Science, medicine, and the future

Infection with HIV-1

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Infection with HIV-1 leading to AIDS has been the greatest biomedical challenge of this century. In December 1997 the World Health Organisation estimated that 11.7 million people had died, more than 30 million were still infected, and 16 000 new infections were occurring each day. Of the 30 million people infected, 27 million are thought to be unaware of their infection. The disease has been especially devastating because of its concentration in young adults, which affects social stability from the level of individual families to national economies.

Improved understanding of HIV-1 biology and its treatment have set the stage for a variety of new scientific initiatives to combat the epidemic.¹⁻⁸ New drug combinations that include protease inhibitors have led to prolonged survival, and a better definition of HIV-1 pathogenesis. Highly active antiretroviral therapy has provided hope that AIDS may one day be conquered, but its high cost has raised new issues, particularly that of equity, with the burden of the epidemic shifting into poorer populations. Scientists, healthcare workers, public health officials, and politicians need to acknowledge that the epidemic is a global problem and that mechanisms for combatting the disease should supersede nationalistic interests.

This article explores three fundamental aspects of HIV-1 biology that will be the focus of intensive research over the next few years and, ultimately, have direct bearing on clinical practice and public health approaches to HIV infection.

Where does HIV-1 hide and can it be eradicated?

In most cases of infection HIV-1 is transmitted across a mucosal barrier. The first cells to be infected are thought to be those of the monocyte lineage, particularly dendritic cells.⁹⁻¹¹ In macaque models of mucosal infection with simian immunodeficiency virus (SIV) the virus can be found in regional lymph nodes within 36 hours and in systemic lymph nodes within four to five days.¹²⁻¹⁵ CD4 lymphocytes and cells of the reticuloendothelial system are infected, including microglial cells in the central nervous system. The heaviest burden of virus is in the germinal centres of lymph nodes, where it resides in both intracellular and extracellular compartments.¹⁴⁻¹⁵

Although infection induces a vigorous immune response that partially clears virus and decreases the

Predicted developments

- Improved survival of patients infected with HIV-1 as a result of more intensive drug treatment
- Changing spectrum and frequency of opportunistic infections and neoplasms and increased frequency of virus resistance in treated patients
- New antiretroviral treatment strategies to inhibit viral entry and integration and new approaches to reverse virus induced immunological damage
- Renewed public health efforts to curb epidemic in developing countries and to identify affordable treatment strategies
- Increased focus on vaccine development and evaluation of candidate vaccines in large scale trials

level of HIV-1 genome detectable in plasma, HIV-1 is rarely if ever eradicated and eventually establishes a dynamic equilibrium in which virus clearance is matched by virus production. This results in a relatively constant level of virus genome detectable in plasma for years, until it again rises (figure).

A major question for patients and clinicians is whether antiretroviral therapy can ever be safely stopped. To achieve this, the reservoirs of HIV-1 must be defined and eliminated. Although antiretroviral therapy combining protease inhibitors with nucleoside and non-nucleoside reverse transcriptase inhibitors can reduce viral loads to undetectable levels, current experience shows that when treatment is stopped virus loads rapidly return to pretreatment levels. The basis for this is the reservoir of HIV-1 in a latent state or a protected site not amenable to standard treatments.

From studies to define the dynamics of virus replication and clearance with highly active antiretroviral therapy, a two compartment model was recognised,¹⁶⁻¹⁷ which is thought to represent rapid clearance of HIV-1 in lymphocytes and slower clearance of HIV in macrophages. Some estimates are that the pool of infected macrophages may take three years or more to die out by natural attrition. Even more problematic is the presence of latently infected cells, in which HIV-1 proviral DNA is integrated into the host cell genome.¹⁻¹⁸ As long as the proviral DNA stays transcriptionally inactive, evidence of infection will not be presented to the immune system and the potential for future relapse will exist. Virus trapped in the extracellular spaces of the lymph node germinal centres can also escape kill-

