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HIV transmission by transplantation of allograft skin: a review of the literature*

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The fear of human immunodeficiency virus (HIV) transmission by means of allograft skin has led to a cautious approach to allograft donor selection. However, no irrefutable diagnostic test exists to determine the possible presence of HIV at the time of donation. In order to find ways of improving HIV donor screening practices for skin banks, we review the presence of HIV in human skin, explore the possible transmission of HIV by transplantation of human allograft skin, and discuss the reliability of existing HIV tests. The use of the polymerase chain reaction (PCR) as a sensitive detection system for HIV infection of skin biopsies, in combination with conventional routine HIV blood screening tests, could lower the risk of transmitting HIV to severely burned patients. © *1997 Elsevier Science Ltd for ISBI. All rights reserved.*

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Introduction

AIDS continues to be spotlighted by the media, putting pressure on health-care workers.

The fear of HIV transmission by means of allograft skin has given rise to a cautious approach to allograft donor selection. Following an incident in 1986, in which HIV seroconversion followed the use of allograft skin for a burned patient, the Burn Unit of Queen Mary's University Hospital in London abandoned the use of human allograft skin obtained from cadavers and other patients[1], and turned to the use of widely meshed autologous skin overlaid with meshed allograft from a parent for resurfacing large-area burns in children[2]. There is clearly a need for a reliable diagnostic test for the presence of HIV at the time of donation.

Human allograft skin

Allograft skin is used as a temporary dressing while awaiting healing of autograft donor areas between multiple harvesting, and is often life-saving. Alternatively, allograft skin can serve as a biological dressing pending definitive surgical treatment of a deep burn or spontaneous healing of partial-thickness skin loss. It will inevitably be rejected. Early excision and grafting with allograft skin avoids mortality due to invasive infection, by reconstituting a barrier to microorganisms. Allograft skin also reduces pain, decreases evaporative water loss, improves the chance of re-epithelialization and leads to better cosmesis[3]. Allograft skin is by far the best alternative to the patient's own skin, and large skin banks have been set up to maintain an adequate supply. The skin bank of the Brussels Burn Wound Center annually collects allograft skin from approximately 100 cadavers for use in their own burn unit and some units at surrounding hospitals.

Alternatives to human allograft skin

Xenografts (e.g. porcine skin grafts[4,5]) and (bio)synthetic materials (e.g. Biobrane[6,7]), in contrast, have been used as temporary wound covering with limited success. These are dressings rather than grafts, as they do not become vascularized. The major difficulties affecting the use of porcine skin are bacterial infection, cost and the theoretical risk of zoonoses such as meningitis[8,9] (*Streptococcus suis*), brucellosis[10] (*Brucella suis*), hydatidosis[11,12] (*Eschinococcus granulosus*), cysticercosis[12,13] (*Cysticercus cellulosae*) and influenza[14,15] (pandemic strains). Pigs raised in a clean laboratory animal house do not appear to carry infections that could be hazardous to man. Non-invasive screening could exclude any significant infectious agent, but biopsy and histological examination of the skin are indicated[16]. It is important that the wound coverings adhere as rapidly as possible and are strong enough to resist loosening during daily patient movement. A wound cover should prevent water loss, as increased water permeability may lead to drying of the underlying tissue with thrombosis of the microcirculation. If water permeability is less than that of normal skin, liquid accumulation and low adherence is usually the result[17]. To date, (bio)synthetic coverings do not supply the necessary functional and cosmetic qualities of normal skin.

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Risk of transmission

Through the application of current screening practices, HIV transmission by transplantation is rare. HIV transmission has been implicated only in transplantation of organs and large vascular tissues (large bone, skin). The risk of transmission may be related to the risk of exposure to infected blood through the transplanted allograft. Lack of transmission from HIV-infected donors of avascular tissues may be due, in part, to tissue processing, which can inactivate HIV[18,19]. Skin processing techniques as applied by the skin bank of the Brussels Burn Wound Centre, cryopreservation with 30 per cent glycerol or 5 per cent DMSO as cryoprotective agent[20], maintain the functional integrity of the allograft but do not inactivate HIV[21]. It is unknown whether allograft processing can inactivate HIV without affecting the functional integrity of the allograft[22,23]. Tissue processing can enhance the safety of the allografts, but cannot replace extensive donor screening[24].

