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# Effect of Irradiation on Incidence of Post-Transplant Lymphoproliferative Disorder after Hematopoietic Cell Transplantation in Miniature Swine

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A B S T R A C T

Post-transplant lymphoproliferative disease (PTLD) is a major complication of clinical organ and cell transplantation. Conditioning and immunosuppressive regimens that significantly impair T cell immunity, including depleting antibodies and calcineurin inhibitors, increase the risk of PTLD after transplantation. Swine PTLD has been shown to closely resemble human PTLD in morphology, histology, and viral-driven reactivation of B cells. Previously, we reported high incidences of PTLD after hematopoietic cell transplantation (HCT) in miniature swine recipients conditioned with thymic irradiation (TI) in addition to T cell depletion and cyclosporine A monotherapy after transplantation. Replacement of TI with 100 cGy of total body irradiation resulted in similar numbers of B cells early post-transplantation, greater numbers of T cells at day 0, and markedly decreased incidence of PTLD, suggesting that a threshold number of T cells may be necessary to prevent subsequent B cell proliferation and development of overt PTLD. Results from this large cohort of animals provide insight into the important effect of irradiation and T cell immunity on the incidence of PTLD after HCT and reinforce the pig model as a valuable tool for the study of PTLD and HCT.



## INTRODUCTION

Post-transplant lymphoproliferative disease (PTLD) is a potentially lethal complication of clinical organ and cell transplantation, as a consequence of prolonged immunosuppression [1]. In humans, PTLD is characterized by an abnormal B cell proliferation related to Epstein-Barr virus primary infection or reactivation. Conditioning regimens that diminish T cell immunity, such as thymoglobulin mediated T cell depletion or long-term calcineurin inhibition, increase the risk of PTLD after transplantation. This risk results from the loss of antiviral function of $CD8^+$ cytotoxic T cells, which are primarily responsible for suppressing Epstein-Barr virus–associated infections [2]. The variability of the human patient population, both clinically and pathologically, complicates the ability to study PTLD. Murine models of PTLD involving immunodeficient mice injected with human PTLD lines and mice infected with murine gamma herpesvirus [3,4] are often unreliable and do not model human disease accurately. Therefore, large animal preclinical models that mimic human PTLD provide an opportunity to identify risk factors and investigate therapeutic approaches to mitigate this frequently lethal condition.

We have previously reported a high incidence (>33%) of PTLD in swine after hematopoietic cell transplantation (HCT) that closely resembles human PTLD both morphologically and histologically [5,6]. In this model recipients were conditioned with T cell depletion using a porcine CD3 immunotoxin and cyclosporine A (CyA) monotherapy with or without thymic irradiation (TI; 700 to 1000 cGy). Risk factors for the development of PTLD in this model, which are similar to those in humans, were also described, including the effects of peripheral blood chimerism levels, T cell depletion, TI, immunosuppression levels, and MHC disparity [6]. We also identified a novel porcine lymphotropic herpesvirus-1 (PLHV-1) associated with PTLD in swine, paralleling human infection with Epstein-Barr virus [7]. These findings further support the pig as a reliable preclinical large animal model of PTLD.

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In an attempt to reduce the incidence of PTLD in this model, we replaced high-dose TI as part of the preparative regimen with low-dose 100 cGy of total body irradiation (TBI). In this study, we report on the effect of irradiation (TBI versus TI) on the incidence of PTLD in our miniature swine model and also demonstrate that serum lactate dehydrogenase (LDH) levels serve as a supportive marker for the diagnosis of PTLD in swine.

## METHODS

### *Animals*

This is a retrospective study of all animals conditioned for HCT at the Transplantation Biology Research Center at Massachusetts General Hospital using a porcine CD3 immunotoxin for recipient T cell depletion and a short course of CyA since 1997. All animals that developed PTLD were diagnosed between 21 and 45 days post-HCT. Therefore, only animals who survived at least 45 days post-HCT were selected for this analysis, unless early death was due to PTLD. Animals were selected from our herd of Massachusetts General Hospital partially inbred MHC-defined miniature swine, which have been previously described [8]. Transplant donors ranged from 4 to 6 months in age and recipient animals from 8 to 12 weeks. All experiments were approved and performed in compliance with the Institutional Animal Care and Use Committee.

### *Irradiation*

When administered, animals received either 100 cGy of TBI or 1000 cGy of TI as previously described [9]. Because only 4 animals received 700 cGy TI in 1997 before the protocol was changed to 1000 cGy TI, we decided to exclude these 4 animals from the PTLD analysis in this study. Animals were sedated, and a cobalt irradiator was used for all regimens. The dose was calculated dependent on the source decay charts.

### *T Cell Depletion and CyA*

Animals receiving no irradiation or TI were administered .05 mg/kg CD3 immunotoxin, pCD3-CRM9 [10], on day −2 for T cell depletion. Animals receiving TBI were either administered .05 mg/kg CD3 immunotoxin, pCD3-CRM9, on day −2 (n = 13) or .05 mg/kg recombinant CD3-immuntoxin (pCD3-rIT) [11] twice daily 8 hours apart beginning on day −4 until day −1 (n = 27). CyA was administered orally through a gastrostomy tube twice daily with target levels of 400 to 800 ng/mL in all animals commencing on day −1 and concluding on either day 30 or 60 for those animals receiving TI or day 30 followed by a 2-week taper for those animals receiving TBI.

