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ORIGINAL ARTICLE

STERILIZATION OF HIV WITH IRRADIATION: RELEVANCE TO INFECTED BONE ALLOGRAFTS

D. G. CAMPBELL AND P. LI

Royal Adelaide Hospital and Institute of Medical and Veterinary Science, Adelaide, Australia

Background: Bone allograft banks commonly sterilize frozen bone by irradiation. The dose–response relationship for HIV is calculated and the dose required to inactivate the bioburden of virus that may be present in allograft bone is determined.
Methods: A virus titre experiment is performed using irradiated frozen HIV. The virus is maintained on dry ice (approximately –70°C) and is exposed to a cobalt 60 source with 0–40 kGy irradiation at 5 kGy intervals. Lymphocyte cell cultures are exposed to serial dilutions of the irradiated virus. The virus titre is quantified by cytological changes of HIV infection and p24 immunofluorescence.
Results: There is a linear relationship between the virus titre and the radiation dose delivered. The inactivation rate of irradiated virus was 0.1134 $\log_{10}$ tissue culture infective doses $_{50}$/mL per kGy (95% confidence intervals, 0.1248–0.1020). The irradiation dose required to inactivate the HIV bioburden in allograft bone is 35 kGy. The irradiation dose required to achieve a sterility assurance level of 10^{-6} is 89 kGy. This dose exceeds current recommendations for sterilizing medical products and the current practice of many bone banks.
Conclusions: It is concluded that gamma irradiation should be disregarded as a significant virus inactivation method for bone allografts.

Key words: allograft, bone, HIV, irradiation, sterilization.

INTRODUCTION

The risk of transmission of HIV from bone allografts has not been completely eliminated by donor screening and methods of virus inactivation are frequently employed. Few suitable methods are available for the inactivation of infectious agents in bone. Chemicals such as ethanol or povidone–iodine are effective *in vitro*,[1–4] but it has been shown that these methods are not as effective when in contact with organic materials such as serum or bone.[5,6] Consequently emphasis has been placed on physical methods such as irradiation.

Bone allograft banks commonly sterilize frozen bone by irradiation to sterilize bacterial contaminants and 15–25 kGy is frequently used. Industry guidelines that do not directly relate to bone allografts have suggested allograft banks use 25 kGy[7] or calculate the sterility assurance level of irradiated bone.[8,9]

Knaepler *et al.* have reported virus inactivation of *in vitro* HIV inoculated bone with 15 kGy irradiation.[5] This observation has not been confirmed by other studies which have suggested that 25 kGy may not be sufficient for sterilization of frozen bone.[10,11]

Withrow *et al.* have examined virus inactivation using a feline leukaemia virus-infected bone model.[12] Irradiation with 29 kGy delayed but did not prevent virus infection of the recipient. Fideler *et al.* have examined gamma irradiation sterilized patella ligament-bone grafts from HIV infected donors with the polymerase chain reaction method.[13] They found doses of 20–25 kGy did not destroy the genes of HIV but the viral nucleic acid was not detectable in grafts treated with 30–40 kGy of gamma irradiation.

The inactivation of viruses by irradiation is dose dependent and there is always a finite probability that an organism may survive. The probability of a single non-sterile item surviving after irradiation is the sterility assurance level.[14] The preferred sterility assurance level is arbitrarily determined but usually a minimum sterility assurance level of 10^{-6} is recommended for sterilization of medical products.[9,14]

The amount of irradiation required to inactivate the virus that may reside in HIV-infected bones remains uncertain and the dose required to achieve an adequate sterility assurance level has not been addressed. We quantified the inactivation of HIV by gamma irradiation. The maximum virus bioburden to be inactivated in a bone allograft was estimated. The aim of the present study is to assess the efficacy of sterilizing HIV-infected bone allografts with irradiation.

METHOD

A virus titre experiment was performed using irradiated frozen HIV virus.