Routine HIV screening tests

Since 1985 all potential blood, tissue and organ donors in Europe and the USA are screened for antibodies to HIV using highly sensitive and specific enzyme-linked immunosorbent assays (ELISA)[25]. Nonetheless, antibody screening does not preclude a small risk of transmission from donors with early HIV infection (false negatives). There is a latency or 'window period', during which antibodies to HIV are not yet present in donor serum (*Table I*)[26]. Transmission of HIV-1 by a seronegative organ and tissue donor has been reported. Seven recipients of organs and unprocessed fresh frozen bone from a seronegative donor, who had no known risk factors for HIV infection, were infected with HIV. HIV-1 was detected retrospectively in donor lymphocytes by viral culture and PCR[27]. The risk of HIV transmission from a seronegative living donor can be reduced by quarantining the tissue grafts for several months until a subsequent negative HIV antibody test confirms that the donor was not infected at the time of tissue removal. Unfortunately, such a quarantine system is not possible for cadaveric skin donations.

The 'window period' can be shortened by testing for the virus itself. Reverse transcriptase PCR (RT-PCR) detection of viral RNA in plasma is the most sensitive HIV screening test, but is complex, rather expensive, and not very reliable. Detection of the p24 antigen in serum is less expensive but, again, not very reliable. After seroconversion, the p24 antigen and the viral RNA can revert to negativity. For the detection of proviral DNA in mononuclear blood cells using PCR, many commercial kits are available (e.g. Roche, Perkin-Elmer, Sorin and Murex). They have proven to be very promising in the detection of early HIV infection. Unfortunately, PCR is strongly inhibited by porphyrin compounds derived from haem and haemoglobin which occur in haemolysed cadaveric blood[21,28,29]. The frequency of false negatives due to this inhibition is estimated to be as high as 15 per cent[30]. The presence of large amounts of haemoglobin or other cellular factors in cadaveric serum can also cause non-specific reactivity (false positives) when using approved commercial antibody detection kits (e.g. Abbott EIA assays)[31,32]. The number of false-positive results depends on the test kits used, sample preparation and local factors. The causes of a false-positive outcome are practically impossible to prevent[33]. To prevent the incorrect exclusion of donors, Patijn et al. recommended that a duplicate repeat be performed when the screening test is positive or intermediate. Since skin transplantation does not imply a sense of urgency, a reconfirmation test can be performed when one or both repeats is again intermediate or positive. To ensure optimal safety measures, the few (false) positive skin donations could even be discarded without confirmation test. Commercial test kits are designed for use in diagnostic testing of living donors, and little is known about the reliability of these tests with haemolysed cadaveric samples. Even HIV antigen or antibody tests of the latest generation (e.g. Abbott AXSYM system) have not been evaluated on post-mortem samples. In the procedures of nearly all tests, it is clearly indicated that the performances of the tests have not been established for cadaver samples.

Table I. Window periods between infective contact and detection by routine HIV screening tests

Screened marker	*Sample*	*Window period**	
		Range	*Mean*
Viral RNA	Plasma	1–45 days	4 days
Proviral DNA	Mononuclear blood cells	1–45 days	1 week
p24 antigen	Serum	1–85 days	2 weeks
IgM antibody	Serum	15–130 days	3 weeks
IgG antibody	Serum	15–145 days	4 weeks

*In ±5 per cent of cases, the window periods are considerably longer than indicated above.

Guidelines for tissue collection

Screening of prospective donors for HIV risk factors is imperative.

Regulations and guidelines for tissue collection are still under development.

