

infection; the explanation is unknown, but susceptibility to the transmission of other viruses is known to vary in these circumstances. Secondly, over two thirds of people who are seropositive for herpes simplex virus type 2 have no history of genital lesions and about one third of new cases of genital herpes are acquired from contacts without symptoms; these data suggest that transmission of the virus could not be eliminated by taking a detailed history and a physical examination.<sup>5</sup>

We need to know—urgently—the most effective methods of screening potential semen donors. This question is very much open to debate. One problem in forming an initial policy was not knowing the incidence of pathogens in potential donors. An investigation has now been conducted, however: pathogenic organisms have been isolated from the urethra of one third of potential donors.<sup>12</sup>

Many groups have formulated guidelines for screening semen donors<sup>2,3,8,11</sup> (the merits have been discussed elsewhere<sup>13</sup>). These guidelines show that there is a consensus on some matters—for example, the exclusive use of frozen semen to allow the serum of the donor to be tested and cleared for HIV antibodies, the use of urethral swabs, and an adequate physical examination and history taking. The use of such guidelines would greatly restrict the transmission of pathogens to recipients. Yet as recently as 1988 there was no systematic approach by donor insemination clinics in Britain to prevent the spread of common sexually transmissible pathogens to recipients.<sup>11</sup> For example, only 9% of clinics carried out urethral tests—suggested as mandatory 15 months earlier by the American Fertility Society.<sup>8</sup> Even in the United States, however, despite specific guidelines a national survey in 1988 also indicated a lack of a structured approach.<sup>14</sup> Over half of the physicians who performed donor insemination were unaware of professional guidelines for recruiting semen donors.

Quite rightly, concern has been growing about the transmission of pathogens by donor insemination. If this concern is to be allayed three steps need to be taken.

Firstly, research is needed to develop new tests for genital pathogens in semen. Ideally every semen sample should be tested for a full range of pathogens. This might lead to recruiting donors from a wider population as well as reducing the risks to recipients.

Secondly, research is needed to provide more data about the transmission of these pathogens.

Thirdly, the guidelines for preventing transmission of infection and for recruiting and testing semen donors should be given more publicity and should be followed by all infertility clinics in both the private and the public sectors.

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## Cultured keratinocytes and keratinocyte grafts

### *Skin grafts from the laboratory can supplement autografts*

The use of cultured epidermal grafts (keratinocyte grafts) to treat patients with life threatening burns was first reported in 1981,<sup>1</sup> and science fiction seemed to be meeting reality. From a small initial biopsy specimen sheets of epidermis had been grown in the laboratory to a size which could cover a wound 10 000 times the area of the biopsy. Apparently technology had produced the ideal dressing for wounds causing extensive loss of skin: the patient's own skin cells. Sadly, however, as other groups from Europe, the United States, and Japan have evaluated the treatment, both in clinical practice and in studies on animals, it has become clear that much remains to be learnt about keratinocyte grafts and wound healing.<sup>2,3</sup>

The breakthrough in culture techniques permitting the generation of epidermal keratinocytes through multiple passages was described in 1975 by Rheinwald and Green.<sup>4</sup> They next suggested that surgeons should explore the use of cultured epithelium to close epidermal defects.<sup>5</sup> Whole skin was treated with trypsin to separate the epidermis from the dermis and to disaggregate the epidermal cells, which were then grown on a feeder layer of lethally irradiated mouse cells in a complex culture medium. Confluent stratified sheets of

epidermal keratinocytes were available for grafting three to four weeks after the biopsy. The first clinical use of sheets of autologous keratinocytes (keratinocyte autografts) was reported from Boston in two adults with extensive burns.<sup>1</sup> Further anecdotal cases and small open series have subsequently been described in the United States,<sup>6,7</sup> Europe,<sup>8</sup> and Japan.<sup>9</sup> In addition, the culture systems have been modified,<sup>10</sup> and a low calcium, serum free medium is available commercially.<sup>11</sup>

