

Virological aspects of human immunodeficiency virus infection

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SUMMARY

The virology of human immunodeficiency virus (HIV) infection is reviewed. The transmission of HIV is restricted to direct contact with the blood or other body fluids of infected human beings. Ordinary social contact with infected individuals holds no risk but in the health care setting all patients must be considered to be potentially infectious and universal precautions taken. The replication of HIV in cells of the immune system carrying the CD4 receptor creates a complex relationship between the virus infection and the host immune response. The pathogenesis and the principles of the laboratory diagnosis of HIV infection are reviewed. Since its discovery HIV has quickly become one of the most studied and best characterized of human pathogens. The diagnosis of HIV infection, because of its implications, has been made more accurate than for any other infection. This understanding has significantly improved treatment but has yet to provide curative therapy, and prevention of infection is still the basis of the fight against AIDS.

Virology

Human immunodeficiency virus (HIV), the causative agent of the acquired immune deficiency syndrome (AIDS), is a retrovirus specifically infecting humans. The HIV viral particle consists of two copies of a 9.6-kilobase RNA genome with associated replicative enzymes: reverse transcriptase, protease, integrase and RNAse H, within a tubular core composed mainly of the p24 capsid protein (1) (Figure 1). Enclosing the core is a lipid envelope through which protrudes the viral glycoprotein receptor gp41 and gp120 complex.

Two distinct HIV strains, HIV-1 and HIV-2, may be distinguished by nucleic acid sequencing or serologically. HIV-2 appears to be less transmissible than HIV-1 and HIV-2-infected individuals take significantly longer to progress to symptomatic disease than those infected with HIV-1. HIV-2 is mainly confined to West Africa but there have been a number of HIV-2 infections in individuals with West African connections elsewhere in the world. It is likely that HIV-1 and HIV-2 arose in Africa between 600 and 1200 years ago by evolution from a closely related virus infecting monkeys: simian immunodeficiency virus.

Transmission

HIV can only be acquired by direct contact with the blood or other body fluids of infected human beings. There is no evidence to support transmission by biting insects such as mosquitoes, nor of animal or environmental reservoirs of infection. Nor is there any evidence that HIV may be transmitted by ordinary social or household contact with an infected person. The main routes of transmission are by sexual contact, by the blood-borne route and from mother to baby (Table 1). Contact of mucous membranes or broken skin with HIV-containing fluids has also rarely resulted in infection. A number of cofactors may increase the risk of acquiring HIV infection, including many sexually transmitted diseases, especially those producing ulcerative lesions.

In the health care setting the risk of HIV transmission may be diminished by adherence to the principles of 'universal precautions' under which blood and other body fluids from all patients are considered to be potentially infectious. Appropriate handwashing and use of protective barriers, safe use and disposal of needles and sharp instruments, and appropriate

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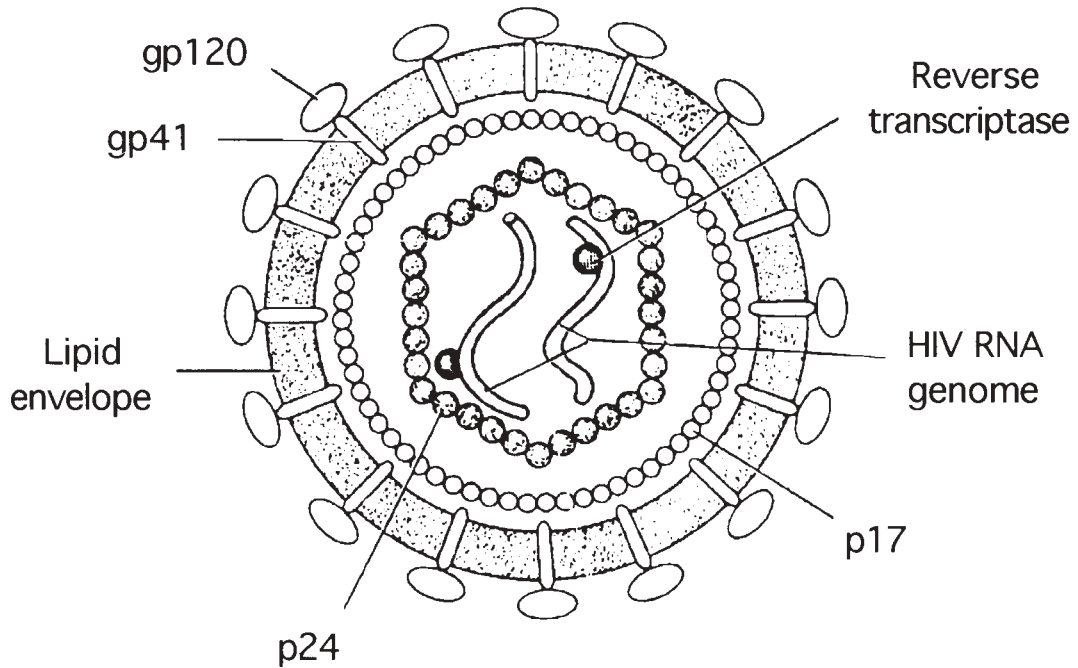