Virus preparation

H3B cells (Institute of Medical and Veterinary Science, Adelaide, SA, Australia) were used as the virus donor cells for virus supernatant. These cells are a clone derived from HIV-infected lymphocytes and were greater than 95% HIV P24 antigen-positive as judged by immunofluorescence and contained an average of two copies of integrated HIV pro-viral DNA per cell.[15]

H3B cells were propagated in RPMI-1640 growth medium (Cytosystems, GIBCO Laboratories, Grand Island, New York) prepared using ultra-pure water supplemented with sodium carbonate 0.85 g/L, hepes 20 mmol/L, L-glutamine 0.29 g/L, penicillin 25 μg/mL, streptomycin 25 μg/mL and phenyl red and further supplemented with 10% heat-inactivated foetal calf serum immediately prior to use. To prepare virus supernatant H3B cells were maintained at a density of 5×10^7 cells/mL with an

Correspondence: Mr D. G. Campbell, The Wakefield Orthopaedic Clinic, 270 Wakefield Street, Adelaide, SA 5000, Australia.

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hourly medium change. Cells were centrifuged at 200 × *g* for 30 min, the supernatant was harvested and the cells resuspended in medium and incubated at 37°C supplemented with 5% carbon dioxide. The supernatant was pooled, chilled on ice and centrifuged at 2850 × *g* for 10 min at 4°C, divided into aliquots of 5 mL and stored at −70°C. One vial was thawed for virus titre (greater than 10^5 tissue culture infective doses ($TCID_{50}$) per mL) and reverse transcriptase activity (greater than 40 000 counts/mL).

Irradiation

Vials of frozen virus maintained on dry ice (approximately −70°C) were exposed to a cobalt 60 source at a commercial facility (Australia Nuclear Science and Technology Organization, Lucas Heights Research Laboratories, Lucas Heights, NSW, Australia). The irradiation dose delivered to samples was calculated by suspending ceric/cerous dosimeters in a receptacle especially prepared for the virus. Dosimeters were irradiated and measured at 20°C, which is the preferred temperature range for dosimeters. Three dosimeters were examined for 2 h 15 min each and the average dose rate measured was 6.39 kGy/h.

Frozen samples were maintained in a polystyrene lined metal can loaded into a gamma pond canister and processed in the cobalt 60 reactor (LC2) for intervals calculated to deliver 0–40 kGy irradiation at 5 kGy intervals.

Virus titres

HUT-78 cells (National Institute of Health AIDS Research and Reference Reagent program) were employed as indicator cells of HIV infection. When infected with HIV they coalesce to form syncytia and immunofluoresce for p24 antigen. HUT-78 cells maintained in subculture in RPMI-1640 growth medium (Cytosystems, GIBCO) supplemented with 10% heat-inactivated foetal calf serum were subcultured 1 day before use and resuspended at a density of 1.5×10^5 cells/mL in 150 cm^2 flasks (Costar, Cambridge, MA, USA).

HUT-78 cells were incubated with 0.001% Diethyaminoethyl-Dextran (DEAE-Dextran) at 37°C for 30 min to enhance viral absorption.[16,17] Cells were pelleted (200 × *g* for 3 min) and resuspended in RPMI-1640 medium and aliquoted 1 mL (1.5×10^5 cells) in eppendorf tubes.

Irradiated and control virus samples were thawed to room temperature and serially diluted 10-fold to 10^{-7} in serum-free RPMI-1640 medium.

HUT-78 cell pellets (1.5×10^5 cells) were resuspended in 600 µL of virus dilution or 600 µL RPMI-1640 for controls and incubated for 2 h. Cells were then washed three times in serum-free medium resuspended in 1200 µL of RPMI-1640 supplemented with 10% foetal calf serum, and aliquots of 200 µL (2.5×10^4 cells) were placed into six wells of a 48-well plate. The plates were sealed and incubated at 37°C.

A further 200 µL of foetal calf serum-supplemented medium was added to each well the following day. Half volume medium changes were made as required.

