Review Article

Application of irradiation as an immunosuppressive agent

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Summary

The concept of using total lymphoid irradiation (TLI) for immunosuppression is based on the prolonged and profound immunosuppressive effects observed after TLI in the treatment of patients with Hodgkin's disease. Pre-operative TLI of allograft recipients has been shown to be immunosuppressive when used alone or together with chemical immunosuppression. Fractionated TLI and allogeneic bone marrow injections produce stable chimaerism without graft-versus-host disease in inbred mice, rats and mongrel dogs and transplantation tolerance of skin and cardiac grafts in rats. In the primate, TLI and bone marrow injection result in significant tolerance of liver and kidney allografts. In 1959 sublethal whole-body irradiation was used as an immunosuppressive agent for the first successful related-human renal allografts between non-identical twins. Despite the dangers of myelosuppression, recent clinical experience has shown TLI to be a useful immunosuppressant for organ transplantation, allowing decreased dosage of concomitant immunosuppressive drugs.

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In the past 10 years total lymphoid irradiation (TLI) has been shown to be a potent immunosuppressive agent with potential for clinical use in organ allotransplantation.[1-3] While indefinite allograft survival with induction of donor-specific tolerance is the desired goal in organ transplantation research, the role of TLI in achieving this end remains controversial. The ability of TLI to ensure prolonged organ allograft survival across minor and major histocompatibility barriers is well established, but studies in experimental models have yielded conflicting results with respect to induction of tolerance.

In this review, the experimental studies and clinical application of TLI will be discussed.

Biological effects of TLI

Most research workers using TLI for immunosuppression have administered electromagnetic photons either as X- or γ-rays. Modern megavoltage radiotherapy is administered either as beams of X-rays produced by linear electron accelerators or as γ-rays produced by cobalt-60 teletherapy units.[4]

The radiations interact with matter to ionise atoms and initiate a cascade of physiochemical reactions during which abnormal chemical bonds may be formed or broken. Cells killed by irradiation die as a result of changes in the configuration of DNA which cause death during attempted mitotic divisions.[4,5] Highly radiosensitive, small, resting lymphocytes die during the interphase before mitosis. Radiation injures small and large molecules indiscriminately and has no selective effect on DNA.[4] The mitotic death is accompanied by chromosomal breaks, translocations, bridges and other structural abnormalities which reflect the production of lesions in chromosomal DNA and which may be single- or double-strand breaks or damage to pyrimidine and purine bases.[4,5] It has been reported that base damage and single-strand breaks are readily repaired enzymatically, whereas double-strand breaks, though less numerous, are much less susceptible to repair and are therefore usually lethal for the cell.

Earlier research workers[6] showed that whole-body irradiation results in lymphocytopenia and suppresses antibody production. About 80% of lymphocytes die a prompt intermitotic death after ionising irradiation, while 20% survive. B cells are quite radiosensitive and undergo both interphase and mitotic death following irradiation, and like them, the sensitive suppressor T-cell precursors may undergo interphase death. The homing potential of cells is also affected by radiation.[6]

It is of interest that the effects of whole-body irradiation are qualitatively and quantitatively different from those of localised or regional radiation.[6]

Immunosuppressive properties of TLI

The rationale for the application of TLI as immunosuppressive agent is based on the findings of suppression of T-cell and preservation of B-cell function without apparent increased susceptibility to infection in irradiated patients with Hodgkin's disease.[5,7,8] The findings of T-lymphocytopenia, B-lymphocytosis, depression of responsiveness in mixed lymphocyte reaction and to phytohaemagglutinin (PHA) mitogenesis, together with a loss of delayed-type hypersensitivity response to rechallenge with skin sensitivity agents has stimulated other workers to consider TLI as a potential immunosuppressive agent in organ allotransplantation.[5] Immunological monitoring after TLI and transplantation have confirmed a sustained and uniform reduction in helper-inducer T cells and in the proliferative responses to PHA, pokeweed mitogen and allogeneic lymphocytes during the first year after grafting. A variable recovery in the absolute number of suppressor-cytotoxic cells and the proliferative response to concanavalin A has been reported. Studies of T-cell recovery in Hodgkin's disease treated with TLI have consistently shown a more rapid recovery of cytotoxic-suppressor cells and a slower recovery of helper-inducer