Figure 1. The structure of the human immunodeficiency virus particle. Modified from Greene (1).

disinfection and sterilization procedures are included in this approach. A fresh 0.5% solution of chlorine-containing disinfectant (1:10 dilution of household bleach) with 10 minutes contact time is recommended for disinfection of surfaces contaminated with HIV preferably with cleaning of visible soiling of the contaminated area before disinfection. No special modifications are required to existing procedures for sterilization, disinfection, housekeeping, laundry or waste-handling to take account of HIV, provided these procedures are adequate to cope with hepatitis B virus. More details may be found in the National Health and Medical Research Council and Australian National Council on AIDS guidelines (2).

Life Cycle

HIV replicates in cells of the immune system carrying the CD4 receptor, most importantly the T-helper lymphocyte and the monocyte/macrophage. The T-helper lymphocyte has a central role in the production of an effective, coordinated immune response. The destruction and disruption of the functioning of this cell by HIV infection is a

key factor in producing the immunosuppression that is the hallmark of HIV/AIDS. There is in vitro evidence that a number of other CD4-positive and CD4-negative cells may be infected with HIV but the significance of this in vivo is not clear.

The CD4 molecule is an immunoglobulin-like cell surface glycoprotein normally involved in signalling processes during T-cell activation. Entry of HIV into a susceptible cell is mediated by interaction between the CD4 molecule and the HIV gp120/41 complex. Binding of the HIV gp120 glycoprotein to the CD4 receptor induces a conformational change in gp120 which brings gp41 into contact with the cell surface. This allows gp41 to fuse the virus envelope with the cell membrane, releasing the viral core into the cell cytoplasm.

Once inside the cell a DNA copy of the HIV RNA genome is made in the cytoplasm by the viral reverse transcriptase enzyme (3) (Figure 2). This DNA copy migrates to the cell nucleus and is inserted into the host cell chromosome by action of the viral integrase enzyme. There it remains as a provirus for the lifetime of the cell. T cells activated in response to antigenic

TABLE 1

IMPORTANT ROUTES OF TRANSMISSION OF HIV

Route

Sexual:	homosexual and heterosexual
Parenteral:	injecting drug use unscreened blood transfusion needlestick injury
Mother/infant:	perinatal breastfeeding

Cofactors

- Other sexually transmitted diseases
- Sexual promiscuity
- Needle sharing
- Highly infectious transmission source