### *Hematopoietic Cell Transplantation*

HCT was performed as previously described [12]. Briefly, transplant donors were mobilized once daily with porcine IL-3 (100 μg/kg/day) and porcine stem cell factor (SCF; 100 μg/kg/day) or with human granulocyte colony-stimulating factor (G-CSF; 10 μg/kg/day) beginning on day −5. Peripheral blood mononuclear cells (PBMCs) were harvested by leukapheresis beginning on day 0. Cytokine injections were continued, and donor animals were leukapheresed daily for up to 3 days until the target cell dose of 1 to $15 \times 10^9$ cells/kg of recipient body weight was collected. After each leukapheresis collection, fresh cells were infused intravenously into recipient animals over 15 to 20 minutes.

### *PTLD Diagnosis*

A diagnosis of PTLD was made based on a combination of flow cytometric confirmation of B cell lymphoproliferation and physical signs and symptoms, including loss of appetite, lethargy, and lymphadenopathy. In the case of death, confirmation of PTLD diagnosis was made by histologic analysis. All recipients that developed PTLD were diagnosed between 21 and 45 days post-HCT.

### *Flow Cytometry*

Flow cytometry was performed on a weekly basis to monitor myeloid and B cell proliferation, chimerism levels, and percent and absolute T cell counts in the peripheral blood. Methods were previously described [5,6]. Briefly, chimerism levels were assessed by the use of pig allelic antigen, a nonspecific cell marker present on donor cells but not on host cells. Donor and hosts were specifically screened before transplant, and only pig allelic antigen–positive donors and pig allelic antigen–negative hosts were selected. B cell lymphoproliferation and absolute B cell numbers were monitored by assessing the $CD3^-/CD16^-$ double-negative population as previously described [5,6].

### *Graft-versus-Host Disease Assessment*

Graft-versus-host disease (GHVD) was assessed daily by physical exam in all animals. Total bilirubin and liver enzymes (alanine and aspartate aminotransferases and alkaline phosphatase) were assessed biweekly (or more often if medically indicated). Any rash was documented, and skin was biopsied and assessed via histopathology if deemed appropriate. Gastrointestinal signs of GVHD were assessed clinically by fecal volume (animals housed in metabolic cages) and consistency (watery, mucoidal, mucohemorrhagic, etc). Additionally, rectal biopsies were taken if clinically indicated and compared with pretransplant samples. Twice weekly, donor and host T cells were assessed via flow cytometry. A Seattle scoring system was developed in swine and used to standardize GVHD assessment (developed in conjunction with Dr. Thomas R. Spitzer- director of the Massachusetts General Hospital unit). A description of this GVHD swine scoring system and the characterization of the miniature swine GVHD is forthcoming (Duran-Struck et al, unpublished data).

### *LDH ELISA*

PBMCs were isolated from heparinized blood by Ficoll gradient centrifugation and cultured for 18 hours with or without phytohemagglutinin (M Form; Invitrogen, Carlsbad, CA, USA). Cells were plated at $1 \times 10^3$ to $5 \times 10^4$ cells per well, and total LDH was determined using the Lactic Dehydrogenase based In Vitro Toxicology Assay Kit (Sigma Aldrich, St. Louis, MO, USA). This assay has been previously described [13].

### *Statistical Analysis*

A 1-sample *t*-test was used to assess whether the leukocyte counts were higher among the PTLD animals than the mean of the normal range under the assumption of unknown variance. A 2-sample *t*-test was used to compare the distribution of LDH levels between the PTLD animals versus healthy controls under the assumption of unequal variances. The *P* values were based on a 2-sided hypothesis test and computed using Stata 7.0 (Stata Corp, College Station, TX). Survival curves were plotted using Kaplan-Meier estimates using a log rank (Mantel-Cox) test. $P < .05$ was considered statistically significant.

## RESULTS

### *Effect of Irradiation on Incidence of PTLD after HCT*

Beginning in 1997, 29 miniature swine received SCF/IL3 mobilized HCTs after conditioning with 1000 cGy TI, T cell depletion with pCD3-CRM9, and a short course of CyA (30 to 60 days), with a PTLD incidence rate of 34.4% (10/29) (Figure 1). In an attempt to reduce post-transplant complications including PTLD in this model of HCT, TI was removed from the protocol, which resulted in a decreased incidence of

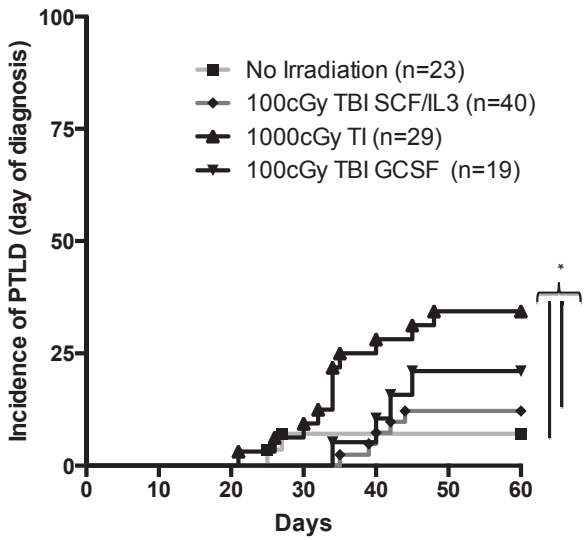


**Figure 1.** PTLD incidence. Incidence of PTLD in animals that received a conditioning regimen with SCF/IL3 + no irradiation (■), SCF/IL3 + TBI (◆), SCF/IL3 + TI (▲), or G-CSF + TBI (▼). *$P < .05$.