patients who can pay for their treatment. As oncologists and surgeons get better at reducing the size of residual tumour (for example, to 10^9 tumour cells, as in a pea sized plasmacytoma[6] or choriocarcinoma[7]) trials of oral lymphopoietic immunostimulants would be justified.

Experience with passive immunotherapy with specific antibodies against tumour targets has shown that human polyclonal antibodies are the safest and the best.[8] This approach has, however, rarely achieved cures when dependent on the host for the final action (such as complement fixation or antibody directed cellular cytotoxicity). The cytotoxic drugs chlorambucil, daunorubicin, and cisplatin have been conjugated with IgG at doses preserving the immunoglobulin's half life while giving effective delivery of the drugs. Such conjugates are best used for tumours retaining their sensitivity to these drugs, and treatment may be given for many months with no ill effects (as much as 67 mg daunorubicin/kg body weight).[8] In one study of children with neuroblastomas that had been reduced in size by other treatment, cultured tumour cells were used to immunise their fathers and prepare human polyclonal conjugates. Three children with advanced disease given the conjugates had complete healing of their bony metastases and were well seven to eight years later.[8] These treatments can really be justified only in children, but in the future human hybridomas might be useful in treating other tumours showing good cross reactions (such as four fifths of neuroblastomas). Anti-idiotype responses against B cell tumours do not really qualify, and only one of 11 anti-idiotypic rodent monoclonals gave good long term results.[9] The results of using mixed hybridomas, such as Campath-1H,[10] are eagerly awaited.

New ways have been found of generating a local toxic environment when conjugated antibodies are delivered.[11] Nevertheless, using rodent monoclonal antibodies increases the risk of human hapten cross reaction, so these must be carefully screened. In general, however, single monoclonal antibodies, like single cytotoxic conjugates, are ineffective. Using a panel of humanised monoclonal antibodies from which to select a cocktail based on the immunohistochemistry of a biopsy specimen of the cancer might be more successful. Similarly, the best conjugates might be selected from in vitro culture studies of the same cancer. Such "tailoring" may again be too expensive for widespread use. When a xenogeneic product is used the host's responses to it can be removed by giving cyclophosphamide 20 mg/kg exactly 24 and 48 hours after the first exposure.[12]

Intravenous infusions of immune lymphocytes harvested from human volunteers and pigs have specific antitumour effects.[13] Such mechanisms account best for the greater success of bone marrow transplants for leukaemia in matched siblings as compared with identical twins.[14] Producing in vitro T cell clones is being explored, but using such allogeneic cells in vivo may be complicated by graft versus host disease and viral transmission.

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1 Currie G. *Cancer and the immune response*. 2nd edn. London: Edward Arnold, 1980:50.
2 Hobbs JR. Thymic factors to treat cancer patients especially those with T-cell deficiencies. In: Schoff-Werner P, Pfitzenmaier K, eds. *Aktuelle Onkologie*. Vol 24. Munich: Zuckschwerdt, 1985:195-204.
3 Salup RR, Back TC, Wiltrout RH. Successful treatment of advanced murine renal cell cancer by bicompartmental adoptive chemoimmunotherapy. *J Immunol* 1987;**138**:641-7.
4 Rosenberg SA, Packard BS, Aebersold PM, *et al*. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. *N Engl J Med* 1988;**319**:1676-80.
5 Nadler SH, Moore GE. Clinical immunologic study of malignant disease: response to tumor transplants and transfer of leukocytes. *Ann Surg* 1966;**164**:482-90.
6 Hobbs JR. Plasma cell tumours of man and mouse. In: Peeters H, ed. *Protides of the biological fluids*. Vol 28. Oxford: Pergamon, 1980:3-13.
7 Bagshawe KD. *Choriocarcinoma*. London: Edward Arnold, 1969.
8 Hobbs J, Melino G, Riches P. Successful uses of tumour markers in man. In: Peeters H, ed. *Protides of the biological fluids*. Vol 31. Oxford: Pergamon, 1984:255-61.
9 Melker TC, Lowder J, Maloney GD, *et al*. A clinical trial of anti-idiotype therapy for B-cell malignancy. *Blood* 1985;**65**:1349-65.
10 Hale G, Dyer MJS, Clark MR, *et al*. Remission induction in non-Hodgkin's lymphoma with reshaped human monoclonal antibody Campath-1H. *Lancet* 1988;ii:1394-9.
11 Bagshawe KD, Springer CJ, Searle F, *et al*. A cytotoxic agent can be generated selectively at cancer sites. *Br J Cancer* 1988;**58**:700-3.
12 Hobbs JR. Outcome of displacement bone marrow transplantation for inborn errors of metabolism. *Acta Paediatr Jpn* 1988;**30**:462-71.
13 Oon C-J, Butterworth C, Elliott P, *et al*. Homologous immunotherapy using immune leucocytes. *Behring Inst Mitt* 1975;**56**:223-7.
14 Hobbs JR. The scope of allogeneic bone marrow transplantation. In: Losowsky MS, Bolton RP, eds. *Advanced medicine Leeds*: Bath: Pitman, 1983:378-91.

