol content[30].

Other parts of the cell cycle may also be susceptible to interference such as post translation modification e.g. proteolytic cleavage, glycosylation, acylation and phosphorylation (Fig. 1). I am unaware of any clinical studies of drugs known to affect this end of the pathway. Tunicamycin is known to interfere with glycosylation and is at present being studied *in vitro*.

What Determines Infectability?

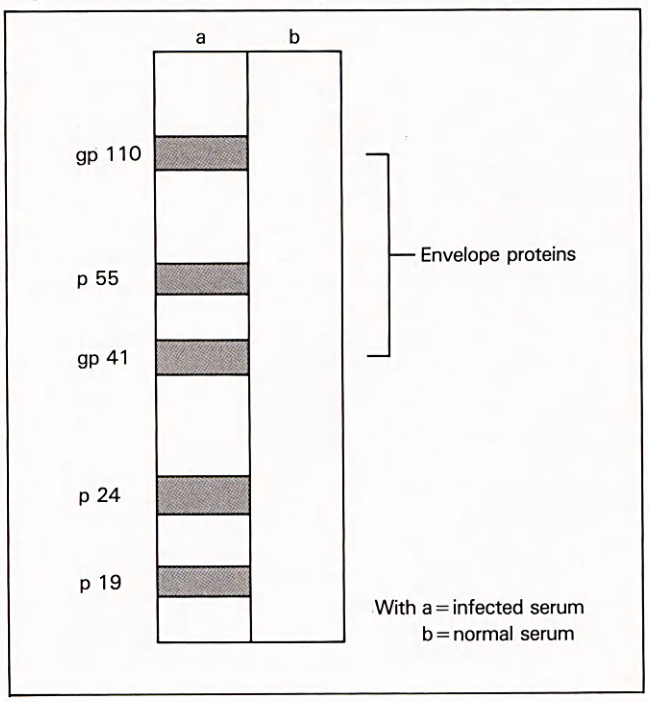
A number of studies are presently looking at sero-negative partners of HIV-positive patients. What is surprising is that some patients have had continual exposure for several years with infected persons and yet remain sero-negative (IVD Weller *et al.*, unpublished observations). Nearly all of these patients including sero-negative haemophiliacs who have had large doses of known infected blood products, have no evidence of virus detectable by virus isolation and molecular hybridisation techniques (E. Miller *et al.*, unpublished observations). It therefore seems likely that some patients are able to 'deal' with the virus before it gets a chance to enter the replication cycle. Human sera have been shown to activate the compliment pathway against most animal retroviruses[31]. The human T cell leukaemic-lymphoma virus (HTLV-I) is not susceptible to destruction by complement[31] and neither is HIV (Clapham *et al.*, unpublished observations).

It would appear that HTLV-I and HIV may be infectious because they are not lysed by the plasma. Clearly it would be interesting to see if there is any difference in susceptibility to complement between sero-positive and sero-negative persons.

Neutralising Antibodies (or lack of)

Following infection and replication antibodies are raised against both core and envelope antigens (Fig. 6). In many virus systems these antibodies are capable of neutralising the virus. Whereas patients infected with HTLV-I have high titres of neutralising antibody[12], patients infected with HIV have low or absent neutralising antibodies (NAB), although they may have high titres of non-neutralising antibodies[12,32]. The small variation in the low titre of neutralising antibodies does not correlate with clinical status and is therefore unlikely to have any protective function. Why then is there no significant neutralisation present in HIV infected patients? A number of possibilities exist. The neutralising epitope may be masked by other epitopes or may be altered by base pair antibodies against other more prominent epitopes. The secondary and tertiary structures of antigens may be altered by base pair changes, far removed linearly from the binding site regions in other virus systems such as polio virus[33] where neutralisation may be changed by a single base pair change up to 300 base pairs away from the binding site. Similarly, the three dimensional structure of virus antigens may contain many epitopic sites as well as binding sites[34] (e.g. influenza virus, see Fig. 7).