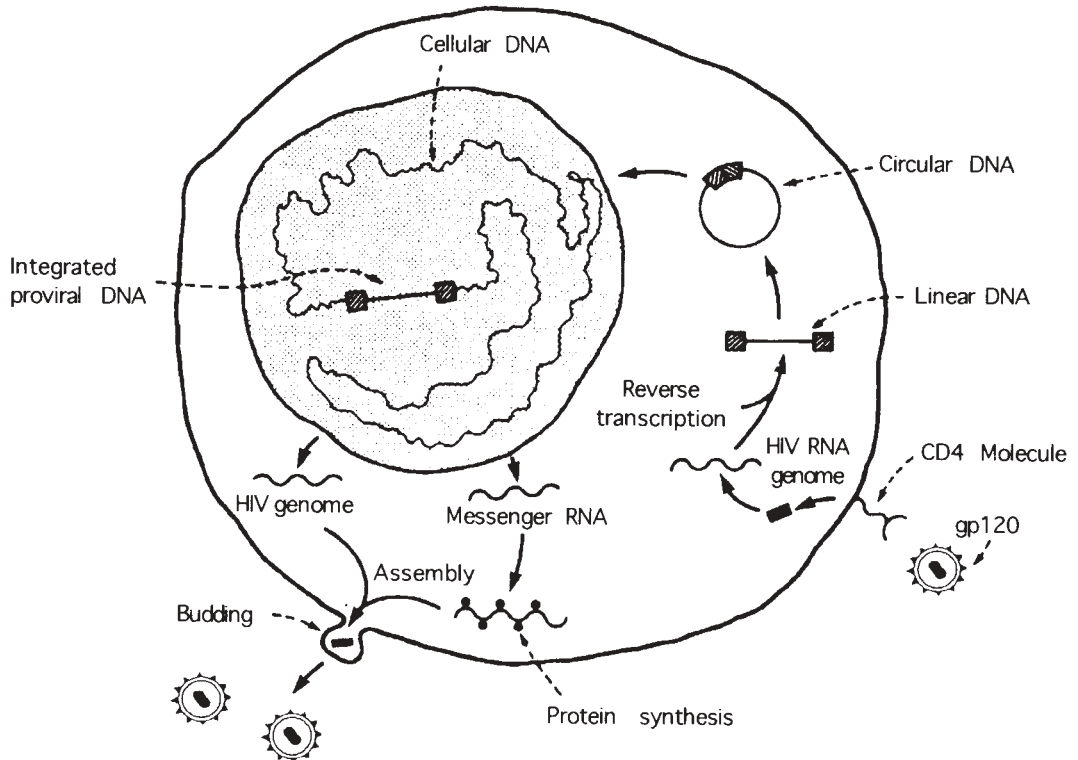


Figure 2. The cellular life cycle of human immunodeficiency virus. Modified from Fauci (3).

stimulation are highly susceptible to HIV infection and replication. Transcription of HIV messenger RNA from the provirus and its translation into HIV proteins occur efficiently in these cells. Assembly of the HIV proteins

and packaging of HIV RNA genomes transcribed from the provirus occur in association with the cell membrane and complete HIV particles exit the cell by budding. Resting T cells are not permissive for