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Predicted effects of interventions on the course of HIV infection and AIDS. **Graph A**—In the natural course of HIV-1 infection plasma viraemia reaches a peak 3-4 weeks after infection and then establishes a plateau, generally between 10^4 and 10^5 genome copies/ml: as the disease progresses over several years immune function declines (both number of CD4 T cells and repertoire of T cell receptor specificities) and virus load increases. **Graph B**—With vaccine induced immunity or immediate treatment with highly active antiretroviral therapy, virus could either be cleared early enough to avoid establishment of viral latency or persistent infection could be established but with very low virus load: in either case immune function would remain intact, and in the future these approaches will be combined to improve chances of preventing persistent infection. **Graph C**—Early treatment with highly active antiretroviral therapy maintains a low virus load, but the reservoir is still present and high virus loads will return if treatment is discontinued. **Graph D**—After early treatment with highly active antiretroviral therapy, periodic immunostimulatory treatment is given to activate latently infected cells: this approach may eventually exhaust the HIV reservoir and allow highly active antiretroviral therapy treatment to be discontinued. **Graph E**—If highly active antiretroviral therapy is initiated late during infection, immune function may not be fully restored without active efforts to reconstitute the repertoire of T cell receptor specificities

ing by antiretroviral drugs until it enters a cell to begin its replication programme.

Another component of the HIV-1 reservoir is infection of the central nervous system, eye, and testes.

These are relatively protected from the normal immune response (immunoprivileged) and are relatively difficult for antiretroviral drugs to penetrate, thereby providing a sanctuary for HIV.

Tackling the HIV reservoir

This problem raises issues about current therapeutic options. While protease inhibitors are highly effective, long term treatment has resulted in unexpected side effects such as lipodystrophy and hyperlipidaemia, as well as the common side effects of highly active antiretroviral therapy, which lead to intolerance in up to 40% of patients (gastrointestinal intolerance, headaches, asthenia, peripheral neuropathy, rash, pancreatitis, and bone marrow suppression). As treatment is prolonged, new side effects and drug interactions will probably appear, and accumulation of mutations in the HIV-1 quasispecies (the spectrum of genetic variants that coexist within the infected host) could eventually lead to drug resistance. Such multiply drug resistant HIV could be transmitted and infect new hosts.¹⁹ Finally, the expense of long term antiretroviral regimens that can suppress viral replication only temporarily is a growing burden for healthcare systems. New approaches to treatment must be sought. Some steps can be taken with currently available agents, but new drugs will also be needed.

Early diagnosis and prompt treatment of infection will reduce the HIV-1 reservoir and decrease the breadth of the genotypic variants in a particular patient. This may increase the chances of eventual cure and decrease the likelihood of resistance developing. Further work on the pharmacology and interactions of current drugs may allow doses to be reduced and suggest combinations that would minimise side effects. Current drugs might also be given in combinations with immunostimulatory treatment (such as interleukin 2, granulocyte-macrophage colony stimulating factor, interleukin 12, tumour necrosis factor α , and antibody to CD3) to promote emergence of HIV-1 from latency. General immune stimulation would be intended to activate latently infected T cells and restart HIV replication, thereby targeting the cells for immune-mediated clearance (figure).

New agents that may be on the horizon include integrase inhibitors, drugs that block entry of HIV-1, and drugs that disrupt HIV-1 transcription. A key goal is designing products that can penetrate the central nervous system and other immunoprivileged sites. The success of current approaches will increase the complexity of evaluating future products, with the need for longer follow up in clinical trials and development of new criteria for treatment failure, including sophisticated analysis of viral reservoirs and HIV quasispecies.¹⁻²⁰

Will an AIDS patient's immune function return to normal after effective treatment?