Fig. 6. *Immunoprecipitation of HTLV III antigens.*



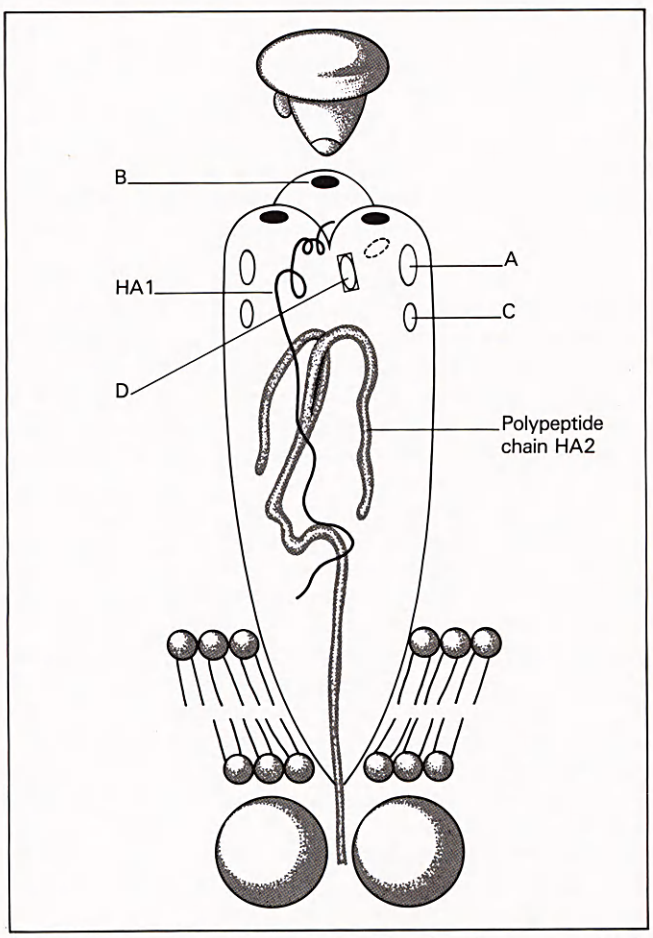


Fig. 7. *Hypothetical illustration showing how an antibody may be raised against a binding site and not neutralising epitopes and vice versa, (based on the haemagglutinin antigen of the influenza virus).*

Antibodies may therefore detect some epitopes but not others which may be masked by being recessed, or masked by antibodies attached to more immunogenic epitopes (or even that several NABs may be required).

There is a recent report of a new mechanism for the neutralisation of enveloped viruses by anti-viral antibody, whereby binding of a flavi-virus to its receptor does not preclude neutralisation at the endosomal acid catalysed fusion step[35]. Clearly, any of these factors could be present in the case of HIV. Another possibility is that of antigen misrepresentation by the antigen presenting cell. HIV is now known to infect monocytes and macrophages (M-M) many of which bear the CD4 antigen [13, Clapham *et al.*, unpublished observations]. Other viruses that are able to reside at low levels in M-M also appear to incite low levels of NAB, i.e. Visna Maedi (VM)[36] and lactate dehydrogenase virus (LDV)[37]. It could be that M-M is able to alter antigen presentation of various membrane epitopes or that M-M represent an immunological privileged site for these viruses. It is interesting to note that these other viruses (VM, LDV) are able to inflict severe neurological damage (such as demyelination) on their hosts. Moreover, brain disease now accounts for the major morbidity of HIV infected persons, ranging from minor neurological dysfunction to severe pre-senile dementia[38–40]. The reasons for this are not clear although in the case of HIV infection many potentially susceptible T4 positive mononuclear cells are present in the brain and some nervous system primary cells also express CD4.

A useful therapeutic strategy would be to induce NAB by infecting mice or similar animals. This has now been done by many groups and although monoclonal ABs to both core and envelope proteins have been prepared, I am not aware of any which have significant neutralising ability. Attempts to change the antigen presentation using ISCOM techniques have increased the number of MCABs raised against the envelope proteins but, again, none have been reported as eliciting neutralisation. Clearly, other antigen presentation modification techniques should be entertained.

Anti-idiotypes

Knowing that the T4 antigen is the virus receptor suggests the possibility of raising an anti-virus antibody by making an anti-idiotype. As the virus binds to T4, the T4 could be used to make an anti-T4 AB (by immunising mice); then repeating the technique using the anti-T4 AB should lead to the production of anti-anti-T4 which could conceivably be active against the virus (Fig. 8). This is theoretically an attractive way of raising what would effectively be a neutralising antibody. However, I am unaware of any successful NABs raised with this technique. Recently, Waldmann and his colleagues[41] have reported that anti-mouse T4 given to mice acts as a tolerogenic umbrella for the immune system. In other words mice become tolerant to antigens given under the cover of anti-mouse T4 antibodies. This has some attractive speculative appeal for HIV. Idiotypes against HIV will include anti-T4 ABs, could they therefore allow the neutralising epitope to be seen as self? Speculation aside, the use of anti-idiotypes as surrogate antigens has already been shown to have a role in vaccination against hepatitis B virus[42] and schistosomiasis[43].