Observations for cytopathic effect were made on days five and seven, when smears for immunofluorescence were made for confirmation of microscopic findings. Microscopy observations were made for cytopathic changes and the formation of multinucleated giant cells, or syncytia, as described by Sodroski *et al.*[18] and Lifson *et al.*[19,20]

p24 antigen immunofluorescence

Cells were washed twice with phosphate-buffered saline (PBS) and resuspended at 10^6 cells/mL in PBS, spotted onto immunofluorescence slides (PH17, Wellcome, Sydney, NSW, Australia) and allowed to air dry. Slides were then fixed in cold 1% formalin for 30 min, followed by 70% ethanol for 1 min.

Slides were washed for 5 min in PBS followed by a 20 min wash in 0.05% non-idet P40 (Boehringer Mannheim, Mannheim, Germany). The slides were then washed a further two times followed by incubation with monoclonal antibody to HIV p24 (Chemicon, Tenecula, CA, USA, cat. no. MAB 880-A), diluted 1/100 in PBS, for 45 min in a humid chamber at room temperature. After washing twice with PBS, fluorescein isothiocyanate conjugate (FITC)-conjugated sheep anti-mouse immmunoglobulin (Silenus Laboratories, Miami, FL, USA), diluted 1/20 in PBS, was added and incubated for 30 min in a humid chamber at room temperature. The cells were washed twice in PBS before coverslip mounting with glycerol.

p24 antigen-positive cells were evaluated using a fluorescence microscope. Non-infected HUT-78 cells were the negative controls and H3B cells were the positive controls.

Calculation of virus inactivation

Virus titres were calculated by the Spearman-Karber method[21] for determination of $TCID_{50}$.

Linear regression analysis was used for the analysis of irradiated virus titres. Microsoft Excel version 4.0 (Microsoft Corporation, Redmond, Washington DC, USA) was used to calculate the slope of the regression line, coefficient of determination of the data (r^2), and P value. The regression line was obtained with the equation:

$$y = a_0 + a_1 x + \text{error}$$

and was illustrated with CA-cricket graph computer software (1990, Computer Associates International Inc, San Diego, CA, USA).

RESULTS

Productive infection was observed in all HUT-78 cell cultures inoculated with positive controls or irradiated samples but varied in the virus titre. There was universal agreement with p24 antigen immunofluorescence and microscopic observations of HUT-78 culture cells harvested for antigen immunofluoresence.

The virus titration results calculated by the $TCID_{50}$ method were subjected to linear regression analysis and values were derived from the regression curves. Twenty-three samples were available for analysis from two independent irradiation episodes, a number of samples were lost due to events independent of the irradiation and titre calculation (bacterial contamination of the HUT-78 cell culture plates).

There was a linear relationship between the logarithm of the biological activity of the virus and the radiation dose delivered, the coefficient of determination was 0.953 and $P < 0.001$. From the radiosensitivity curve of HIV-infected HUT-78 cells (Fig. 1) the inactivation rate was −0.1134 $\log_{10}$ $TCID_{50}$/mL per kGy (95% confidence intervals, −0.1248 to −0.1020). The radiation dose that provides a 90% reduction of biological activity (D_{10} value) was 8.82 kGy.

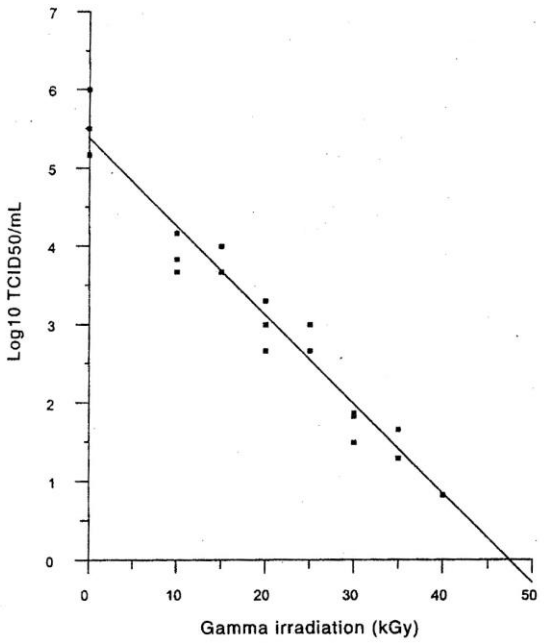

Fig. 1. Virus titre of gamma irradiated HIV determined by HUT-78 cell culture inoculation.

