Transfusion Medicine, 1996, 6, 261–271

Guidelines on gamma irradiation of blood components for the prevention of transfusion-associated graft-versus-host disease

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Received 21 December 1995; accepted for publication 8 February 1996

INTRODUCTION AND TERMS OF REFERENCE

Transfusion-associated graft-vs.-host disease (TA-GVHD) is a rare but usually fatal complication of transfusion. The American Association of Blood Banks survey of 1990 revealed 12 cases in the context of $13{\cdot}8 \times 10^6$ nonirradiated components transfused (Anderson *et al.*, 1991). The risk associated with an individual transfusion depends on the number and viability of contaminating lymphocytes, the susceptibility of the patient's immune system to their engraftment and the degree of immunological (HLA) disparity between donor and patient. There is relatively little scientific information in the literature on which to base guidelines for clinical practice, and no precise estimates of TA-GVHD risk in different clinical settings. Gamma irradiation of cellular blood components has been the mainstay of TA-GVHD prevention, but surveys of blood banks in both the USA and UK have revealed wide variations among centres in irradiation dosage, clinical indications and quality control (Anderson *et al.*, 1991).

This guideline document will therefore consider: (i) the frequency, clinical features and diagnosis of TA-GVHD in a variety of clinical settings, with recommendations of patient groups for whom prevention of TA-GVHD should be considered; (ii) prevention of TA-GVHD by gamma irradiation of blood components, and the components which should be so treated; (iii) the implications of gamma irradiation for blood component function, storage and labelling, and any possible hazards to recipients of such components; (iv) provision and quality control of equipment and dosimetry for the gamma irradiation of blood components.

The document will not discuss ultraviolet irradiation of blood products as a means of preventing HLA alloimmunization, nor will therapy of TA-GVHD be considered.

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PATHOGENESIS, CLINICAL FEATURES AND DIAGNOSIS OF TA-GVHD

Pathogenesis and clinical features

TA-GVHD is a potential complication of transfusion of any blood component containing viable T lymphocytes where there is a degree of disparity in histocompatibility antigens between donor and patient. There appears to be a particular risk when donor and patient share an HLA haplotype, as occurs within families or in populations with restricted haplotypes. Under certain circumstances, these cells engraft and proliferate in the patient. Interaction between donor T lymphocytes and recipient cells carrying either class I or class II HLA antigens results in cellular damage which may be natural killer (NK) cell mediated. Major target tissues include skin, thymus, gastrointestinal tract, liver, spleen and bone marrow. The risks of TA-GVHD are highest in recipients with immunodeficiency or immunosuppression, although TA-GVHD has not been described in patients infected with the human immunodeficiency virus. In immunocompetent individuals, sharing of an HLA haplotype with the donor appears to be a major contributory factor. Since the onset of clinical features is delayed for 1–2 weeks after transfusion, a high index of suspicion is

necessary. The classical early features of fever, maculopapular skin rash, diarrhoea and hepatitis with or without jaundice may be attributed to other causes in immunosuppressed patients. Neonates may demonstrate early hepatosplenomegaly and lymphadenopathy followed by lymphoid regression. Bone marrow involvement produces severe hypoplasia with profound pancytopenia. The symptoms and signs are particularly difficult to differentiate from primary infection in premature or congenitally immunodeficient neonates. The disease generally follows a downhill course, with death, usually due to infection, in > 90% of cases (Sazama & Holland, 1993). A further rare complication, namely blood donor-mediated rejection of transplanted marrow, has also been reported in two cases.

Diagnosis and incidence

The most rapid way to make the diagnosis is by skin biopsy, although the histological features may be supportive rather than pathognomonic. It is therefore useful to have additional evidence of persistence of donor lymphocytes by cytogenetic or HLA analysis. DNA analysis by restriction fragment length polymorphism digestion followed by radiolabelled DNA probes allows identification of transfused cells from small-volume blood samples. However, their presence alone does not necessarily indicate TA-GVHD, since donor lymphocytes can persist for at least 1 week in adults, up to 6–8 weeks in neonates after exchange transfusion and for up to 2 years after intrauterine transfusion, without the development of TA-GVHD. TA-GVHD is almost certainly under-diagnosed, making it impossible to state with certainty the frequency and risk of the problem in any clinical situation, particularly since the indications for irradiated components are not consistent across the UK. In addition, the real incidence may change, as newer methods of blood component production result in reduced lymphocyte contamination. It has also been suggested that some donors may be radio-resistant.

There is currently no formal reporting system in the UK whereby cases of TA-GVHD can be collated, although a system for notification of *all* serious complications of transfusion is under investigation. Details will be published separately.

Recommendation. All cases of TA-GVHD should be notified through the national reporting system.