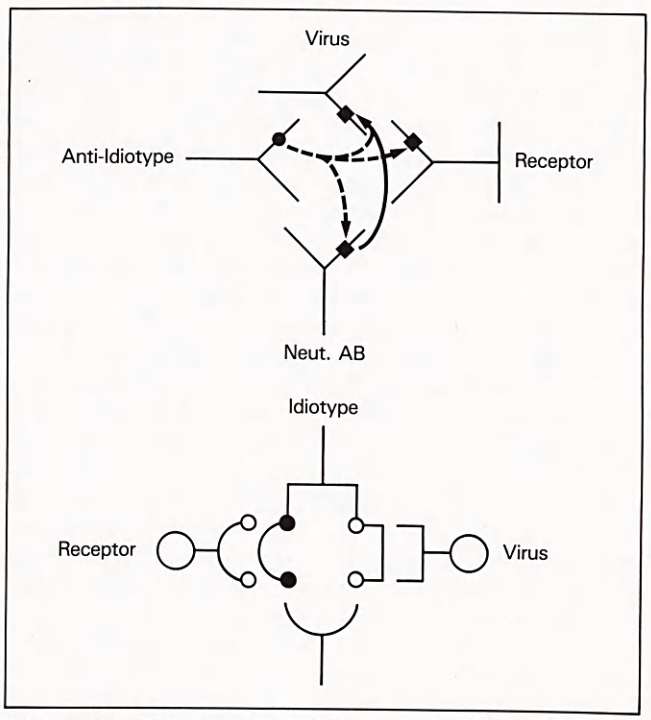


Fig. 8. *Anti-idiotypes: Two different presentations of the same theme; antibody to the receptor will itself act as an antigen in its variable region raising an antibody which should also react with the virus.*

The T Cell Response

The immune system's other arm of defence after B cells and their antibodies is the T cell system. Unfortunately the T cell system in HIV infection is severely disturbed. T4 + cells are destroyed and even those not known to be infected have impaired function[44]. Many retroviruses have a conserved transmembrane protein called P15E[45] which is known to be immunosuppressive in some T cell response assays. Other soluble sustances may be produced by infected cells which interfere with T cell function[46]. The clearance of many viruses is dependent on the activity of virus-specific cytotoxic T lymphocytes (CTL) restricted by Class II molecules of the major histocompatibility complex. Indeed, in some animal model systems, persistent infection occurs if there is an absence of, or decrease in the production of these cells[47]. It will be interesting to see if CTL cells are induced against HIV infected cells and whether or not the natural killer (NK) cells or lymphokine activated killer (LAK) cells have any role.

Immunotherapy

The T4 lymphocyte effectively acts as the conductor of the immunological orchestra[48], and hence there is probably no more effective way of vandalising the immune system than killing T4 positive cells, which is how HIV acts *in vitro* and *in vivo*. Therefore therapeutic stratagems designed to replace the body's flagging immune defences have been studied, and, although they may have an important role to play in the overall

treatment plan, it is unlikely that immune restoration alone will succeed while the virus is able to replicate unhindered.

The numerous immunological abnormalities described in HIV infection are beyond the scope of this article [reviewed in 49]. Suffice it to mention however that the most severe defect appears to involve cellular immunity.

Therapeutic stratagems have therefore focused on the following potential therapies: interferons, thymic replacement hormones, lymphokines and cytokines, bone marrow transplantation, isoprinosine and cimetidine, intravenous immunoglobulin therapy and plasmapheresis.

Interferons are known to have a viral function and the availability of recombinant human interferon has enabled *in vitro* and *in vivo* studies in AIDS patients.

There are two types of interferons: alpha, produced by leukocytes and fibroblasts and gamma, produced by lymphocytes and monocytes. Studies *in vitro* showed that alpha interferon suppresses the replication of HIV[50]. *In vivo* studies have unfortunately been disappointing with the exception of patients with Kaposi's sarcoma (KS). As vinblastine also has considerable single agent activity against KS, several centres are now studying combined therapy using regimes containing alpha interferon and vinblastine. Clinical trials with gamma-interferon, which should be theoretically superior to alpha-interferon, were delayed due to the lack of sufficient material. Preliminary studies suggest that gamma-interferon may not be as useful as alpha in the treatment of KS. However numerous centres are intensively studying gamma-interferon in AIDS and will report in the near future.

Thymic replacement therapies in patients with AIDS are unfortunately both anecdotal and uninspiring. Whereas it is easy to overlook the lack of reported clinical success, it should be noted that numerous different thymic peptides are now available and some, or a combination of them, may be useful in some cases. A recent report that antibodies to thymosin also neutralise HIV demands further investigation[51].