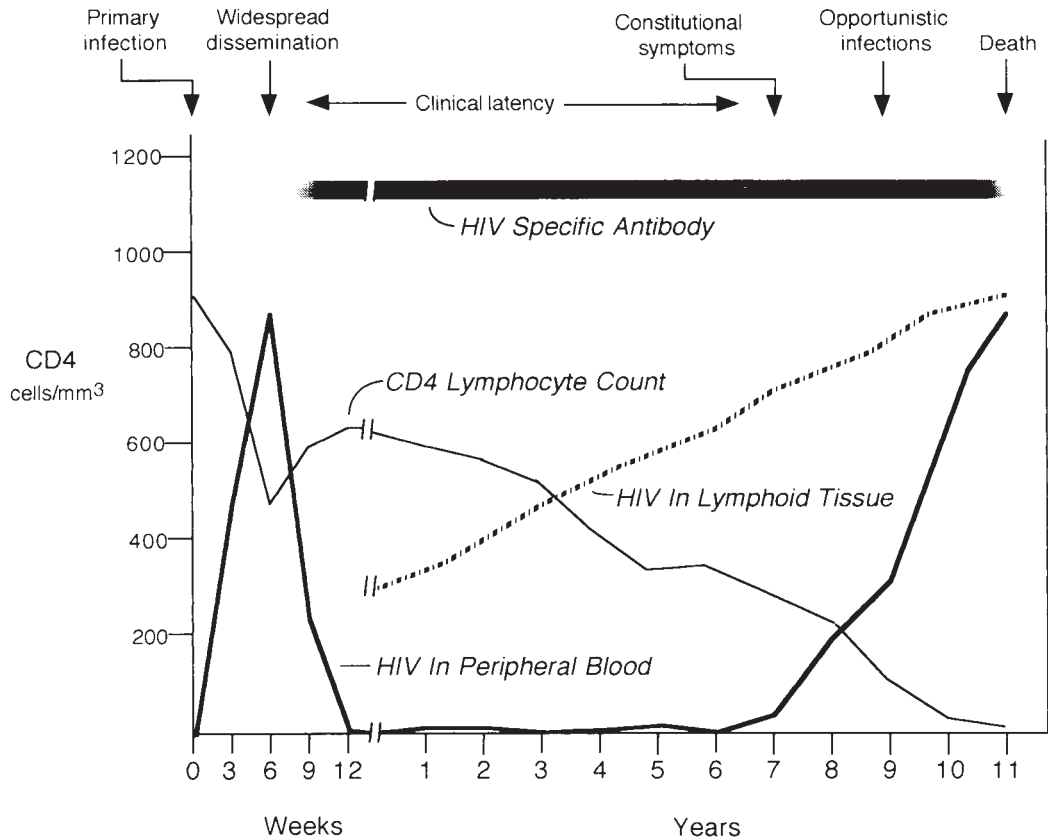


Figure 3. Virological and immunological events during human immunodeficiency virus infection. Modified from Fauci (5).

HIV replication and after integration the provirus may remain in a state of latency without active replication. When T lymphocytes latently infected with HIV are activated in response to an antigen, latent HIV proviruses are stimulated to replicate. It seems that cell signalling molecules responsible for T-cell activation are also used by HIV as triggers for replication.

Pathogenesis

In the weeks following initial infection there is a transient burst of high titre viraemia which seems to be important in seeding the virus widely to lymphoid tissue in the body and perhaps the brain (4,5) (Figure 3). Infected macrophages are probably an important vehicle for this seeding, and seem to be the cell in which HIV infection becomes established at the site of entry to the body. This burst of HIV in the peripheral blood quickly clears, probably due to the appearance of a neutralizing

antibody response and cell-mediated immunity, and trapping of the virus within lymph nodes. Development of measurable antibodies to HIV generally occurs within 1-3 months of infection and seropositivity is usually life-long, although antibodies to the viral core (p24) are frequently lost with the development of AIDS. In up to 50% of patients the initial viraemia is associated with a transient mononucleosis-like 'seroconversion illness'.

Subsequently for an average of 7 to 11 years there is a period of clinical latency with no symptoms and low levels of HIV-infected cells circulating in the peripheral blood, mostly latently rather than actively infected. Beginning at the time of initial infection and progressing through the period of clinical latency there is an inexorable decline in the number of circulating CD4-positive T-helper lymphocytes. Eventually the CD4 count declines to below 200 cells/mm³(µl) and the patient begins to experience the opportunistic

infections and malignancies that define AIDS, the symptomatic final phase of HIV infection. A number of mechanisms have been proposed by which T-helper lymphocytes may be destroyed by HIV infection, but their relative contribution to the decline in CD4 numbers is still not completely clear. Destruction of infected cells by the cytotoxic arm of the immune system together with direct HIV cell cytotoxicity are probably the most important mechanisms.

For some time it was hard to reconcile the decline in numbers of T-helper lymphocytes with the relatively low levels of HIV-infected cells circulating in the peripheral blood during clinical latency. It is now known that during this period active replication of HIV is taking place within the lymphoid tissue, where 98% of the body's helper T cells are located. This is also the site of antigen presentation and T-cell activation and therefore of a population of cells highly susceptible to HIV infection. Recent evidence suggests that the prolonged period of clinical latency represents a relative balance between the destructive effects of HIV replication and the regenerative capacity and antiviral action of the immune system. Very large numbers of HIV particles (about 10^8 - 10^9) are apparently newly produced each day, and each day are destroyed by the immune system. Likewise immense numbers of T-helper cells (2×10^9) appear to be destroyed by HIV replication and replaced by the haemopoietic system each day. New HIV particles produced within the lymph node are efficiently trapped within the tentacle-like processes of the antigen-presenting follicular dendritic cells (FDC) but serve to infect new generations of activated T cells during the process of antigen presentation. This almost equal struggle between the immune system and virus initially contains HIV replication within the lymphoid tissue and maintains the CD4 lymphocyte count. However, in the long term HIV is able to evade the destructive effects of the immune response, and in turn the damaging effects of prolonged HIV infection in key immune cells and tissues gradually wears down the body's capacity to fight the virus.