The most obvious application for keratinocyte grafts was in patients with burns damaging more than half of the body surface. Such patients have too few donor sites to provide enough split skin grafts to resurface the area of the burn after surgical excision. The usual practice is for such wounds to be covered with biological dressings, such as pigskin, or synthetic dressings until the donor sites have healed and may be reused. In these circumstances the results of keratinocyte autografting have been variable and disappointing. Factors that have proved important include the preparation of the wound bed (a freshly excised wound being better than a chronic granulating wound), the presence of infection, and even the centre

performing the surgery. Because of the delay between taking a biopsy specimen from the patient and the keratinocyte autograft becoming available sheets of allogenic epidermal cells from unrelated donors have been used as keratinocyte allografts.<sup>12</sup> The initial reports suggested that these allografts survived for a long time—possibly due to loss of the Langerhans cells (antigen presenting cells) in culture, but this proved incorrect: it is now accepted that the cells must be of autologous origin to survive transplantation.<sup>13 14</sup>

Split skin grafts are used in other conditions in which keratinocyte grafts are possible. Reports of conditions treated with keratinocyte grafts have slowly diversified to include chronic leg ulcers,<sup>15-17</sup> junctional epidermolysis bullosa,<sup>18</sup> excisions of giant congenital naevi (G Gallico, personal communication), tattoo excisions,<sup>13</sup> and split skin graft donor sites.<sup>19</sup> Grafts of both autologous and allogenic origin have been used, but burns and giant naevi have been treated almost exclusively with keratinocyte autografts. Clinical experience with these conditions was presented at a recent meeting in New Orleans. It has become clear that keratinocyte allografts (and probably autografts) have beneficial effects on wound healing apart from providing cover: these include production of growth factors and extracellular matrix proteins.

The disappointing clinical results in deep burns wounds and the fact that the epidermal keratinocyte sheets grown by the method of Rheinwald and Green contain no dermal elements have led to the investigation of complex cultures using some form of dermal equivalent or substrate to support the growth of the keratinocyte layer. The keratinocytes are usually grown directly on to the chosen substrate<sup>20 21</sup> but may also be placed on the substrate after it has been applied to the wound. The substrates used have varied from simple collagen gels of bovine and rat origin (with and without a cellular component), through complex cross linked matrices of animal<sup>22</sup> or human collagen, to whole dermis—both after cryopreservation and as a live cadaveric allograft.<sup>23</sup> Clinical results of collagen gel bearing grafts have been disappointing in patients with congenital naevi (L Dubertret personal communication). The use of cryopreserved dermis has been studied by Cuono and his coworkers, who treated patients with burns with whole cadaveric allografts after aggressive early removal of the eschar.<sup>24 25</sup> They removed by dermabrasion the highly antigenic epidermis of the cadaveric allograft when the keratinocyte autografts were ready (24-30 days)—before any signs of allograft rejection. The dermabraded bed proved ideal for the placement of grafts, with a good take and excellent cosmetic results. DNA analysis five weeks after grafting, however, suggested that progressive cellular replacement had occurred in the dermis.<sup>26</sup> A recent European Community workshop on skin equivalents in Lyons discussed these substrates at length, concluding that although stabilised matrices of human collagen are being developed, these still need rigorous testing in wound healing.

Replacing the dermis by using a live or cryopreserved human dermis overcomes the need to devise a material of suitable composition and structure but introduces the risk of transmission of disease—including infection with HIV.<sup>27</sup> This risk should not, we believe, exclude the use of allograft dermis with adequate screening of the donors.

Furthermore, there is still some controversy about the requirement for a substrate at all. Studies of early wounds showed incomplete basement membranes and defective anchoring fibrils<sup>28</sup>; long term follow up of early patients treated with keratinocyte autografts in Boston showed that eventually a normal dermis was regenerated beneath keratinocyte grafts when they were placed directly on muscle fascia.<sup>29</sup> This would argue against the need for any substrate,

even though the current optimal skin graft in surgical use—the split skin graft—does contain dermal elements.

Perhaps the most exciting future research on cultured keratinocytes concerns the diffusible products they secrete.<sup>30</sup> These accelerate epithelialisation of wound healing in animals (transforming growth factor alpha),<sup>31</sup> and in vitro they alter the growth, movement, and contraction of wound fibroblasts.<sup>32</sup> This may explain the dramatic beneficial response of lesions such as leg ulcers to treatment with keratinocyte allografts.<sup>15</sup> The secretion of such autologous and paracrine factors survives deep freezing (R G C Teepe, personal communication), and cryopreserved keratinocyte allografts taken from storage banks may become widely applicable.

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