Interleukin-2 (IL-2) is a lymphokine which has been shown to reconstitute deficient *in vitro* immune responses[52]. Although IL-2 may improve some immune responses *in vivo,* no dramatic response has been reported from numerous clinical studies. Moreover, it has been argued that as IL-2 augments the number of T4 + cells it may in fact be adding fuel to the fire by adding further infectable cells. Indeed virus production may be 'hotted up' *in vitro* by adding fresh T4 + uninfected cells. Nevertheless IL-2 may yet have a role to play in combination with other agents such as reverse transcriptase inhibitors.

Marrow transplants and lymphocyte transfusions have been performed in a few cases and do not appear to help the immune status. Again one is adding fresh uninfected cells to an 'infected' *in vivo* culture.

Isoprinosine and cimetidine are both able to improve cellular immunity *in vivo* with varying degrees of success. Encouraging results with these agents in melanoma, systemic lupus erythematosus and rheumatoid arthritis have led to controlled trials in patients with AIDS and related conditions, which have yet to be reported.

Intravenous immunoglobulin therapy may have a role in the management of ill HIV infected patients. It is unlikely that sufficient neutralising antibody would be present to have a specific anti-HIV effect. Early trials in children are said to be encouraging.

Anecdotal experience with plasmaphoresis does not suggest that this will be a useful therapeutic modality in AIDS.

Whereas there may be some cautious ground for optimism using some modality in combination, it should be noted that improvements in *in vivo* immune function and clinical status remain curiously uncoordinated in the majority of trials and reports reviewed.

Vaccines

Studies of the epidemiology of HIV infection suggest that the only certain barrier to further spread of the disease is a vaccine.

Previous viral epidemics which have inflicted significant mortality and morbity, e.g. paralytic poliomyelitis and smallpox, have been successfully contained by mass vaccination. Hepatitis B virus (HBV), the causal agent of 'serum' hepatitis and responsible for the high incidence of chronic liver disease, and hepatocellular cancer (one of the most common cancers in some parts of Africa and Asia), has recently been the target of a successful vaccination programme using antibodies against the envelope antigen of (HBsAg)[53]. The vaccine has been prepared directly from infected persons by cloning the envelope gene and, more recently, by preparing an anti-idiotypic antibody vaccine[42].

The Epstein Barr Virus (EBV) is causally linked with Burkitt's lymphoma in Africa and nasopharyngeal carcinoma in Asia. Recently a vaccine, prepared against the surface antigen (glycoprotein 340), has been shown to protect against EBV induced lymphomas in cottontop tamarins[54].

The only retrovirus for which a successful vaccine has yet been developed is the feline leukaemia virus (FeLV). This was also the first retrovirus to be identified as being exogenously transmitted in animals, when Prof. Jarrett and his colleagues reported the transmissable nature of lymphosarcoma in cats[55]. He was able to isolate a retrovirus (FeLV) and showed that infection may be accompanied by the development of a lymphosarcoma in some cats, or acquired immunodeficiency (manifested by wasting and death from opportunistic infections) in others, while some cats remained asymptomatic[56]. The development of a vaccine was far from straightforward. The first prototype, although inducing protective antibodies, did not protect against the development of malignant disease and indeed may have worsened the outcome[57]. However, the later use of an inactivated vaccine prepared from a high titre supernatant in such a way as to preserve the envelope glycoproteins, which was then combined with an adjuvant, protected against laboratory challenge and natural exposure[58]. This preparation is now commercially available. Although the successful development of this retrovirus vaccine is encouraging, making a vaccine for AIDS is unlikely to be as

easy because protective neutralising antibodies are not known to be effectively induced *in vivo*[32] as they are in FeLV infected cats.

HIV has now been cloned and many isolates have been extensively characterised[59]. It is clear that there is considerable variation in the genes coding for the envelope antigens, and any vaccine would have to depend on conserved regions. Such regions are being sought in numerous different isolates from different parts of the world. In the meantime the envelope gene has been subcloned and inserted into a vaccinia vector which readily induces envelope antibodies to the glycoproteins gp. 110, 120[60,61]. Unfortunately these antibodies are not as yet known to have any neutralising or protective function. No doubt trials using subunits of the same protein as well as different proteins alone or in combination are now in progress.

Another approach which could achieve the same end is the use of synthetic peptides manufactured from known base pair sequence information. Sequence peptides, however, are not necessarily good antigens, as studies on the tobacco mosaic virus (TMV) have shown[62]. Peptides having the correct amino acid sequence do not necessarily have the correct conformation at the region of binding. In contrast to the relatively stable structure of a protein in solution, small peptides are thought to exist in a multiplicity of transient conformational states in dynamic equilibrium. The conformation of the binding site may change to improve structural complementary with the peptide ligand. X-ray crystallography studies have revealed rotations and translations of aromatic side chains and expansion of the binding cavity by movement of various hypervariable loops[62]. Furthermore, the immunogenicity of a peptide may be increased by coupling it with the purified protein derivative of tuberculin (PPD) in animals presensitised with BCG[63].