PREVENTION OF TA-GVHD

Techniques of lymphocyte disarmament

The 'threshold' dose of lymphocytes required for TA-GVHD in humans is unknown, but may depend on the recipient's ability to reject transfused lymphocytes. At least one case has been reported after transfusion of only 8×10^4 lymphocytes kg^{-1}. Successful prevention therefore depends either on physical removal of donor lymphocytes or on destruction of their proliferative capacity. Current filtration technology cannot consistently produce the levels of lymphocyte removal required, and at least one case of TA-GVHD has been seen after transfusion of filtered blood products. As filtration technology advances, however, this situation may change. The mainstay of prevention therefore continues to be gamma irradiation to prevent lymphocyte proliferation, despite theoretical concerns about long-term carcinogenicity.

Recommendation. Gamma irradiation is currently the only recommended method for TA-GVHD prevention. Leucodepletion by current filtration technology is inadequate for this purpose.

Dose of gamma radiation

Experience has revealed the importance of the selection of an effective dose of gamma radiation, validation of the dose actually delivered throughout the irradiation field and some form of assurance that a given component has been irradiated. Initial work based on abolition of MLC reactions suggested that a dose of 15 Gy was sufficient to inactivate lymphocyte responses. TA-GVHD has, however, since been reported following components irradiated with 20 Gy. Techniques of residual T lymphocyte growth detection have led to the recommendation of 25 Gy as the appropriate dose.

With commercial irradiators, the dose of gamma radiation delivered can vary from the centre of the container to the periphery by up to 35%, and along the central axis by up to 30%. Thus it is important to specify whether the recommended dose is an average value or the minimum dose to any point of the container. In the USA, the Food and Drug Administration require a central dose of 25 Gy and a minimum of 15 Gy to any other point in the container (J. Fratantoni, oral communication, April 1993). In the UK, a *minimum* of 25 Gy is recommended (NBTS/NIBSC, 1993). To ensure by dosimetry that this dose distribution is achieved, consultation with supporting physicists is recommended.

Recommendation. The minimum dose achieved in the irradiation field should be 25 Gy, with no part receiving > 50 Gy.

Standard blood components which should be gamma irradiated

Lymphocyte viability is retained in stored red cells for at least 3 weeks, and TA-GVHD has developed following transfusion of whole blood, red cells, platelets and granulocytes. Fresh unfrozen plasma, containing only 10^4 lymphocytes kg^{-1}, has been implicated only in the context of congenital immunodeficiency and in any case is now never administered. Transfusion of granulocytes poses a particular risk, on account of both freshness and number of contaminating lymphocytes, and the likelihood that the recipient is immuno-incompetent. TA-GVHD has not been described following transfusion of frozen deglycerolized cells, which are in any case thoroughly washed free of leucocytes after thawing.

TA-GVHD has *not* been described following transfusion of cryoprecipitate or fractionated plasma products such as clotting factor concentrates, albumin and intravenous immunoglobulin. Only one case has been ascribed to transfusion of fresh *frozen* plasma but this infant (with thymic hypoplasia) had already received several transfusions of red cells (albeit irradiated) and it is possible that these may have been the source of the viable lymphocytes. The likelihood of any lymphocytes surviving freezing and thawing in the absence of a cryoprotectant and possessing intact proliferative potential appears remote and is only of potential significance in the context of congenital immunodeficiency.

Recommendation. For at-risk patients, all red cell, platelet and granulocyte transfusions should be irradiated, except cryopreserved red cells after deglycerolization. It is not necessary to irradiate fresh frozen plasma, cryoprecipitate or fractionated plasma products.

Donations from family members and HLA-selected donors

Because of the sharing of HLA haplotypes, donations from family members pose a particular risk of TA-GVHD, especially when the recipient is a neonate, e.g. maternal platelets to treat perinatal alloimmune thrombocytopenia. Red cells, granulocytes and fresh plasma have all been implicated in TA-GVHD after transfusion from family members. There is an increased risk from donations from both first- and second-degree relatives while consanguineous relationships increase the risk (McMilin & Johnson, 1993).

Several cases of TA-GVHD have been reported from Japan, where fresh blood is not infrequently used, and where common HLA haplotypes increase the chance of a transfusion recipient receiving blood from a haploidentical donor, often homozygous. Two additional cases were from family donations and one case had an HLA haploidentical donor. These observations are of particular relevance for patients receiving HLA-selected platelet concentrates from nonfamily members because of refractoriness to random donor platelets. This would be expected to increase the risk of TA-GVHD, especially if the platelet donor is homozygous for one of the recipient's HLA-haplotypes, since this is analogous to donations within families or within racial groups of limited genetic diversity. A case of TA-GVHD following transfusion of blood components from an unrelated HLA homozygous donor was recently reported. It does not appear to be common practice in the UK at present to irradiate platelets in this setting, other than for recipients of allogeneic BMT. The risk from HLA-selected platelets where the donor is *not* homozygous is uncertain. However, many transfusion centres now specifically maintain panels of homozygous donors for refractory patients, and in practice it is probably more reliable to recommend irradiation of *all* HLA-selected platelets, rather than risk the misallocation of some donations.

Recommendation. All transfusions from first- or second-degree relatives should be irradiated, even if the patient is immunocompetent. Likewise, all HLA-selected platelets should be irradiated, even if the patient is immunocompetent.