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cells. T-cell maturation or recovery after completion of TLI may reduce its potency as an immunosuppressive agent, hence the importance of early allografting after completion of TLI.[9] In experimental models the administration of cyclophosphamide between the completion of TLI and allografting prevents the recovery of peripheral blood OKT4- and OKT8-reactive lymphocytes.[10] In these studies T-cell subsets were identified by indirect immunofluorescent antibody techniques using monoclonal antibodies OKT4 and OKT8 as the primary antibodies.[10]

Experimental studies

Slavin *et al.*[1] were the first to show significant skin-graft survival after TLI in H_2-incompatible strains of mice. They showed considerable skin-graft survival in Balb-C mice conditioned with 200 rad/d 5 times per week to a total of 3 400 rad. In addition, these authors showed that a single injection of bone marrow produced stable chimaeras in similarly TLI-treated recipients.[1] Of importance in these early experiments was that chimaeras could be established by transfer of fully allogeneic bone marrow cells without induction of graft-versus-host disease.

Subsequent studies have shown impressive long-term cardiac allograft survival in mongrel dogs and rhesus and cynomolgus monkeys.[11–13] Concomitant administration of azathioprine (AZA), anti-human thymocyte globulin (ATG) and cyclosporin A (CSA) was needed to ensure consistent and prolonged survival.[11–13] Myburgh *et al.*[2,14–16] and Smit *et al.*,[17] using TLI in a major study in primates, showed long-term kidney allograft survival either with or without concurrent bone marrow transplantation. Full tolerance for kidney and liver allografts was obtained with cumulative doses ranging from 600 to 2 800 rad.[2] However, radiation-related deaths occurred with increasing frequency with cumulative doses of 2 000 rad and more. The administration of CSA in combination with suboptimal TLI in the same baboon kidney transplantation model resulted in inferior graft survival rates compared with TLI alone. A synergistic or additive effect could not be demonstrated.[2]

Du Toit *et al.*[18–20] showed poor pancreatic allograft survival after TLI in a baboon allograft model. However, the administration of 800 rad subtotal marrow irradiation together with CSA resulted in considerable and consistent pancreatic allograft survival, results previously not achieved with TLI alone.[21] In contrast, other workers[22,23] showed minimal pancreatic allograft survival after TLI in mongrel dogs and primates.

Fields of irradiation

Significant prolongation of organ allograft survival in experimental models has been achieved with whole-body and subtotal marrow irradiation.[24–29] In all the experimental models the fields irradiated have been much more extensive than the mantle and inverted-Y fields used in patients with Hodgkin's disease. In the baboon, extensive fields are needed if prolonged engraftment of pancreas, kidneys and liver are to be achieved.[2] This has necessitated irradiation of the entire trunk below the base of the skull and includes thorax, abdomen, pelvis, proximal femurs and humeri, and tail.[2,21] In rats, dogs and monkeys, similar extensive fields are needed.[13,24,27] Current studies in man have indicated, however, that the conventional fields used in the treatment of Hodgkin's disease are adequate.[3,30,31]

Need for concomitant immunosuppression

Although TLI alone is adequate for the establishment of marrow graft acceptance in outbred mongrel dogs, further immunosuppression is needed to obtain consistent engraftment of the heart, pancreas and kidney in cynomolgus monkeys, baboons and man.[3,11,13,21,32] In extensive studies over 15 years, Myburgh *et al.*[2,15,16] showed extended renal and liver allograft survival in baboons treated with fractionated TLI only. Many animals in that series have survived for more than 5 years with no need for concomitant immunosuppression.[2]

Nevertheless, the current consensus is that maintenance immunosuppressive therapy is indicated after TLI and engraftment in human allograft recipients.[3,5] Rejection has frequently been reported after cessation of maintenance chemical immunosuppression in TLI allograft recipients.[9]