Highly active antiretroviral therapy can increase the number of circulating CD4 T cells even in patients with advanced AIDS. This is associated with prolonged survival and a diminished rate of common opportunistic infections such as by *Pneumocystis carinii*, *Mycobacterium avium-intracellulare*, and cytomegalovirus. The

increased number of CD4 T lymphocytes is the result of both regeneration of T cells and redistribution of T cells sequestered during periods of active HIV-1 replication. Importantly, the repertoire of T cell receptors for both CD4 and CD8 lymphocytes remains skewed despite the greater number of circulating T cells.

The T cell receptor is a heterodimeric molecule composed of α and β subunits. The V region of the receptor gene determines the V β subtype of the T cell receptor. Each V β subtype contains unique sequences that allow the receptor to recognise a broad spectrum of peptide epitopes in the context of a type I major histocompatibility complex molecule. In HIV infected patients some V β subtypes are overrepresented and others are underrepresented.²¹ The spectrum of antigen recognition sites within each V β family is also altered. Genetic techniques (heteroduplex mobility assays or methods based on the polymerase chain reaction) have revealed oligoclonal populations, presumably the result of selective clonal expansion and deletion of T cells with particular receptor specificities. This means that the ability of a patient's immune system to recognise certain antigens, pathogens, or neoplasms may be altered or absent despite an overall increase in the number of circulating T cells.

The altered T cell repertoire may lead to new patterns of autoimmunity, opportunistic infections, and AIDS related malignancies. While rates of Kaposi's sarcoma and B cell lymphomas have diminished with the advent of highly active antiretroviral therapy, other neoplasms such as squamous cell carcinoma of skin, testicular cancer, myeloma, Hodgkin's disease, and head and neck cancers may be increasing among treated patients. The causes and consequences of "holes" in the T cell repertoire are being studied, including whether the diversity of the repertoire will naturally expand or require active reconstitution.²² Initial indications are that the diversity of T cell receptor V β genes can increase in patients with undetectable virus load for more than six months, suggesting that regeneration of naive precursors is possible.

Is it possible to prevent HIV-1 infection?

Ultimately, preventive vaccination will be the most efficient and cost effective approach to stop the HIV-1 epidemic. Efforts to develop a vaccine have been impeded by a lack of defined immune correlates for protection or recovery from HIV infection. Recent studies in people who remain uninfected despite multiple high risk exposures have provided important information about potentially effective immune mechanisms.

A small subset of sex workers in the Gambia and in Kenya were shown to have a reduced risk of HIV-1 infection if they had remained uninfected after six years of high risk exposures.²³⁻²⁴ While these subjects had no evidence of virus infection by culture, polymerase chain reaction, or HIV specific antibody, peripheral blood mononuclear cells taken from them showed HIV specific CD8 cytotoxic T lymphocyte activity.²⁴

People without detectable virus or HIV-specific antibody after substantial occupational exposure to contaminated material also show HIV-1-specific lymphoproliferative activity²⁵ and HIV specific cytotoxic T lymphocyte responses.²⁶ These data suggest that transient infection may occur and be cleared by

Candidate vaccines evaluated in phase I clinical trials

- Purified recombinant envelope subunit proteins gp120 and gp160
 - Derived from multiple HIV-1 strains
 - Produced from mammalian, insect, or yeast cells
 - Formulated with multiple adjuvants
- Peptides vaccines based on envelope or gag sequences
 - Produced as multiple antigenic peptides, lipopeptide conjugates or combination of peptides containing multiple epitopes and formulated with a variety of adjuvants
 - Delivered by multiple routes
- Live recombinant vector vaccines expressing env alone or multiple antigens including env
 - Recombinant poxvirus vectors including vaccinia and canarypox by multiple routes
 - Recombinant salmonella given orally
 - Given in series or in combination with recombinant envelope subunit proteins
- Particle vaccines
 - HIV-1 p24 expressed as a fusion protein with the self assembling Ty protein
- Nucleic acid vaccines encoding multiple genes and delivered by multiple approaches

natural immune defences, particularly T cell mediated responses.