MANUFACTURING ASPECTS

Undertaking the irradiation of blood components constitutes a manufacturing process. The responsible department is therefore expected to comply with relevant aspects of the *EC Guide to Good Manufacturing Practice* (Commission of the European Communities, 1992).

(1) Effect of irradiation on blood components

The use of gamma irradiation in the prevention of TA-GVHD aims to inactivate T lymphocytes whilst preserving the function of other blood cells.

(i) Red cells

(a) *Recovery*. There is evidence that gamma irradiation results in reduced post-transfusion red cell recovery but only after prolonged storage. Red cells irradiated within 24 h of collection and subsequently stored for 28 days show a reduced 24-h recovery

compared with nonirradiated controls but still above the minimum acceptable 75%. It has also been suggested that red cells can be irradiated up to 14 days after collection and stored for at least a further 14 days. Loss of viability was the same whether the cells were irradiated on day 1 or day 14 (FDA, 1993). It is clear that many different combinations of pre- and post-irradiation storage times can still produce an acceptable red cell component. Irradiation has no clinically significant effect on red cell pH, glucose consumption, ATP and 2,3 DPG levels. Supernatant-free haemoglobin levels are increased.

(b) *Potassium.* Gamma irradiation of red cells increases the level of extracellular potassium. The potassium level in irradiated units is approximately twice that of nonirradiated controls, a ratio which persists throughout storage, although the rise in the first 24 h may be more than double that of nonirradiated controls. In considering the clinical significance of this, both the speed and volume of the transfusion, as well as the age of the blood, must be taken into account. Previous recommendations of a 1-day shelf-life for large-volume transfusion to neonates and a 4-day shelf-life to other patients, may be overly prescriptive.

It has been calculated that 'top-up' transfusions, when given at standard flow rates, do *not* constitute a risk of hyperkalaemia, even when given to premature neonates. For example, red cells, even when stored for 14 days after irradiation, when given as a 10-mL-kg^{-1} 'top-up' transfusion, will provide less than half the daily potassium requirements of 2 mmol kg^{-1}, and the amount given ($\approx$0·05 mmol h^{-1}) will be rapidly distributed throughout the total body water (Strauss, 1990).

In contrast, potassium load may become clinically important in rapid large-volume transfusions such as exchange transfusion, and in particular intrauterine transfusion. In the latter situation, infusion of large volumes of 90% haematocrit-irradiated red cells may present the fetus with a total potassium influx of $\approx$9·3 mmol L^{-1} into a central vein, a procedure sometimes associated with otherwise unexplained bradycardias.

Similar considerations should apply to exchange transfusion, large-volume transfusions via a central line to children and adults, and where there is pre-existing hyperkalaemia. The routine removal of supernatant plasma and washing of irradiated red cells has been advocated, but is not considered necessary and such manipulation simply increases the risk of error and contamination.

Recommendation. Blood may be irradiated at any time up to 14 days after collection, and thereafter stored for a further 14 days from irradiation. Where the patient is at particular risk from hyperkalaemia, e.g. intrauterine or exchange transfusion, it is recommended that red cells be transfused within 24 h of irradiation.

(ii) Platelets

Gamma irradiation below 50 Gy has not been shown to produce significant clinical changes in platelet function.

Recommendation. Platelets can be irradiated at any stage in their 5-day storage and can thereafter be stored up to their normal shelf life of 5 days after collection.

(iii) Granulocytes

The evidence for irradiation damage to granulocyte function is conflicting, but in any case granulocyte products should be transfused as soon as possible after preparation.

Recommendation. Granulocytes for all recipients should be irradiated as soon as possible after production, and thereafter transfused with minimum delay.

(II) Potential hazards of irradiation of blood components

(i) Radiation-induced malignant change

Concern has been expressed at the potential for radiation-induced malignant change in nucleated cells capable of survival in the recipient. No such cases have been reported and it is likely that the dose of gamma irradiation delivered to blood components significantly exceeds the lethal dose for such cells at high dose rates (3–4 Gy min^{-1}), resulting in complete cell death rather than transformation.

(ii) Reactivation of latent viruses

Gamma irradiation can activate latent viruses and could theoretically result in transfusion-transmitted infection of the recipient. Again no such cases have been reported and the doses routinely delivered are likely to exceed significantly those associated with such activation.

(iii) Leakage of plasticizer

Leakage of plasticizer is a theoretical risk for the recipients of large-volume transfusions of irradiated components and for neonates in particular. No increase in the rate of plasticizer leaching was found

in one study of traditional PVC bags, but the effect of irradiation on the multiplicity of new plastics and plasticizers needs to be determined.

Recommendation. Irradiated components not used for the intended recipient can safely be returned to stock to be used for recipients who do not require irradiated components. The reduction in shelf life must be observed.

(III) Labelling and documentation requirements

1 Irradiated components must be identified by an approved overstick label. The label should be permanent and include the date of irradiation and any reduction in shelf life. Approved bar code labels should be used.