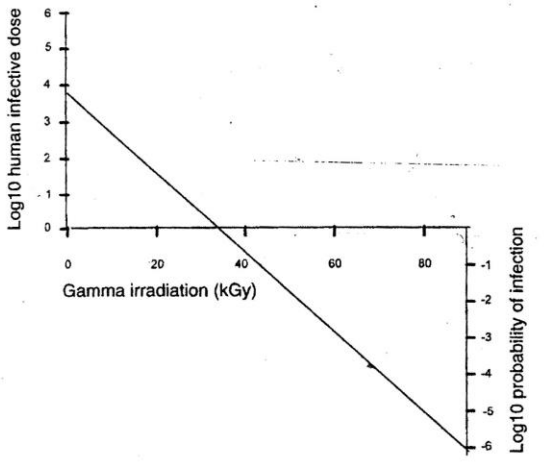

Fig. 2. Dose–response curve of an HIV-infected femoral head treated with gamma irradiation. The bioburden is an estimate of the human-infective dose that may occur in an acutely infected femoral head allograft donor during the window period (prior to HIV-antibody production). We have used a worst case scenario assuming the HIV bioburden in bone to be equivalent to the maximum known concentration of HIV in serum.

DISCUSSION

Unlike chemical agents gamma irradiation is not dependent on physical contact. It is, therefore, possible to determine the dose-related effectiveness of irradiation as a sterilizing agent for HIV. The constant nature of irradiation inactivation of viruses makes it possible to calculate the dose–response relationship for HIV and mathematically determine the dose required to inactivate a known bioburden of virus.[22] If the bioburden of HIV in allograft bone is known it should be possible to calculate the dose required to achieve a certain probability of the virus surviving the treatment. Our calculation of the dose of irradiation required to achieve a sterility assurance level of less than one per million HIV-infected bone allografts is shown in Fig. 2.

Irradiation of HIV in serum maintained on dry ice at approximately –70°C resulted in an inactivation rate of 0.113 $TCID_{50}$ dose/mL per kGy over a range of irradiation doses that are clinically relevant to bone allograft specimens. The results correspond to those of Hiemstra *et al.* who report the largest previous study of 11 HIV samples.[23] The decimal reduction value (D_{10} value) of the current study was 8.82 kGy and is comparable to the D_{10} value of 8 kGy calculated by Hiemstra *et al.* Studies with smaller sample sizes have suggested the D_{10} value to be 6.10 kGy[24] and 'greater than 4 kGy'.[25] The D_{10} values for HIV approximates other viruses and retro-viruses[26–28] but HIV is one of the more radio-resistant viruses.

The $TCID_{50}$ assay is a direct measurement of viral infectivity and has several advantages over alternative methods. Reverse transcriptase assay or antigen-capture assays were used in early reports of HIV radiosensitivity and erroneously reported the virus to be unusually radiosensitive.[29,30] The $TCID_{50}$ assay is several orders of magnitude more sensitive than either reverse transcriptase assay or antigen-capture assays and is measured over a greater range.[31]

The virucidal effectiveness of gamma irradiation is directly related to damage to the virus genome[32] and radiation inactivation has been used to determine the size of the virus genome. Hiemstra *et al.* estimated the size of HIV to be 3×10^3 kDa[23] which closely approximates the known size of the genome[33,34] suggesting the inactivation rate and D_{10}-value to be correct. Because the efficacy of virus inactivation by irradiation is related to the size of the genome, and not physical or biological properties, the D_{10}-value obtained is also applicable to other HIV strains and quasispecies with a similarly sized genome.[32]