Data obtained in chimpanzees suggest that through a process of mutation and natural selection HIV may successively escape the action of neutralizing antibody and evade an

otherwise adequate immune response. The reverse transcriptase of HIV allows a relatively high mutation rate and over time diverse viral subpopulations accumulate within the infected individual. Neutralizing antibody probably provides a selection advantage to subpopulations of HIV that resist antibody binding due to different genetic sequences in the neutralizing epitopes of the gp120 'V3 loop'. A resistant subpopulation may be selected in this manner to become the dominant HIV strain, until replaced in its turn by a new resistant subpopulation when a neutralizing antibody response develops against this dominant strain. There is preliminary evidence of a similar process occurring in the immune escape of HIV from cytotoxic T-cell responses.

While evading the antiviral effects of the immune response, HIV infection in turn has an adverse impact on the body's ability to mount effective immune responses. As discussed above the main cell infected and killed by HIV is the T-helper lymphocyte, which has a central coordinating role in the immune system. Decline in numbers of this cell correlates with impaired immune functioning of the host. However, HIV infection also appears to actively induce a state of chronic overactivation of immune cells. B lymphocytes from HIV-infected individuals spontaneously secrete high amounts of tumour necrosis factor (TNF) and interleukin-6 (IL-6) as well as other pro-inflammatory cytokines, but appear to be defective in elaboration of immunoregulatory cytokines important for T-cell help. This imbalance contributes to a nonspecific state of activation of monocytes and T cells which facilitates HIV replication, while at the same time disrupting the T-helper cell functions important for effective immune functioning. Over time the chronic maintenance of immune cells in an activated state probably diminishes their functional capability. In addition, the adverse effects of prolonged HIV infection gradually cause a degeneration of the network of antigen-presenting FDC and of lymphoid tissue. Eventually during advanced HIV disease the FDC network is destroyed and lymph node architecture severely disrupted. Antigen presentation and the coordinated functioning of immune cells are severely disrupted by this stage and HIV particles, no longer trapped within the lymph node, begin to circulate in high titres in the blood stream.

About 5% of people infected with HIV remain healthy and do not experience the decline in CD4 lymphocyte count characteristic of progression toward AIDS. These people are the focus of great interest in case lessons can be learned from them that would improve HIV treatment. However, it appears that they are a heterogeneous group. No consistent feature of their immune responses has yet been identified that would explain their nonprogressive disease, and apart from isolated cases it has not been possible to identify a defect in the infecting strain of HIV. One interesting recent study from Melbourne's Macfarlane Burnet Centre for Medical Research has described a deletion in the gene coding for an HIV accessory protein (*nef*) in a cohort of long-term survivors. This may have implications for the production of an HIV vaccine.

Laboratory Diagnosis

Serological detection of specific antibody is the mainstay of HIV laboratory diagnosis, although detection of circulating viral antigen, isolation of virus in cell culture, or detection of viral genetic material in cells or plasma may be useful in certain clinical situations (6). A variety of serological test formats are available. In countries such as Australia and the USA the standard diagnostic approach is a screening enzyme immunoassay (EIA) followed by Western blot confirmation of specimens reactive on EIA. In Papua New Guinea the usual strategy is screening using a particle agglutination assay and confirmation of reactive specimens by EIA.

Both EIAs currently licensed for HIV diagnosis in Australia are combined HIV-1 and HIV-2 assays. These employ antigen from the *gag* region of HIV, which crossreacts extensively with HIV-2, and distinct antigens from the *env* region of HIV-1 and HIV-2. These HIV proteins coat wells or beads in a plastic EIA tray and will bind HIV-specific antibody present in patient serum pipetted into the well. This bound HIV-specific antibody may be detected by adding an antibody specific for human immunoglobulin which has been complexed to an enzyme; alternatively HIV antigen labelled with enzyme may be used for the same purpose. Subsequent addition of the enzyme's substrate produces a coloured

product and signals the presence of the patient's HIV-specific antibody bound in the well. Washes at each step in the assay remove all material not specifically bound to the solid phase. Specificity and sensitivity of the current generation of EIAs is extremely high: 99.8% and 99.5% respectively. The sensitivity and specificity of the particle agglutination assay employed for HIV screening in Papua New Guinea is comparable to that obtainable with HIV EIAs. In this test gelatin particles coated with HIV viral components are agglutinated by HIV-specific antibodies in patient serum or plasma to give a visible reaction. Although strictly speaking an HIV-1 assay, this gelatin particle agglutination assay has detected the small number of HIV-2-positive sera encountered in Australia to date.