2 Labels which are sensitive to gamma rays and change from 'NOT IRRADIATED' to 'IRRADIATED' are available and are considered a useful indicator of exposure to gamma rays. The dose at which the label changes to 'IRRADIATED' must be marked on the label. As a minimum, a label should be included with every batch. However, it is not necessary to attach a label to every pack in a batch *provided* that the irradiation procedure follows a validated, documented and well-controlled system of work that is integrated to component labelling and release mechanisms and permits retrospective audit of each stage of the irradiation process. Clear physical separation of nonirradiated and irradiated units is essential. In practice, the presence of a radiation-sensitive label on every pack will be of reassurance to staff subsequently handling the product. Batch control can also be performed using thermoluminescent dose meters. The use of radiation-sensitive labels does not replace the need for regular and precise dosimetry.

3 There should be a permanent record of all units irradiated. This should include details of irradiation batch and donation numbers, component type, the site of irradiation, when irradiation was performed and by whom.

Recommendation. All irradiated units should be labelled as such using an approved bar code label. Each batch of one or more units should be monitored using a radiation-sensitive device, and should be permanently recorded, manually or by computer.

EQUIPMENT, DOSIMETRY AND MAINTENANCE

Laboratories performing irradiation of blood components must work to a clearly defined specification and are strongly recommended to work closely with a medical physicist. The collaboration starts with the initial delivery and installation procedures and continues with the required in-house validation of the defined irradiation procedure followed by regular monitoring of blood unit dosimetry and the laboratory environment.

A set of technical guidelines is provided in Appendix 1. These have been written for dedicated blood irradiation machines, an approach which is encouraged. If the irradiations are done on a radiotherapy machine, these guidelines should be shown to the radiotherapy physicist who will draw up an equivalent protocol.

CLINICAL INDICATIONS FOR GAMMA-IRRADIATED BLOOD COMPONENTS

Many patients who will require irradiated products are treated by a 'shared care' approach involving more than one hospital. To reduce the likelihood of nonirradiated products being given inadvertently, it is suggested that patients be issued with a laminated card, like a blood group card, to indicate the need for irradiated products.

PAEDIATRIC PRACTICE

The newborn may be at particular risk of TA-GVHD either because of possible physiological immune incompetence or because of an underlying congenital T-cell immunodeficiency. There is indirect clinical evidence that neonates may not be able to reject transfused allogeneic lymphocytes and that transfusion, at least in large volumes, may result in either immunological tolerance or further immune suppression. Normal circulating donor lymphocytes have been found 6–8 weeks after routine exchange transfusion (ET) and maternal cells have been detected after intrauterine transfusion (IUT) for haemolytic disease of the newborn (HDN) 2–4 years after transfusion in otherwise healthy newborns. The majority of cases of TA-GVHD reported in apparently immune competent infants have occurred in the setting of IUT followed by ET, suggesting transfusion-induced tolerance or immune suppression. Also, neonates who have had an ET with fresh blood will not reject a skin homograft from the same donor.

Categories of neonates at risk of TA-GVHD

Two surveys of current practice in the US have revealed wide differences in neonatal practice (Sanders & Graeber, 1990; Anderson *et al.*, 1991).

Intrauterine and exchange transfusions (IUT/ET)

(a) IUT alone. Despite the lack of reported cases of TA-GVHD following IUT alone from unrelated donors, it is difficult not to recommend irradiation in this setting, combining as it does a large-volume transfusion of fresh blood with a recipient of considerable immaturity. The absence of reported cases may represent a combination of the rarity of the disorder, under diagnosis, and/or the already established practice of irradiation in this situation. However, irradiation alone will not prevent the possible immunomodulatory effects of contaminating leucocytes, prevention of which would require leucodepletion also (Royal College of Physicians of Edinburgh, 1993).

(b) IUT and subsequent exchange transfusion. The majority of TA-GVHD in apparently immunocompetent infants have been reported in the setting of ET following IUT for HDN in pre-term and term infants. Although reports are scarce, the published evidence supports irradiation of blood for IUT and any subsequent ET such babies may receive (Parkman *et al.*, 1994).

(c) Exchange transfusion alone. Only two cases of TA-GVHD have been reported following ET alone, one in a pre-term infant and one in a term infant, but in the latter an immune defect could not be excluded. Therefore, the argument for irradiation of blood for ET in either pre-term or term infants is not compelling at the present time. However, like IUT, ET represents a large-volume transfusion of relatively fresh blood. Thus, while irradiation may represent the counsel of perfection, particularly for premature neonates, the risks of TA-GVHD must be balanced against those of any delay in transfusion while irradiation is performed.

Recommendation. All blood for IUT should be irradiated. It is essential to irradiate blood for ET if there has been a previous IUT, or if the donation comes from a first- or second-degree relative. For other ET cases, irradiation is recommended provided this does not unduly delay transfusion. For IUT and ET, blood should be transfused within 24 h of irradiation, and in any case at 5 days or less from collection.