Against this background the development of a procedure for the rapid synthesis of a solid support of a large number of peptides by Geysin and colleagues[64] has exciting implications for developing peptides potentially useful in AIDS. Using this technique he has been able to develop a neutralising antibody to a putative discontinuous epitope of foot and mouth disease virus[65]. Taking as a template a non-neutralising antigenic epitope (VPI), peptides of increasing length were synthesised and assayed for antibody binding after addition of each residue, some of which were β-alamines to allow structural flexibility. Finally, a neutralising peptide was eventually obtained. Such a technique could be used against the binding site of HIV and the T4 molecule.

Vaccine Evaluation

Once a successful vaccine or peptide has been prepared it will be necessary to establish an evaluation programme that will document safety and efficacy whilst minimising the time needed for approval. A primate model has been developed using the chimpanzee which is susceptible to HIV infection [for review of vaccine development see 66]. Chimpanzees have already been used successfully to test HIV vaccines[53]. A cheaper primate that was infectable and had a shorter life cycle would be an advantage, and one may yet be discovered.

Once satisfactory protection is shown in other primates human trials would then follow. Initially, trials will need to investigate immunogenicity safety and protection against infection; it will be necessary to decide whether or not volunteers from the AIDS 'at risk' group or others should be used. Larger scale pilot studies would follow and then mass vaccination—but for whom? Obviously those in 'at risk' groups will need to be given the vaccine but what about the general population? Increasing reports of heterosexual transmission in the West[67,68] and the epidemic nature of AIDS-like illnesses in Africa[69,70] could suggest that all sexually active persons be included in the at risk groups.

Genetic Approaches to Treatment

Following the successful cloning and sequencing of several isolates much has been learnt about HIV (Fig. 9). Initially, it was thought to be very closely related to HTLV-I in that both are human retroviruses with T cell trophism for T4 + lymphocytes, and both have standard retrovirus genomic structure with genes encoding for core or structural proteins (GAG), reverse transcriptase (POL) and membrane proteins (ENV) without any known oncogenes but with the addition of a gene capable of transactivation (TAT gene) and regulating the rate of virus replications. Homologous sequences between HTLV-I and III for the GAG, POL, ENV and TAT genes have been reported[71]. It is now known that HIV is a considerably more complicated virus than HTLV-I. It has at least two extra genes[72,73] (Fig. 9). These are known as the short open reading (SOR) frame which may regulate the TAT gene and the open reading frame at the 3 prime end of the virus (3'ORF) which may be responsible for the cytopathogenicity of the virus. This structure is very similar to that of the Visna-Maedi virus in sheep and HTLV-III is now regarded as being more akin to the lenti viruses than to HTLV-I[72, 73].

Recent studies have shown that the TAT gene, which lies between SOR and ENV, is able to mediate activation in a trans configuration of the genes linked to HIV long terminal repeat (LTR) sequences. Biological clones of HIV with the TAT gene deleted do not replicate until the TAT gene is restored or by introduction of the TAT III protein itself[74,75].

The gene which may encode the cytopathic potential for HIV (3'ORF) is under similar investigation. How then might these genetic Achilles' heels lead to further therapeutic strategies? The construction of clones encoding genes the wrong way round so that they make mirror image or anti-sense RNA has been reported in several systems[76–78]. A recombinant AIDS virus with the TAT gene reversed (or 3'ORF) is obviously an attractive theoretical proposition[79]. But could it be given *in vivo?* A coupled gene encoding responsiveness to a drug that could control replication *in vivo* would be a desirable addition (e.g. DHFR gene responsive to methotrexate)[80] which would be the minimum control needed to assuage fears of what recombinant AIDS-viruses might