A group of people who remained HIV seronegative despite living with HIV infected partners have also been found to show HIV specific production of interleukin 2 by peripheral blood mononuclear cells and evidence of HIV specific IgA in mucosal secretions,²⁷ suggesting that induction of immunity at mucosal sites may be important.

These results, and the evidence that CD8 cytotoxic T lymphocytes are important mediators of reducing viral load after primary infection with HIV²⁸⁻³⁰ or SIV,³¹⁻³³ present a compelling argument for designing vaccine strategies with the potential to induce HIV specific CD8 cytotoxic T cell responses in both the systemic and mucosal compartments. Studies in primate models of lentivirus infection suggest that immune mediated protection from infection is possible and that preventing infection at a mucosal site is less difficult than prevention of infection from intravenous inoculation. Because HIV-1 transmission is relatively inefficient and probably results from a small number of virions in most cases, development of an efficacious vaccine should be within our reach.

Candidate vaccines

A variety of candidate vaccines have been evaluated in human trials (see box). In general, they have had excellent safety profiles, with side effects being limited to transient local reactions.³⁴⁻³⁵ Optimal immunogenicity has not yet been attained, but elements of the induced immune responses are promising.

Neutralising antibody is an important determinant of vaccine induced immunity for many vaccines against other viral agents, and it is thought to be important for a successful AIDS vaccine. Current vaccine candidates have been able to induce neutralising antibody responses against strains of HIV adapted to cultured T cell lines that are homologous to the strain from which the vaccine antigen was derived. The size of response induced by the most immunogenic formulations are still 5-10 fold lower than those produced by HIV-1

infection. The responses are type specific with a relatively short half life and are unable to neutralise primary isolate viruses that are characteristic of transmitted strains.

Lymphoproliferative responses have been induced by all vaccine approaches and range in size from stimulation indices of >100 (for some subjects vaccinated with recombinant vaccinia vectors and boosted with purified recombinant envelope proteins) to indices of 5-10 (for subjects primed with recombinant canarypox). Recombinant poxvirus vectors can induce HIV specific CD8 cytotoxic T cells in most subjects, and the response is often detectable for more than 12 months. Unlike antibody responses, vaccine induced cytotoxic T cells are broadly reactive.³⁶ Cytotoxic T cells induced by recombinant canarypox vectors have been shown to lyse target cells infected with primary HIV-1 isolates from multiple clades.³⁶ In addition, recombinant canarypox vaccines have induced suppression of HIV-1 replication by non-cytolytic mechanisms mediated by CD8 cells.³⁷

Thus, current vaccine candidates can induce durable CD8 cytotoxic T lymphocyte responses with killing activity across different strains, but they are unable to induce neutralising antibody with activity against typical transmitted virus. Efforts are ongoing to identify vaccine formulations that can induce neutralising antibody against primary HIV-1 isolates. Modern vaccine concepts—including various systems of delivering live vectors, nucleic acid vaccines, and cytokine adjuvants—are finding their way into clinical trials, and innovations in antigen formulation, targeted antigen delivery, and modulation of immune responses are creating new vaccine strategies.

Conclusions

Antiretroviral therapy will continue to improve and will be a key factor in the distribution of healthcare resources to minimise polarisation of the epidemic between those who can afford new interventions and those who cannot. Treatments that eradicate HIV-1 are likely to require new drugs with novel mechanisms of inhibiting HIV-1 proliferation. Integrase inhibitors and products that block HIV-1 entry will be particularly important. Successful treatment of AIDS patients is likely to produce a new spectrum of complications, particularly an increased frequency of neoplasms.

The T cell repertoire of AIDS patients will probably be partially restored if virus replication can be suppressed for more than two years, but it may never regain its full spectrum of antigen recognition without active reconstitution. Earlier diagnosis and treatment, where available, will reduce this problem.