Top-up transfusion

(a) Pre-term infants. The pre-term infant is commonly multiply transfused yet there are only two case reports of TA-GVHD, one following three transfusions from an unrelated donor and one following a single transfusion. Whilst the risk appears small, the scenario of repeated donations from a single donor is increasing, since blood donations are now often divided into many aliquots dedicated to a single neonate in an attempt to reduce donor exposure.

(b) Term infants. With increasing gestational age the ability of transfusions to induce tolerance decreases and the term or near-term infant seems capable of responding appropriately to transfused cells. Even in the setting of multiple transfusions associated with extracorporeal membrane oxygenation (ECMO), there has been only one case of TA-GVHD and therefore these infants appear not to be at risk.

Recommendation. There is no necessity to irradiate blood for routine 'top-up' transfusions of premature or term infants unless either there has been a previous IUT or the blood has come from a first- or second-degree relative, in which case the blood should be irradiated.

Platelet transfusions

There have been no reported cases of TA-GVHD following platelet transfusion alone, but since platelets are also contaminated with small numbers of lymphocytes, the recommendations for red cell transfusion should also apply to platelets.

Recommendation. Irradiation should be performed on platelets transfused in utero to treat alloimmune thrombocytopenia, and on platelet transfusions given after birth to infants who have received either red cells or platelets in utero. However, there is no need to irradiate other platelet transfusions for pre-term or term infants, unless they have come from first- or second-degree relatives.

Granulocyte transfusions

As with platelets, there have been no cases of TA-GVHD clearly attributed to granulocytes. However, since these products are heavily lymphocyte contaminated, transfused extremely fresh and prescribed for infants who are already severely ill, it would be reasonable to irradiate all granulocyte transfusions.

Recommendation. All granulocyte transfusions should be irradiated for babies of any age, and transfused as soon as possible after irradiation.

Cardiac surgery

There have been no published reports to date of TA-GVHD occurring in immunocompetent neonates undergoing cardiopulmonary bypass surgery or any other surgical procedure. However, there is an unreported case of TA-GVHD following cardiac surgery

Table 1. Congenital immunodeficiency states with predominant defect of cell-mediated immunity

In which TA-GVHD has been reported:
SCID, not otherwise classified
SCID, with dwarfism
3rd and 4th arch/pouch syndrome (Di George's)
Wiskott–Aldrich syndrome
Purine nucleoside phosphorylase deficiency
Cell-mediated immunodeficiency, not otherwise classified
Reticular dysgenesis
In which TA-GVHD has not been reported:
Adenosine deaminase deficiency
MHC Class I deficiency
MHC Class II deficiency
Leucocyte adhesion deficiency
Immunodeficiency with eosinophilia (Omenn's syndrome)
Ataxia telangiectasia
Chronic mucocutaneous candidiasis

Abbreviations: SCID, severe combined immunodeficiency; TA-GHVD, transfusion-associated graft vs. host disease.

in an infant with Di George syndrome in association with a second congenital malformation of the head or neck and a heart defect (personal communication from Dr Sheela Amin, Harefield Hospital, Middlesex). There needs to be a high index of suspicion concerning coexisting cardiac defects and immunodeficiency. Dysmorphic features, anomalies of ear, lip or palate, hypocalcaemia, and absolute lymphopenia ($< 2 \times 10^9\ L^{-1}$) are all suggestive of an immunodeficiency syndrome. If in doubt, blood should be irradiated until a definitive diagnosis is made. If Di George syndrome is confirmed, then irradiated products are essential.

Recommendation. There is no need to irradiate red cells or platelets for infants undergoing cardiac surgery unless clinical or laboratory features suggest coexisting immunodeficiency.

Congenital immunodeficiencies in infants and children

To date, TA-GVHD has been reported in children with a number of congenital immunodeficiencies (Table 1). These immunodeficiency states have in common a defect of T-cell function with many also manifesting B-cell defects. The occurrence of TA-GVHD in this patient group following transfusions of fresh plasma containing very few lymphocytes suggests that the degree of immunodeficiency is critical in determining susceptibility.

A number of other congenital disorders whose features include a clinically significant degree of T-cell dysfunction are recognized (Table 1) but have not been reported in association with TA-GVHD. With the exception of chronic mucocutaneous candidiasis (CMC), the immunological features of these diseases are very similar and these patients may therefore also be at risk of TA-GVHD. In CMC the lymphocyte response to allogeneic cells appears intact and it is unlikely that these patients would develop TA-GVHD. However, as there is no clear laboratory parameter which will distinguish those who are certainly at risk of GVHD from those who are not, products should be irradiated from the time an immune disorder is suspected. In the newborn infant the presenting features of immunodeficiency syndromes may be unrelated to the immune defect (e.g. cardiac disease, hypocalcaemia, thrombocytopenia, eczema) and a high index of suspicion is required, particularly in infants less than 6 months old with recurrent chest infections. Confusion may arise as similar clinical features may be present in such patients due to acute or chronic infection, and these may be difficult to distinguish from GVHD, even at a histological level. HLA typing of lymphocytes is advised whenever TA-GVHD is suspected.