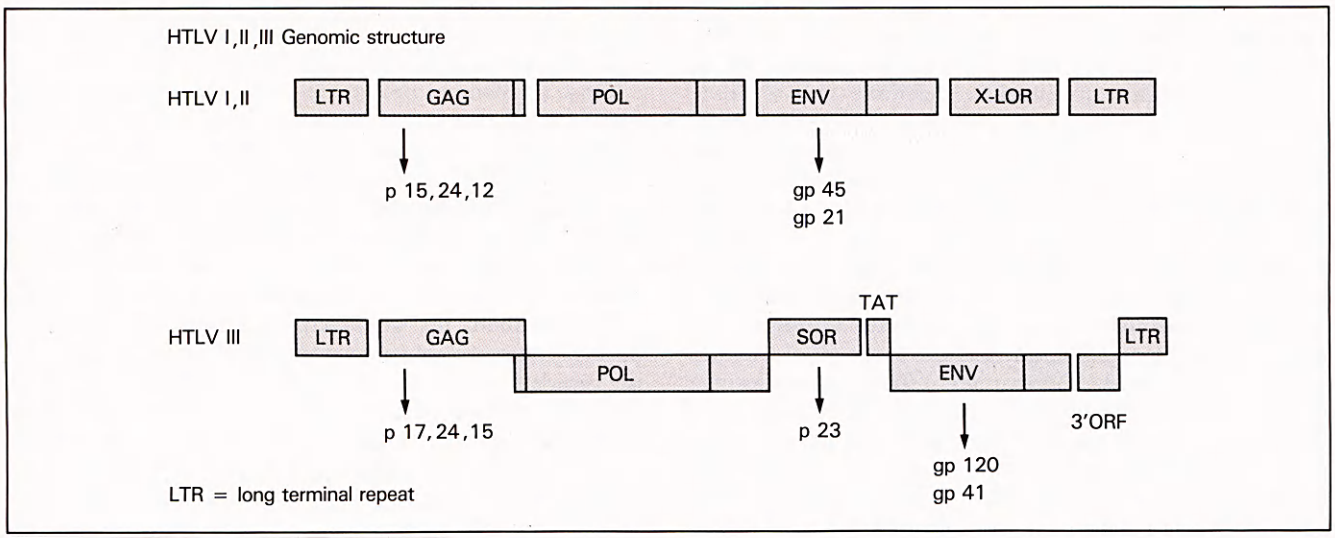


Fig. 9. *The genetic structure of HTLV-I and HTLV-III (HIV) showing the extra genes (SOR, TAT, ORF 3') of HIV which make the virus more closely related to the lenti viruses. The X or LOR of HTLV-I also has a transcriptional activational (TAT) function.*

inflict *in vivo*. Whereas this approach will be regarded as scientific fiction for some time, it should not obscure the tremendous amount of hard scientific fact that has been accumulated about this virus (viruses) which has allowed the development of a rational approach to therapeutic options summarised here.

In spite of the many problems encountered in isolating and trying to understand HIV there are certainly grounds for optimism that we will eventually be able to effectively treat if not to eliminate AIDS and its related conditions.

It cannot be over-emphasised how much of what has been done in the way of combating the AIDS problem was only possible because of the strong base of scientific research covering many disciplines such as animal retrovirology, immunology, cell and molecular biology, which were able to be rapidly recruited to identify the causal agent and subsequently attack this disease. Although there is no present cure, it should be remembered that the rate of progress in AIDS research has no historical equal.

Addendum

Since this paper was prepared, one or possibly two new HIV like viruses have been announced. Montaignier and colleagues report a virus from West African AIDS patients which they call LAV-2, whilst Essex and colleagues report a virus from healthy Senegalese prostitutes which they call HTLV-4 and which is claimed to be apathogenic. Both these viruses have considerable differences to known strains of HIV and may be more similar to the T lymphotropic viruses.

Acknowledgements

I would like to thank Professor Robin Weiss, in whose laboratory some of the quoted work was performed, and also Paul Clapham, Mark Marsh, Quentin Sattentau and Peter Beverley with whom we worked on these projects.

This article is based on a paper read at the Conference on AIDS held at the Royal College of Physicians in May 1986.