Specimens positive in the above screening assays are generally repeated in the same test to exclude laboratory error and if positive again are called 'repeat reactors'. However, further confirmatory testing of repeat reactors is required. This is because the seroprevalence of HIV in many countries is extremely low; even lower than the fractions of a percent of false positive reactivity that such high quality EIA or agglutination screening tests will produce. Some false reactivity will occur in any laboratory test and cannot be completely avoided. In serological tests false reactivity may have many causes including crossreacting antibodies produced to other pathogens, autoantibodies and antibodies to components of the test kit.

A hypothetical example illustrates how low frequency of HIV infection may adversely affect the positive predictive value of even the most accurate test: at an HIV seroprevalence in the community of 0.01%, 1 true positive HIV infection would be detected for every 10 000 patient specimens tested. If the screening test was 99.9% specific, 10 false positive reactions would occur for every 10 000 specimens tested. In this example false positive reactions would outnumber detection of true infections 10:1 if only a single screening test were used in laboratory diagnosis. This would clearly be unacceptable, and therefore confirmatory testing to distinguish true positive from false positive reactivity is performed on all specimens 'repeat reactive' by screening test.

In countries such as the USA and Australia Western blot is the confirmatory assay usually employed. Western blots consist of a nitrocellulose strip down the length of which all the component proteins of HIV are bound in distinct bands, albeit not visible to the naked eye. This nitrocellulose strip is prepared by the manufacturer by 'blotting' to transfer HIV proteins from an electrophoretic gel of HIV-infected cultured lymphocytes. In the diagnostic laboratory patient serum is incubated with the nitrocellulose strip and the presence of HIV-specific antibody detected with an enzyme-labelled detector antibody exactly as described for an EIA above. A positive serum will contain antibodies to many HIV proteins and their presence bound to the nitrocellulose strip and labelled with precipitated enzyme product is seen as a series of dark bands which form a fingerprint characteristic of HIV, like a computer bar code. In Australia the criteria for a positive HIV Western blot are the presence of a glycoprotein band (gp41, gp120/160) and at least three other bands. A specimen is not considered to be positive for HIV antibody until a positive Western blot result has been obtained. This algorithm of screening EIA and Western blot confirmation gives positive and negative predictive values that approach 100%.

Occasionally sera will give repeat reactivity on screening EIA but will not fulfil the criteria for positivity by Western blot. Such sera are regarded as indeterminate and may represent partially complete seroconversion to HIV or nonspecific reactivity. Experience at the Australian National HIV Reference Laboratory suggests that most true seroconverters will show progression to a fully positive Western blot within 4 weeks of an indeterminate Western blot result, and effectively all will have done so within 9 weeks. Therefore serial bleeds for a 3-month period of follow-up may be used to distinguish HIV seroconverters from false reactors, who will typically show no change in reactivity during this period.

However, the Western blot is an expensive and technically demanding assay and resolution of the indeterminate results that it sometimes produces can be onerous. Recent improvements in accuracy and ease of use of EIAs and other rapid tests have made it possible for the World Health Organization to

develop HIV testing strategies which do not involve Western blot confirmation and are at least as effective (7). Such alternative strategies are particularly appropriate in countries where resources are limited. The use in Papua New Guinea of particle agglutination and EIA as screening and confirmatory assays respectively is an example of such a strategy. The testing algorithm employed is analogous to that outlined above involving EIA and Western blot. Briefly, serum samples negative when tested with the initial screening assay are considered not to contain antibody to HIV. Specimens repeat reactive when tested by the screening assay are confirmed positive by testing with the second assay. It is important that both assays have different antigen preparations or different test principles (e.g. agglutination versus EIA) to increase the independence of results obtained with each. Normally the more sensitive assay is used for screening and the more specific for confirmation.

Conclusion

In the relatively short time since its discovery, HIV has become one of the most studied and best characterized of human pathogens. Laboratory diagnosis of HIV infection is arguably more accurate than that of any other infectious agent, but our detailed understanding of this complex virus has yet to translate into truly effective therapy. At least for the foreseeable future prevention of infection will underpin the fight against AIDS.

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