There have been no reports of TA-GVHD occurring in patients with isolated defects of humoral immunity.

Recommendation. It is recommended that all the immunological deficiency states outlined in Table 1, with the exception of CMC, should be considered as indications for irradiation of cellular blood products. Once a diagnosis of immunodeficiency has been suspected, irradiated products should be given while further diagnostic tests are being undertaken.

Acquired immunodeficiency states in childhood

Transient defects of T-cell function can occur following a number of common childhood viral infections and as a complication of tuberculosis and leprosy. This is also a feature of autoimmune disorders, malnutrition and burns. Nevertheless, TA-GVHD has not been recognized in these cases and irradiation of blood products is not recommended, even if immunological testing has demonstrated a defect. Despite the profound T-cell defect which develops in infection with HIV, no cases of TA-GVHD have been described in children or adults, perhaps because donor lymphocytes also become infected.

Recommendation. There is no indication for the irradiation of cellular blood components for infants or

children who are HIV antibody positive, or who have AIDS. However, this should be kept under review.

ACUTE LEUKAEMIA AND BONE MARROW TRANSPLANTATION IN CHILDREN AND ADULTS

Acute leukaemia

There are very few published reports of TA- GVHD in patients receiving intensive chemo(radio)therapy without bone marrow transplantation. A review of the world literature revealed 14 adult cases (nine AML, five ALL) prior to publication of US guidelines in 1987. Since 1988, there has been only one adult case in AML (Sazama & Holland, 1993). In children, there have been only eight ALL and two AML cases reported in the world literature. In surveys of adult and paediatric practice in the UK, no centres routinely irradiate products for acute leukaemia without transplantation, and no cases of TA-GVHD were reported.

Recommendation. It is not necessary to irradiate red cells or platelets for adults or children with acute leukaemia, except for HLA-matched platelets or donations from first- or second-degree relatives.

Allogeneic bone marrow transplantation

It has been common practice to gamma-irradiate blood products given to bone marrow transplant (BMT) recipients during the last 20 years. There is no consensus as to the duration of such treatment, current practice in the UK ranging from 2 months to indefinitely, depending on ease of access to irradiation facilities. There are no unequivocal scientific data to indicate when irradiation of blood products can safely be withdrawn after allogeneic BMT. It seems prudent to continue irradiation at least until the immunosuppressive therapy such as cyclosporin-A is withdrawn (i.e. at least 6 months in most cases). Since chronic GVHD can also be significantly immunosuppressive, irradiated products should be considered for patients with active chronic GVHD.

Recommendation. All recipients of allogeneic BMT should receive gamma-irradiated blood products from the time of initiation of conditioning chemo/radiotherapy. This should be continued while the patient remains on GVHD prophylaxis, i.e. usually 6 months, or until lymphocytes are $> 1 \times 10^9\ L^{-1}$. *It may be necessary to irradiate blood products for SCID patients for considerably longer, up to 2 years, and for patients with chronic GVHD, if there is evidence of immunosuppression.*

Donors of allogeneic bone marrow

There have been two reports of TA-GVHD associated with graft rejection, apparently mediated by third-party lymphocytes, putatively from transfused blood.

Recommendation. To prevent this, blood transfused to bone marrow donors prior to or during the harvest should be irradiated.

Autologous bone marrow/peripheral blood stem cell recipients (ABMT/PBSC)

Virtually all UK centres currently irradiate products for autologous BMT (ABMT) recipients and 30% of centres have a policy of use of irradiated products given to potential ABMT recipients before and during 'harvesting' of marrow or peripheral blood stem cells. This is to prevent the harvesting of allogeneic T lymphocytes which might cause TA-GVHD after re-infusion. As with allogeneic transplants, current knowledge does not allow precise guidance on when irradiation can be safely discontinued. Many patients need prolonged periods of red cell and, particularly, platelet support after ABMT. We would recommend, as a minimum, continuing to use irradiated blood products until there is unequivocal evidence of haemopoietic engraftment and lymphoid reconstitution. In most patients this would mean at least 3 months of treatment. If, however, the patient has received total body irradiation (TBI), this may take up to 6 months.

Recommendation. Patients undergoing bone marrow or peripheral blood stem cell 'harvesting' for future autologous re-infusion should only receive gamma-irradiated cellular blood products during and for 7 days before the bone marrow/stem cell harvest to prevent the collection of viable allogeneic T lymphocytes which could withstand cryopreservation. All patients undergoing ABMT or PBSCT should then receive gamma-irradiated cellular blood products from the initiation of conditioning chemo/radiotherapy until 3 months post-transplant (6 months if TBI used).