The United States Public Health Service recommends that potential donors with HIV-related risk factors be excluded from donation, regardless of antibody status. Additionally, the use of screening tests that may be more sensitive in early infection, such as p24 antigen tests or PCR, may be considered, based on such factors as their positive predictive value in the donor population, availability, cost and timeliness with which results can be made available[18]. The Belgian Health Service recommends anti-HIV-1 and -HIV-2 screening tests. For living donors, an additional antibody test 3 months after removal of the tissues is required[34]. The American Association of Tissue Banks and the European Association of Tissue Banks recommends that blood samples should be

tested for antibodies to both HIV-1 and HIV-2, and for HIV p24 antigen. They state that HIV DNA detection by PCR is too time consuming to be useful for organ transplantations, but is valid in testing tissues which can be conserved for several months or years[35]. Skin transplantation does not imply a sense of urgency, thus allowing more time for thorough donor control. It is ethically unacceptable unless all possible measures to minimize the risk of transmission have been taken.

Targets for HIV infection in skin

HIV uses the CD4 molecules of host cells as a receptor for entry. Human lymphocytes are the major site of HIV-1 replication[36]. Human epidermal Langerhans' cells (LC) are HLA-DR$^+$/DQ$^+$, CD1$^+$, CD4$^+$, dendritic antigen-presenting leucocytes which renders them as theoretical targets for direct infection by HIV (*Figure 1*). In vitro infection of cell cultures enriched for LC with laboratory strains of HIV has been reported[37–39].

To date, conflicting results have been published concerning the in vivo infection of epidermal cells (EC) by HIV. In 1984, Belsito et al. described a strong reduction in LC numbers during HIV infection[40]. This was confirmed by Oxholm et al. in 1986[41]. In 1988, Rappersberger et al. Observed that in certain HIV-1-infected individuals, LC are the only epidermal cells to react with monoclonal antibodies (MAb) against HIV-1 core proteins p17 and p24. Furthermore, electron-microscopic analysis of skin and mucosal biopsies showed that HIV-1 replicates in LC, and is released from them[42]. Dréno et al. demonstrated a correlation between the numbers of LC and CD4$^+$ lymphocytes. Numbers of LC were lower in patients with Centers for Disease Control (CDC) disease stages III and IV than in those with disease stage II[43]. In 1989, Kanitakis et al. observed no reactivity of LC with MAb to HIV p18, p24, and gp120. No significant correlation could be established between the number of LC and the number of peripheral blood CD4$^+$ lymphocytes or the CDC disease stage[44]. In 1990, Chesebro et al. showed that human keratinocytes are susceptible to HIV infection[45]. In 1991, Kalter et al. suggested that a role for Langerhans' cells as a principal viral reservoir or vector of transmission is highly unlikely. They analysed skin from 28 HIV-seropositive subjects at various clinical stages by transmission electron microscopy, immunofluorescent staining, in situ hybridization, PCR and by direct virus isolation. With these techniques, demonstration of HIV in the epidermis of infected patients was equivocal and, even then, infrequent. In contrast, viral DNA was detected from the dermis of the same skin samples (26 of 28 samples)[46]. The number of LC of infected patients were within normal limits. Zambruno et al. demonstrated HIV-1 proviral DNA in LC-enriched, but not in LC-depleted, epidermal cells[47] and, using RT-PCR, Kanitakis et al. found HIV-1 mRNA in the epidermis of one of 12 patients[48]. Näher et al. found that genetic variants of HIV can evolve independently from one another and can coexist in blood and epidermis from the same patient[49]. It is unclear whether the evolution of those different genotypes occurs in the epidermis or in the progenitor cells of the LCs. In 1994, Henry et al. detected mRNA for regulatory (*tat, rev, nef*) and structural (*env*) genes in EC enriched for LC, but not in LC-depleted EC[50]. Cimarelli et al. quantified HIV-1 proviral DNA in LC of infected patients using a competitive PCR assay. They concluded that 1 per 1000 to 1 per 100 LC is infected[51]. Since there are $\pm$700 LC per mm^2 [52], one biopsy (0.8 mm) would contain 35–350 HIV genomes.