References

1. Barré Sinoussi, F., Chermann, J. C., Fey, F. *et al.* (1983) *Science,* **220,** 868.
2. Gallo, R. C., Salahuddin, G. Z., Popovic, M. *et al.* (1984) *Science,* **224,** 500.
3. Sarngadharan, M., Popovic, M., Bruch, L., Schupback, J. and Gallo, R. C. (1984) *Science,* **224,** 506.
4. Chiengsong-Popov, R., Weiss, R. A., Dalgleish, A. G. *et al.* (1984) *Lancet,* **ii,** 477.
5. Poeisz, B. J., Ruscetti, F. W., Gazdar, A. F. *et al.* (1980) *Proceedings of the National Academy of Science USA,* **77,** 7415.
6. Kalyanaraman, U. S., Sarngadharan, M. G., Robert Guroff, M. *et al.* (1982) *Science,* **218,** 571.
7. Levy, J. A., Hoffmann, A. D., Kramen, S. M., *et al.* (1984) *Science,* **225,** 840.
8. Coffin, J. C., Haase, A., Levy, J. A. *et al.* (1986) *Nature,* **321,** 10.
9. Norman, C. (1985) *Science,* **230,** 518.
10. Weiss, R.A., Teich, N., Varmus, H. and Coffin, J. (eds) (1985) *RNA Tumour Viruses.* New York; Cold Spring Harbor Laboratory.
11. Nagy, K., Clapham, P., Chiengsong-Popov, R. and Weiss, R. A. (1983) *International Journal of Cancer,* **32,** 321.
12. Clapham, P., Nagy, K. and Weiss, R. A. (1984) *Proceedings of the National Academy of Science USA* **81,** 3083.
13. Dalgleish, A. G., Beverley, P. C. L., Clapham, P. *et al.* (1984) *Nature,* **312,** 763.
14. Maddon, P. J., Littman, D. R., Godfrey, M. *et al.* (1985) *Cell,* **42,** 93-104.
15. Maddon, P. J., Dalgleish, A. G., Clapham, P. *et al.* (1986) *Cell,* (in press).
16. McDougall, J. S., Kennedy, M. S., Sligh, J. M. *et al.* (1986) *Science,* **231,** 382.
17. Hahn, B., Shaw, G. M., Arya, A. K. *et al.* (1984) *Nature,* **312,** 166
18. March, M. (1984) *Biochemical Journal,* **218,** 1.
19. Öberg, B. (1983) *Pharmacology and Therapeutics,* **19,** 387.
20. Balzarini, J., Mitsuya, H., De Clerq, E. and Broder, S. (1986) *International Journal of Cancer,* **37,** 451.
21. Mitsuya, H., Popovic, M., Yarchoan, R. *et al.* (1984) *Science,* **226,** 172.
22. Sandstrom, E. G., Byington, R. E., Kaplan, J. C. and Hirsh, M. S. (1985) *Lancet,* **ii,** 1480.