Risks of donor insemination

Guidelines on control of infections need further publicity

The risk of transmission of pathogens to recipients of donor insemination was highlighted by Stewart *et al* in 1985, when they documented the transmission of HIV to four out of eight recipients of cryopreserved semen from a donor without symptoms.[1] This account stimulated several reviews of mainly anecdotal reports of the transmission of genital pathogens including ureaplasma, HIV, and neisseria to recipients of donor semen.[2,3] Two more recent reports of transmission of cytomegalovirus and herpes simplex virus type 2 have again highlighted the risks of donor insemination and the problem of screening semen donors.[4,5]

Hammitt *et al* showed that cytomegalovirus could be recovered from the semen of one of four donors seropositive for cytomegalovirus even after the semen had been frozen (−196°C) for up to nine months.[4] This finding has two important implications: it has confirmed that freezing does not inactivate herpes simplex virus[6] (and HIV and chlamydia can also survive freezing[1,7]), and it has provided further support for the belief that all semen donors should be seronegative for cytomegalovirus.[8] This viewpoint is, however, contentious: such a commitment would effectively reduce the donor pool by 40%.[9,10]

At present three quarters of donor insemination clinics in Britain do not even perform serological tests for cytomegalovirus.[11] Clearly a policy for dealing with cytomegalovirus needs to be formulated, but we first need to know, for example, what the risk of infection is in couples in whom the man is seropositive but the woman is seronegative. In such cases can the use of donors seropositive for cytomegalovirus be justified?[9] What is the incidence of reactivation of the virus? Are the various strains of equal pathogenicity and equally resistant to freezing? Do men who are seropositive secrete the virus in their semen only intermittently, as the data of Hammitt *et al* would suggest?[4]

The transmission of herpes simplex virus type 2 has been proved by finding identical restriction enzyme patterns in a recipient of the donor semen and the donor.[5] The virus was transmitted to only one of the two recipients of fresh semen from a donor who was without symptoms.[5] This report raises two issues. Firstly, only one of the recipients contracted an

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.[5]

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.[12]

Many groups have formulated guidelines for screening semen donors[2 3 8 11] (the merits have been discussed elsewhere[13]). 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.[11] For example, only 9% of clinics carried out urethral tests—suggested as mandatory 15 months earlier by the American Fertility Society.[8] Even in the United States, however, despite specific guidelines a national survey in 1988 also indicated a lack of a structured approach.[14] 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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1 Stewart GJ, Tyler JPP, Cunningham AL, *et al.* Transmission of human T-cell lymphotropic virus type III (HTLV III) by artificial insemination by donor. *Lancet* 1985;ii:581-5.
2 Greenblatt RM, Handsfield HH, Sayers MH, *et al.* Screening therapeutic insemination donors for sexually transmitted diseases: overview and recommendations. *Fertil Steril* 1986;**46**:351-64.
3 Mascola L, Guinan ME. Screening to reduce transmission of sexually transmitted diseases in semen used for artificial insemination. *N Engl J Med* 1986;**341**:1354-9.
4 Hammitt DG, Aschenbrenner DW, Williamson RA. Culture of cytomegalovirus from frozen-thawed semen. *Fertil Steril* 1988;**49**:554-7.
5 Moore DE, Ashley RL, Zarutskie PW, Coombs RW, Soules MR, Corey L. Transmission of genital herpes by donor insemination. *JAMA* 1989;**261**:3441-3.
6 Sherman JK, Menna JH. Cryosurvival of herpes simplex virus-2 during cryopreservation of human spermatozoa. *Cryobiology* 1986;**23**:383-5.
7 Sherman JK, Jordan GW. Cryosurvival of Chlamydia trachomatis during cryopreservation of human spermatozoa. *Fertil Steril* 1985;**43**:664-6.
8 American Fertility Society. New guidelines for the use of semen for donor insemination. *Fertil Steril* 1986;**46**:95-110.
9 Chauhan M, Barratt CLR, Cooke S, *et al.* Screening for cytomegalovirus antibody in a donor insemination programme: difficulties in implementing the American Fertility Society guidelines. *Fertil Steril* 1989;**51**:901-2.
10 Chauhan M, Barratt CLR, Cooke S, *et al.* A protocol for the recruitment and screening of semen donors for an artificial insemination by donor programme. *Hum Reprod* 1988;**3**:873-6.
11 Barratt CLR, Monteiro EF, Chauhan M, *et al.* Screening donors for sexually transmitted disease in donor insemination clinics in the UK. A survey. *Br J Obstet Gynaecol* 1989;**90**:461-6.
12 Monteiro EF, Spencer RC, Kinghorn GR, *et al.* Sexually transmitted disease in potential semen donors. *Br Med J* 1987;**295**:418.
13 Barratt CLR, Chauhan M, Cooke ID. Donor insemination: a look to the future. *Fertil Steril* (in press).
14 Office of Technology Assessment. *Artificial insemination. Practice in the United States.* Washington, DC: Congress of the United States, 1988:113.

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,[1] 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.[2 3]

The breakthrough in culture techniques permitting the generation of epidermal keratinocytes through multiple passages was described in 1975 by Rheinwald and Green.[4] They next suggested that surgeons should explore the use of cultured epithelium to close epidermal defects.[5] 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.[1] Further anecdotal cases and small open series have subsequently been described in the United States,[6 7] Europe,[8] and Japan.[9] In addition, the culture systems have been modified,[10] and a low calcium, serum free medium is available commercially.[11]

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