There are indications that the presence of the CD4 antigen is not a prerequisite for HIV infection and that accessory cells such as interdigitating cells and macrophages are also targets of HIV infection[63]. Heng et al. reported in vivo HIV-1 infection of keratinocytes, which lack the CD4 molecule, in tissues coinfected with herpes simplex[54]. In 1992, Kim et al. reported that the HIV *tat* gene can efficiently transform human keratinocytes in culture[55]. Recently, Ramarly et al. demonstrated that normal cultured human keratinocytes can be infected in vitro by and HIV-1 mono- and lymphotropic strain and may transmit HIV-1 to bystander lymphoid cells[56].

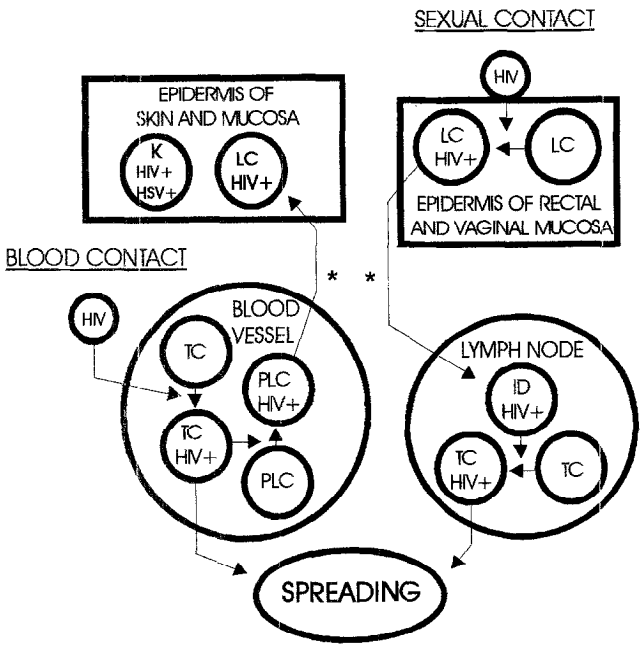

Figure 1. Hypothetical spread of HIV infection. LC = Langerhans cell; ID = interdigitating cell; TC = T cell/lymphocyte; ETC = epidermal T cell; PLC = progenitor cells of LC (derived from the bone marrow); K = keratinocytes; HSV = herpes simplex virus; * = migration and transformation.

Discussion

There is still no satisfactory replacement for human allograft skin, and detection of HIV in cadaveric blood is not very reliable. it is hampered by porphyrins and haemoglobin which occur in haemolysed blood. Haemolysis is inevitable and sets in immediately after death. Nevertheless, it is planned

to adapt PCR methodology for reliable detection in haemolysed cadaveric blood using a procedure adapted for forensic samples[57]. By contrast, proviral DNA can easily be detected in cadaveric skin using PCR without inhibition of porphyrins. Due to the controversy about the actual site of HIV infection in skin, it is probably safer to detect HIV in full-thickness skin samples, rather than in LC-enriched EC, and it has the additional advantage of being less complicated. Enrichment of LC from EC using immunomagnetic microspheres[58] or by discontinuous density gradient centrifugation[59] is rather complex and time consuming. The use of a sensitive method for detection of HIV in the engrafted skin itself, independent of the serology, can add additional safety to the grafting of allograft skin or cultured epithelium. Furthermore, it can be useful for the detection of HIV in organ or tissue donors, when no pretransfusion blood sample is available. Transfusions given to a potential organ or tissue donor prior to HIV screening dilutes the blood, which may result in a false-negative test result[60].

The positive predictive value of PCR-based HIV detection in skin from the cadaveric tissue donor population (e.g. heart valve, bone and arteries) will have to be evaluated and compared with approved, routine screening tests.

Finally, it is highly recommended that blood samples routinely collected upon admission to the donor hospital be used, whenever possible, for the HIV screening test. Ideally, donor hospitals should be encouraged to preserve blood samples of all patients susceptible of becoming a donor.

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