In most studies ATG, steroids and AZA have been used as adjunctive drugs,[3,24] but in some patients severe myelosuppression has followed the use of AZA and TLI.[9] The efficacy of TLI together with CSA remains to be shown in man although results in a baboon pancreatic transplantation model have been encouraging.[21]

Human experience

To date approximately 50 patients have been treated with fractionated TLI before renal transplantation.[3,10] In most studies TLI has resulted in effective immunosuppression for organ transplantation. The majority of patients have been renal allograft recipients and have needed concomitant immunosuppressive treatment to prevent rejection.[3,10] In one study, cessation of adjunctive treatment resulted in rejection of the kidney.[9] The observation underlines the importance of maintenance immunosuppression after TLI or the need for 'topping-up' irradiation either pre- or postoperatively.[5] In most studies modest complications were observed during and after TLI, sometimes necessitating interruption of the radiation therapy.[3] Nearly all patients suffered from nausea, vomiting, increased fatigue, leucopenia and thrombocytopenia.[3,10] An increased susceptibility to infection, particularly herpes simplex and cytomegalovirus infections, was observed in some patients.[3,10] The majority of patients received irradiation to mantle and inverted-Y fields.[3,10,30] In Najarian *et al.*'s[3] study, optimal results were achieved with 2 500 rad delivered in 100-rad fractions followed by transplantation within 2 weeks, followed by a tapering prednisone schedule and maintenance AZA. In addition the administration of donor bone marrow at the time of transplantation did not produce chimaerism.

Disadvantages of TLI include the need for additional pharmacological immunosuppression and the problem of maintenance of patients in a state of readiness over the period between the end of TLI conditioning and organ transplantation.

In view of the recent advances in pharmacological immunosuppressive therapy, particularly with CSA, TLI seems unsuitable for use solely as a means of routine nonspecific immunosuppression.

Radiation-related complications

Apart from the interference with host defence mechanisms, TLI has a profound effect on the bone marrow. Common complications include leukopenia and thrombocytopenia, resulting in an increased susceptibility to infection and a bleeding diathesis.[3,10] An increase in complications can be expected with the addition of concomitant chemical immunosuppression.

Gastro-intestinal complications of TLI include anorexia, nausea and vomiting, and may necessitate interruption of the preparative radiation therapy, hospitalisation and nutritional support with intravenous hyperalimentation.[3] Degrees of weight

loss and anaemia have been reported in experimental models and in man.[3,21] Diabetic patients tolerate radiation less well and seem to experience more severe weight loss and gastrointestinal symptoms than their non-diabetic counterparts.[3]

Infections frequently reported after TLI include herpes simplex, herpes zoster and cytomegalovirus infections.[3,10] Improvement has been reported after treatment in selected cases with the antiviral drug acyclovir. In many patients, cultures for Epstein-Barr virus are positive.[3,10]

Radiation-induced mutagenesis is of concern in all patients receiving TLI.[6] However, the risk of leukaemia or lymphoma is not increased in patients with Hodgkin's disease treated with 4400 rad TLI alone.[9] Unfortunately, the effects of uraemia and the addition of ATG or CSA to TLI are not known.[9] Nevertheless, the development of lymphoma in a small number of renal allograft recipients has recently been reported by Najarian *et al.*[3] Pennock *et al.*[13] have also reported the occurrence of metastatic lymphoma after TLI and CSA immunosuppression in cynomolgus monkeys which received cardiac allografts. The development of lymphoma after TLI in baboons has never been reported.[2,21]

On balance, the side-effects of TLI seem to compare favourably with those of other immunosuppressive regimens.

Conclusion

TLI has been shown to be a potent immunosuppressive agent in experimental models and in man. The need for administration of donor bone marrow remains to be demonstrated. In man, the addition of concomitant pharmacological immunosuppression is a prerequisite if prolonged survival of allografts is to be ensured. TLI produces stable chimaeras in rodents and full tolerance for kidney and liver allografts in primates. Laboratory and clinical experience suggest that optimal results are achieved by immediate organ engraftment after completion of TLI. Frequently, the clinical use of TLI is compromised because a suitable allograft is not available at the time a potential recipient completes a course of TLI.

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