The irradiation dose required to sterilize an HIV-infected allograft is determined by the inactivation rate and the amount of virus in the bone. The amount of virus in the bone is termed the bioburden. Sterilization of bone grafts infected with HIV is most important during the early stages of infection before antibodies can be detected and the donor eliminated by routine screening tests (window period). During the window period the bioburden in serum is a maximum of 10^4 $TCID_{50}$/mL.[35] Human bone-derived cells are not susceptible to productive HIV infection[36] and it is unlikely that the bioburden in bone will exceed serum. The most common bone allograft used is a femoral head (average 50 mm diameter) and the maximum bioburden would be 5.8 $\log_{10}$ TCID if an equivalent blood volume is assumed. The actual HIV bioburden in allograft bone will vary with the temporal relation of HIV infection and the amount of red marrow/blood present in the bone. We have assumed a worst case scenario using calculations to include or exceed the maximal possible bioburden.

An additional factor is the minimum number of surviving organisms required to cause an infection in the allograft recipient. Even if one or a few organisms are eluted from the bone the potential of causing an infection is minimal. Only very few of the most virulent micro-organisms are capable of initiating infection

when a single cell gains access to sub-epithelial tissues. Most other organisms require 1000 or more organisms to initiate an infection and most bacteria require a critical initial number of cells even to initiate growth *in vitro*.[9] The *in vivo* infectious dose of HIV is unknown but the simian immunodeficiency virus (SIV) is an approximate animal model for HIV and the *in vivo* infectious dose of SIV has been evaluated. Infection occurs in rhesus monkeys challenged with SIV at a dose of 100 $TCID_{50}$.[37] If the SIV model is assumed, the *in vivo* infective dose of HIV is 1/100 of the TCID. Therefore, the 5.8 $\log_{10}$ TCID of an HIV-infected femoral head is equivalent to an allograft recipient infective dose of 3.8 $\log_{10}$. The human-infection dose response curve for a gamma irradiated femoral head infected with HIV was calculated (Fig. 2).

From our calculations the irradiation dose required to inactivate the HIV bioburden is 35 kGy. The irradiation dose required to achieve a sterility assurance level of 10^{-6} is 89 kGy and this dosage exceeds current recommendations for routine radiation sterilization of bone allografts. Achieving a sterility assurance level of 10^{-6} with 89 kGy would result in graft destruction. It is likely that a similar or greater dose is required to inactivate other serious virus infections including Hepatitis B and Creutzfeldt-Jakob disease.[38,39] The bioburden and D_{10}-value for Hepatitis C is unknown.

The HIV sterilization dose of 89 kGy exceeds current recommendations for sterilizing medical products and the current practice of bone banks which use gamma irradiation for secondary sterilization. It is concluded that gamma irradiation is not a reliable method of HIV inactivation of bone allografts. Rigorous donor screening remains essential to minimize the risk of virus transmission to allograft recipients. Allograft irradiation has mostly been used as a method of secondary sterilization to prevent bacterial infection and the present study does not detract from this long-standing and effective practice.