OTHER PATIENT GROUPS

Lymphoma

TA-GVHD has been reported in all forms of lymphoproliferative disease (Spitzer *et al.*, 1990; Anderson *et al.*, 1991). Twenty cases associated with Hodgkin's disease (HD) have been reported, almost certainly an underestimate. TA-GVHD has occurred during treatment with chemotherapy alone or with radiotherapy,

and the risk of TA-GVHD appears not to be influenced by the stage of the disease. TA-GVHD has also been described in children with HD. There are fewer reports of TA-GVHD in non-Hodgkin's lymphoma (NHL) despite this being a more common disease than HD. The majority have been high grade and there has been at least one case of TA-GVHD in T-cell NHL. NHL represents a lower risk of TA-GVHD than HD and it is probably not necessary to use irradiated blood products for NHL patients. With careful surveillance it may be possible to separate a subgroup with higher risk, e.g. T-cell NHL. TA-GVHD has also been reported in children with NHL, and the incidence of TA-GVHD in this situation should be carefully monitored.

The purine antagonists fludarabine, 2-chlorodeoxyadenosine (CdA, cladribine) and 2′-deoxycoformycin (DCF) induce profound lymphopenia, with low CD4 counts which persist for several years (Cheson, 1995). Case reports have appeared of TA-GVHD following treatment of low-grade B-cell malignancies with fludarabine and cladribine, and several other unpublished cases have occurred (personal communications from Drs B. Woodcock, J. Z. Wimperis and M. E. Wood). Considering that these patients do not require intensive transfusion support, this association is considered significant.

Recommendation. We recommend that all adults and children with Hodgkin's disease at any stage have irradiated red cells and platelets, but this is not necessary for adults or children with non-Hodgkin's lymphoma. However, this should be kept under review. Patients treated with purine analogue drugs (fludarabine, cladribine and deoxycoformycin) should have irradiated cellular components.

Solid tumours

A dozen cases of TA-GVHD have followed treatment of a variety of solid tumours, ranging from rhabdomyosarcoma, Ewing's sarcoma and neuroblastoma in the young to renal carcinoma, cervical carcinoma and glioblastoma in patients in their 60s. In relation to the number of patients with cancer, it is a rare occurrence. However, the effect of dose escalation of chemotherapy regimes in children and young adults is unknown.

Organ transplantation

GVHD following solid organ transplantation has been reported after pancreas and spleen, heart, liver and renal transplantation. Considering the immunosuppression used post-operatively, the use of cyclosporin A which can predispose to GVHD, and the previous use of family-directed transfusion in renal transplants, it must be a rare occurrence. However, it is usually due to the transfer of viable donor lymphocytes within the transplanted organ. No prophylactic treatment of blood products is therefore necessary, but early recognition of GVHD may lead to prompt appropriate treatment. The role of blood components in this context has not been established.

Acquired immunodeficiency and aplastic anaemia

There are no reports of HIV-infected patients developing TA-GVHD despite the immunodeficiency. We are unaware of reports in patients with aplastic anaemia or those treated with anti-lymphocyte globulin or CAMPATH antibodies.

Recommendation. It is not necessary to irradiate blood components for patients with solid tumours, organ transplants, HIV or aplastic anaemia. However, the effects of new regimes of chemo- and immunotherapy must be monitored.

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APPENDIX 1

Technical aspects of irradiation of blood components

Choice of blood irradiators. The equipment will contain a long half-life, gamma-emitting source, probably Cs-137. The activity must be specified by the manufacturer on an appropriate certificate to ± 20%. The source must be double encapsulated. Its size and shape should be specified together with the dimensions of the outer housing and details of the thicknesses and nature of all housing materials. This information is necessary to demonstrate adequate containment of the source and may be required for accurate dosimetry.

Adequate shielding must be provided to ensure that dose rates are as low as reasonably achievable at all accessible points in all service modes of operation. Your Radiation Protection Adviser will advise on whether this has been achieved.

Because so much lead is used for shielding, the equipment could be top-heavy. Mechanical stability is essential, and possibly strengthening of the floor. The control panel must be clearly laid out and the function of each control explained fully in the manual. Desirable features include: a safety interlock to ensure the hand cannot get trapped whilst loading, a means of retrieving samples manually and a means of detecting failure of the turntable mechanism.

Commissioning and dosimetry. The manufacturer, or their agent, should commission the irradiator and provide a calibration certificate, traceable to National Standards, for the dose rate at a specified point in the canister. To achieve the minimum recommended dose of 25 Gy in a reasonable time requires a central axis dose rate of at least 3 Gy min^{-1} (Leitman, 1993). The timing mechanism for the irradiator should also be checked.

Commissioning would include the provision of generic isodose charts for that type of equipment. However, a thorough survey of the dose distribution throughout the irradiated volume must then be made as there are literature reports of marked variations with commercial equipment (Masterson & Febo, 1992). The isodose distribution should be determined with the canister full of blood equivalent material. Appropriate checks can be made on doses and dose distribution using thermoluminescence dosemeters or commercial dose mapping systems.

If the minimum dose of 25 Gy results in a maximum in excess of 50 Gy, seek advice on improving the dose uniformity. Spacers may be useful to avoid underdosing the bottom of the pack.