23. Sarin, P. S., Taquchi, Y., Sun, D. *et al.* (1985) *Biochemical Pharmacology,* **34,** 4075.
24. Broder, S., Yarchoan, R., Collins, J. M. *et al.* (1985) *Lancet,* **ii,** 627.
25. Rozenbaum, W., Dormont, D., Spire, B. *et al.* (1985) *Lancet,* **i,** 450.
26. Yarchoan, R., Klecker, R. W., Weinhold, K. J. *et al.* (1986) *Lancet,* **i,** 575.
27. McCormick, J. B., Setchell, J. P., Mitchell, S. W. and Hicks, D. (1984) *Lancet,* **ii,** 1367.
28. Blanche, S., Fischer, A., LeDeist, F. *et al.* (1986) *Lancet,* **i,** 863.
29. Pompidou, A., Zagury, D., Gallo, R. C. *et al.* (1985) *Lancet,* **ii,** 1423.
30. Sarin, P. S., Gallo, R. C., Scheer, D. I., Crews, F. and Lippa, A. S. (1985) *New England Journal of Medicine,* **313,** 1289.
31. Hoshino, H., Tanaka, H., Minra, M. and Okada, H. (1984) *Nature,* **310,** 324.
32. Weiss, R. A., Clapham, P., Chiengsong-Popov, R. *et al.* (1985) *Nature,* **316,** 69.
33. Diamond, D., Jameson, B. A., Bonin, J. *et al.* (1985) *Science,* **229,** 1090.
34. Patterson, S. and Oxford, J. S. (1986) *Vaccine* **4,** 79.
35. Gollins, S. W. and Porterfield, J. S. (1986) *Nature,* **321,** 244.
36. Peluso, R., Haase, A., Stowring, L., Edwards, M. and Ventura, P. (1985) *Virology,* **147,** 231.
37. Murphy, W., Nawrocki, J. F. and Pease, L. R. (1983) In: *Immunology of Nervous System Infections, Progress in Brain Research,* Vol. 59 (ed P. O. Behan). Amsterdam: Elsevier.
38. Shaw, G. M., Harper, M. E., Hahn, B. H. *et al.* (1985) *Science,* **227,** 177.
39. Levy, R. A., Shimabukuro, J., Hollander, H., Mills, J. and Kaminsky, L. (1985) *Lancet,* **ii,** 586.
40. Goldwater, P. N., Synek, B. J. L., Koelmeyer, T. D. and Scott, P. J. (1985) *Lancet,* **ii,** 447.
41. Benjamin, R. J. and Waldmann, H. (1986) *Nature,* **320,** 449.
42. Kennedy, R. C., Gidberg, J. W., Lanford, R. E. and Dreesman, (1986) *Science,* **232,** 220.
43. Grzych, J. M., Capron, M., Lambert, P. H. *et al.* (1985) *Nature,* **316,** 74.
44. Murray, H., Rubin, B. Y., Masur, H. and Roberts, R. (1984) *New England Journal of Medicine,* **310,** 883.
45. Cianciolo, G., Copeland, T. D., Oroszlan, S. and Synderman, R. (1985) *Science,* **229,** 453.
46. Ruddle, N. (1986) *Immunology Today,* **7,** 8.
47. Flyer, D. C., Burakoff, S. J. and Faller, D. V. (1983) *Nature,* **305,** 815.
48. Dalgleish, A. G. (1986) *Immunology Today,* **7,** 142.
49. Gottlieb, M., Wolfe, P. R. and Mitsuyasu. (1985) In *Advances in Host Defense Mechanisms* Vol. 5. (eds J. Gallin and A. S. Fauci, New York: Raven Press.
50. Ho, D. D., Hartshornk, L., Rota, J. R. *et al.* (1985) *Lancet,* **i,** 662.
51. Sarin, P., Sun, D. K., Thornton, A. H., Naylor, P. H. and Goldstein, A. L. (1985) *Virology,* **232,** 1135.
52. Lifson, J. D., Benike, C. J., Mark, D. F. and Koths Engleman, E. G. (1984) *Lancet,* **i,** 698.
53. Zuckerman, A. J. (1985) *British Medical Journal,* **290,** 492.
54. Epstein, M. A., Morgan, A. J., Finerty, S., Randle, B. J. and Kirkwood, J. K. (1985) *Nature,* **318,** 287.
55. Jarrett, W. F. H. (1975) *Comparative Leukaemia Research,* **43,** 209.
56. Jarrett, W. F. H., Crawford, E. M., Martin, W. B. and Davie, F. (1964) *Nature,* **202,** 567.
57. Olsen, R. G., Hoover, E. A., Schaller, J. P. *et al.* (1977) *Cancer Research,* **37,** 2082.
58. Lewis, M. G., Mathes, L. W., Olsen, R. G. (1981) *Infect. Immunol.,* **34,** 888.
59. Wong Staal, F., Shaw, G. M., Hahn, B. H. *et al.* (1985) *Science,* **229,** 759.
60. Chakrabarti, S., Robert-Guroff, M., Wong-Staal, F., Gallo, R. C. and Moss, B. (1986) *Nature,* **320,** 535.
61. Shiu-Lok, Hu., Kosowski, S. G. and Dalrymple, J. M. (1986) *Nature,* **320,** 537.
62. Ada, K. and Skehel, H. (1985) *Nature,* **316,** 764.
63. Sia, D. Y., Lachmann, P. J. and Leung, K. N. *Immunology,* **51,** (4), 755.
64. Geysin, H. M., Melol, R. H. and Barteling, S. (1984) *Proceedings of the National Academy of Science USA,* **81,** 3998.
65. Geysin, H. M., Barteling, S. J. and Meleon, R. H. (1985) *Proceedings of the National Academy of Science USA,* **82,** 178.
66. Frances, D. P. and Petricciani, J. C. (1985) *New England Journal of Medicine,* **313,** 1586.
67. Redfield, R., Markham, P. D., Salahuddin, S. Z. *et al.* (1985) *Journal of the American Medical Association,* **254,** 2094.
68. *Mortality and Morbidity Weekly Report* (1985) **34,** 37.
69. Serwadda, D., Mugerwa, R. D., Sewankaribo, N. K. *et al.* (1985) *Lancet,* **ii,** 849.
70. Biggar, R. J. (1986) *Lancet,* **i,** 79.
71. Arya, S. K., Gallo, R. C., Hahn, B. H. *et al.* (1984) *Science,* **225,** 927.
72. Wong-Staal, F. and Gallo, R. C. (1985) *Nature,* **317,** 395.
73. Sonigo, P., Alizon, M., Staskus, K. *et al.* (1985) *Cell,* **42,** 369.
74. Fisher, A. G., Fernberg, M. B., Josephs, S. F. *et al.* (1986) *Nature,* **320,** 367.
75. Sodroski, J., Goh, W. C., Rosen, C. *et al.* (1986) *Nature,* **321,** 412.
76. Izant, J. G. and Weintraub, H. (1985) *Science,* **229,** 345.
77. Melton, D. A. (1985) *Proceedings of the National Academy of Science USA,* **82,** 144.
78. Rosenberg, U. B., Preiss, A., Seifert, E., Jäckle, H. and Knipple, D. C. (1985) *Nature,* **313,** 703.
79. Mariman, E. C. M. (1985) *Nature,* **318,** 414.
80. Tellier, R. and Weber, J. M. (1985) *Nature,* **318,** 414.