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REFERENCES

1. Martin LS, McDougal JS, Loskoski SL. Disinfection and inactivation of the human T lymphotropic virus type III/Lymphadenopathy-associated virus. *J. Infect. Dis.* 1985; **152**: 400–3.
2. Sattar SA, Springthorpe VS. Survival and disinfectant inactivation of the human immunodeficiency virus: A critical review. *Rev. Infect. Dis.* 1991; **13**: 430–47.
3. Resnick L, Veren K, Salahuddin SZ, Tondreau S, Markam PD. Stability and inactivation of HTLV-III/LAV under clinical and laboratory environments. *JAMA* 1986; **255**: 1887–91.
4. Kaplan JC, Crawford DC, Durno AG, Schooley RT. Inactivation of human immunodeficiency virus by Betadine. *Infect. Control.* 1987; **8**: 412–14.
5. Knaepler H, Koch F, Bugany H. Untersuchungen zur HIV-Inaktivierung in allogenen Knochentransplantaten durch chemische Desinfektion und radioaktive Bestrahlung. *Unfallchirurgie* 1992; **18**: 1–6.
6. Dahners LE, Hoyle M. Chemical sterilization of bacterially contaminated bone without destruction of osteogenic potential. *J. Orthop. Trauma* 1989; **3**: 241–4.
7. Van Winkle W Jr, Borick PM, Fogarty M. Destruction of radiation-resistant micro-organisms on surgical sutures by 60Co-irradiation under manufacturing conditions. In: *Radiosterilization of Medical Products. Proceedings of a Symposium, Budapest, 5–9 June 1967 and Recomended Code of Practice.* Vienna: International Atomic Energy Agency, 1967; 169–180.
8. International Organization for Standardization. *Sterilization of Health Care Products. Requirements for Validation and Routine Control. Radiation Sterilization. ISO/DIS 11137.2.* International Standards Organization, 1993.
9. Gaughran ERL. Sterility assurance vs. safety assurance. In: Harris LE, Skopek AJ (eds). *Sterilization of Medical Products. Proceedings of an International Symposium on Advances in Sterilization of Medical Products.* Sydney: Johnson & Johnson, 1985; 119–27.
10. Conway B, Tomford WW, Hirsch MS, Schooley RT, Mankin HJ. Effects of gamma irradiation on HIV-1 in a bone allograft model. *Trans Orthop. Res. Soc.* 1990; **15**: 225.
11. Campbell DG, Li P, Stephenson AJ, Oakeshott RD. Sterilization of HIV by gamma irradiation. A bone allograft model. *Int. Orthopaedics* 1994; **18**: 172–6.
12. Withrow SJ, Oulon SA, Suto TL *et al.* Evaluation of the anti-retroviral effect of various methods of sterilizing/preserving corticocancellous bone. *Orthopaedic Research Society* 1990; 226.
13. Fideler BM, Vangness T, Moore T, Li Z, Rasheed S. Effects of gamma irradiation on the human immunodeficiency virus. A study in frozen human bone-patella ligament-bone grafts obtained from infected cadavera. *J. Bone Joint Surg.* 1994; **76A**: 1032–5.
14. International Atomic Energy Agency. *Sterility Assurance Level.* In: *Guidelines for Industrial Radiation Sterilization of Disposable Medical Products (Cobalt-60 Gamma Irradiation). IAEA-TECDOC-539.* Vienna: International Atomic Energy Agency, 1990; 39.
15. Li P, Burrell CJ. Synthesis of human immunodeficiency virus DNA in a cell-to-cell transmission model. *AIDS Res. Human Retroviruses* 1992; **8**: 253–9.
16. Duc-Nguyen H. Enhancing effect of diethylaminoethyl-dextran on the focus-forming titre of a murine sarcoma virus (Harvey strain). *J. Virol.* 1968; **2**: 763–6.
17. Levy JA, Shimabukuro J, McHugh T, Casavant C, Stites D, Oshiro L. AIDS-associated retrovirus (ARV) can productively infect other cells besides human T helper cells. *Virology* 1985; **147**: 441–8.
18. Sodroski J, Goh WC, Rosen C, Campbell K, Haseltine WA. Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. *Nature* 1986; **322**: 470–4.
19. Lifson JD, Reyes GR, McGrath MS, Sttein BS, Engleman EG. AIDS retrovirus induced cytopathology: Giant cell formation and involvement of CD4 antigen. *Science* 1986; **232**: 1123–7.
20. Lifson JD, Feinberg MB, Reyes GR *et al.* Induction of CD4-dependent cell fusion by the HTLV-III/LAV envelope glycoprotein. *Nature* 1886; **323**: 725–8.
21. Dougherty RM. Animal virus titration techniques. In: Harris RJC (ed.). *Techniques in Experimental Virology.* New York: Academic Press, 1964; 183–6.
22. Gardiner JF, Peel MM. *Introduction to Sterilisation and Disinfection.* Melbourne: Churchill Livingstone, 1986.
23. Hiemstra H, Tersmette M, Vos AH, Over J, van Berkel MP, de Bree H. Inactivation of human immunodeficiency virus by gamma radiation and its effect on plasma and coagulation factors. *Transfusion* 1991; **31**: 32–9.
24. Kitchen AD, Mann GF, Harrison JF, Zuckerman AJ. Effect of gamma irradiation on the human immunodeficiency virus and human coagulation proteins. *Vox Sang.* 1989; **56**: 223–9.
25. Conway B, Tomford W, Mankin HJ, Hirsch MS, Schooley RT. Radiosensitivity of HIV-1: Potential application to sterilization of bone allografts. *AIDS* 1991; **5**: 608–9 (English abstract).