Large scale trials of candidate HIV vaccines will be performed, and products that can induce durable responses by CD8 cytotoxic T cells are likely to have partial efficacy and provide a benchmark for future studies. New approaches to formulation and delivery will improve vaccine effectiveness, which in combination with early antiretroviral treatment will make a substantial impact on the epidemic in countries with sufficient resources and healthcare infrastructure.

Meanwhile, it is essential that traditional public health approaches to prevention be employed with increased resolve and that new initiatives in education,

behaviour modification, and barrier protection be pursued aggressively.

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Understanding controlled trials

Randomisation methods in controlled trials

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The main purpose of randomisation is to avoid bias by distributing the characteristics of patients that may influence outcome randomly between treatment groups so that any difference in outcome can be explained only by treatment. These characteristics might be demographic ones such as age or prognostic factors such as clinical history or disease severity. For example menopausal status may influence outcome of treatment for breast cancer.

The most elementary form of randomisation is, in the case of two treatments, equivalent to allocating treatment by tossing a coin. Lists for allocating patients by simple randomisation may be constructed with tables of random numbers or random functions on pocket calculators or statistical software. Treatments may then be allocated to patients in sequence using numbered opaque envelopes containing treatment allocations or remotely by phone.

While such simple randomisation will on average allocate equal numbers to each arm, even in quite large trials simple randomisation can result in groups of different sizes. In small trials there may be substantial differences in group sizes that will reduce the precision of estimates of the difference in treatment effect and hence efficiency of the study.

One method to prevent unequal treatment group sizes is block randomisation. This guarantees that at no time will the imbalance be large and at certain points the numbers of participants in each group will be equal. If, for example, we choose blocks of four, there are six sequences to which we can allocate treatments A and B: AAB, ABAB, ABBA, BAAB, BABA, and BBAA. One of the six arrangements is selected randomly and then four participants assigned accordingly. The process is then repeated as many times as is needed for the required sample size.

With simple randomisation or block randomisation substantial imbalance in prognostic characteristics can, nevertheless, arise by chance and can bias the analysis of outcome. One method to achieve balance between groups for a prognostic variable is stratified randomisation, in which separate randomisation lists are used for each prognostic subgroup. For example, in a study of alternative treatments for breast cancer it would be advantageous to stratify on menopausal status. Separate randomisation lists would be prepared

for each stratum using a block randomisation. It should be noted that using simple randomisation with each stratum would defeat the purpose of stratification as the resulting randomisation would be no different from simple randomisation. A standard practice in multicentre trials is to stratify randomisation by treatment centre.

Stratification may be extended to two or more factors, although the number of separate randomisation lists rapidly becomes very large. For example, if one was to stratify on three prognostic variables, with each having just two levels, eight separate randomisation lists would be required for each combination of factors. In practice therefore it is rarely feasible to go beyond two factors.

Stratified randomisation makes the process more elaborate and brings with it the risk of mistakes that the simpler methods might prevent. If there is uncertainty about which patient characteristics may influence the outcome of treatment it may be prudent to proceed without stratification.¹

An alternative method of obtaining treatment groups that are comparable in prognostic variables is minimisation. This achieves balance on a set of prognostic factors, although not for each combination. Even in small trials it will provide groups that are very similar on several prognostic factors. For all levels of each prognostic factor on which the investigator wishes to maintain balance, a running total is kept of how many patients have been assigned to each treatment. At the start of the trial treatment is randomly allocated to the first patient. Subsequent patients are assigned using a randomisation weighted towards the group to which assignment would minimise the imbalance. After each patient is entered the relevant totals for each factor are updated ready for the patient. Details with examples of the minimisation method are discussed by Pocock¹ and Altman.²

Whatever method of allocation is used, the process of allocation needs to be done in such a way that the randomisation cannot be deciphered, a topic discussed in a forthcoming note.

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² Altman DG. *Practical statistics for medical research*. London: Chapman and Hall, 1991.

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