Following calibration/recalibration, a table should be produced which gives irradiation times for specified doses for a set period (e.g. 1 year for Cs-137).

Operation. Both the dose rate and the dose distribution should be checked upon installation, annually, and after any source change or mechanical alteration, particularly to the rotating turntable. Results falling outside these guidelines should be discussed with the manufacturer and usage of the machine should cease pending the outcome of an investigation. In routine use bags of blood should be packed closely together with any remaining air space filled with dummy bags of water. The small residual air spaces will cause only a tiny additional dose (1–2% maximum). Do not allow any bags to protrude above the upper rim of the canister.

Quality control of procedures. All operators must have been adequately trained in the use of the equipment. The names of authorized operators should appear in the local rules (see later) or in a suitable log book. Standard operating procedures (SOPs) for all laboratory aspects of irradiation must be followed by all staff performing irradiation. There should be a defined person who documents the periodic review of all data relating to the use of the irradiator.

Maintenance. Wipe tests must be carried out at regular intervals to check for leakage of radioactive contamination (every 26 months is statutory in the UK; every 6 months is recommended). The wipe test must be done according to an SOP in the manner specified by the manufacturer and the swabs counted in a low-background area with an appropriate scintillation detector, as recommended by the local Radiation Protection Adviser (RPA).

The maximum permissible activity removed from surfaces likely to be contaminated is 0·18 kBq (0·005 μCi). Action to be taken in the event of a raised count rate must be specified, for example as follows.

1 If the count rate is above the permissible level, all movements in the vicinity of the irradiator must cease to prevent possible dispersal of radioactivity and specialist radiation protection advice should be called at once.
2 If the count rate is below the permissible level but consistently above background, the RPA and manufacturer should be informed.

Check weekly that dose rates are below upper limits previously agreed with your RPA on all external surfaces both when the irradiator is in use and when it is not in use.

Make suitable mechanical and electrical checks as recommended by the manufacturer and in accordance with the Electricity at Work Regulations (1989). Check operational procedures every 6 months. A list of possible causes of malfunction in the operator's manual is very helpful.

Legislation and official guidance. In addition to general legislation relating to health and safety, e.g. 'Radiation Safety for Operators of Gamma Irradiation Plants' and 'Approved Code of Practice', the following specific legislation will apply.

1 Radioactive Substances Act (1993) – the radioactive source must be registered with HM Inspectorate of Pollution.
2 Ionizing Radiations Regulations (1985) – these are formulated under the Health and Safety at Work Act. A Radiation Protection Supervisor (RPS) must be appointed in writing and will be responsible to the employer for ensuring that all work is carried on in accordance with the regulations. There must be written local rules and documentation.
3 Transportation and disposal of the radiation source are covered by the Radioactive Substances Act (1993), the Radioactive Substances (Carriage by Road Great Britain Amendment Regulations, 1985), the Road Traffic Training of Drivers of Vehicles Carrying Dangerous Goods Regulations (1992). New Radioactive Substances (Carriage by Road) regulations are imminent.
4 Electricity at Work Regulations (1989)

This list should be updated annually. If in doubt about any aspect of equipment, dosimetry, maintenance or protection, consult your RPA.

Local rules and documentation. Local rules and documentation for work with a blood cell irradiator should define:

1 *Responsibilities and personnel.* To include the employer, head of department, Radiation Protection Supervisor (RPS), Radiation Protection Adviser (RPA), staff authorized to operate the irradiator, and 'outside workers', e.g. service engineers.
2 *Introduction and code of practice.* All persons who intend to use the irradiator must, before starting, read these departmental rules and sign that they have understood and agree to abide by the regulations. The Ionizing Radiations Regulations 1985 and 'Approved Code of Practice' are available for consultation from the RPS and/or RPA.

Any project involving the use of radioactive materials must be discussed with the RPS and Head of Department.

The location of the irradiator should be a supervised area.
3 *Monitoring.* The location and details of a radiation monitoring device must be documented near the irradiator. The monitor should be calibrated annually and the calibration of the meter reading available from the RPS.

The gamma cell irradiator must be monitored weekly as per the instructions in the log book, drawn up in consultation with the RPA. A record of radiation monitor readings will be kept in the log book. If any measurement exceeds a previously agreed level, the RPA must be contacted immediately.

Staff who work frequently with the irradiator will wear whole body monitors and extremity monitors on the fingers if either is advised by the RPA.
4 *General operations procedures.* This should include manufacturer, contact supplier, source and activity, authorized users and access. A list of named users is located in the irradiator logbook which must also include a record of the daily usage of the irradiator. Radiation exposure will be reduced if the log book can be filled in away from the irradiator.

The irradiator must be regularly serviced. This includes leak testing every 6 months.
5 *Contingency plan.* (i) In the event of sticking of the turntable (or source, depending on the system), the operator must contact the RPS or RPA immediately and prevent access to the area until assistance arrives. A predetermined plan should then be followed. (ii) In the event of fire a member of staff must appraise the Fire Officers of the presence of the source and assist in checking that the shielding remains intact.