26. Bassin RH, Duran Troise G, Gerwin BI, Rein A. Abrogation of Fv-1b restriction with murine leukemia viruses inactivated by heat or by gamma irradiation. *J. Virol.* 1978; **26**: 306–15.
27. Latarjet R. Inactivation du virus Leucemogere de gross par les radiations UV, X et Y. *Int. J. Cancer* 1970; **6**: 31–9 (English abstract).
28. Rainbow AJ, Mak S. DNA strand breakage and biological functions of human adenovirus after gamma irradiation. *Radiat Res.* 1972; **50**: 319–33.
29. Bigee PD. Inactivation of human immunodeficiency virus (AIDS virus) by gamma and X-ray irradiation in body fluids and forensic evidence. *FBI Law Enforcement Bull.* 1988; 8–9.
30. Spire B, Dormont B, Barre-Sinoussi F, Montagnier L, Chermann JC. Inactivation of lymphadenopathy: Associated virus by heat, gamma rays, and ultraviolet light. *Lancet* 1985; **1**: 188–9.
31. McDougal JS, Cort SP, Kennedy MS *et al.* Immunoassay for the detection and quantitation of infectious human retrovirus, lymphadenopathy-associated virus (LAV). *J. Immunol. Meth.* 1985; **76**: 171–83.
32. Ginoza W. Inactivation of viruses by ionizing radiation and by heat. In: Mararorosch K, Koprowski H (eds). *Methods in Virology*, Vol. 4. New York: Academic Press, 1968; 139–209.
33. Ratner L, Haseltine W, Patarca R *et al.* Complete nucleotide sequence of the AIDS virus, HTLV-III. *Nature* 1985; **313**: 277–84.
34. Wain Hobson S, Alizon M, Montagnier L. Relationship of AIDS to other retroviruses. *Nature* 1985; **313**: 743.
35. Daar ES, Moudgil T, Meyer RD, Ho DD. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. *N. Engl. J. Med.* 1991; **324**: 961–4.
36. Campbell DG, Stephenson AJ, Li P, Oakeshott RD. Human immunodeficiency virus infection of human bone-derived cells. *Clin. Orthop.* 1996; **331**: 291–9.
37. Murphey-Corb M, Martin LN, Davison-Fairburn B *et al.* A formalin-inactivated whole SIV vaccine confers protection in Macaques. *Science* 1989; **246**: 1293–7.
38. Mikhailov MI, Samoilenko II, Zairov GK, Anan'ev VA. Vliianie gamma-izlucheniia na komponenty virusa gepatita B i del'ta-antigen [Effect of gamma radiation on the components of hepatitis B virus and the delta antigen]. *Vopr. Virusol.* 1987; **32**: 68–71.
39. Gibbs CJJ, Gajdusek DC, Latarjet R. Unusual resistance to ionizing radiation of the viruses of kuru, Creutzfeldt-Jakob disease, and scrapie. *Proc. Natl Acad. Sci. USA* 1978; **75**: 6268–70.