On the crossroad between tolerance and posttransplant lymphoma
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The role of the Epstein-Barr virus in the development of post-transplant lymphomas is well established. However, not all lymphomas that arise in these patients contain Epstein-Barr virus, suggesting that other cofactors are involved in tumor pathogenesis. We propose that immunologic interactions that result from the introduction of immunocompetent donor cells during transplantation contribute to a lymphomagenic environment in the host. Murine models of lymphoma that arises following transfer of allogeneic hematopoietic cells are discussed and are related to the transplant setting. One contemporary viewpoint of transplantation immunology holds that interactions between the host and donor components of the immune system determine the ultimate degree of tolerance or reciprocal immunoreactivity (eg, rejection, graft-versus-host disease) within the transplant patient. We conclude that host-donor immunologic microchimerism may also be an overlooked factor in the development of posttransplant lymphomas.

At first glance, the title of this paper appears to be an effort to link two entirely unrelated topics. After all, a preponderance of studies have established the Epstein-Barr virus (EBV) as the primal driving force within post-transplant lymphoproliferative disorders (PTLD) [1], and a depressed host antiviral immune response apparently completes the list of factors necessary for production of these tumors. Indeed, direct reestablishment of immunologic control [2], most specifically by means such as adoptive transfer of anti-EBV-specific, HLA-matched cytotoxic T cells [3–5], has been associated with tumor regression. Further, some patients who are treated by withdrawal of immunosuppression may also reject their grafts and thus by definition have not achieved donor-specific tolerance. What, then, is the impetus that leads us to suggest an intersection between tolerance and posttransplant lymphomas?

On reflection, several features of PTLD are not easily explained by a disease model restricted to understanding the interactions between EBV-induced B-cell lymphoproliferation and defective host immunologic controls. For example, not all PTLDs contain EBV, nor are they necessarily even of B-cell origin [6,7+]. Conversely, other EBV-associated tumors, such as nasopharyngeal carcinoma, do not occur in transplant patients with the same frequency as does PTLD. We also do not know why a large number of PTLDs are extranodal, and a virocentric perspective does not fully explain why a disproportionate number of PTLDs occur in the allograft [8]. The occasional donor cell–derived PTLD in the organ recipient [9] may be a probabilistic phenomenon, but it might also reflect other, poorly understood processes. For all of these reasons, it may be worthwhile to first acknowledge the importance of EBV and the associated host anti-EBV immune response in the pathogenesis of these tumors, and then ask where we might seek other, cryptic, cofactors that could explain additional aspects of posttransplant lymphoid neoplasia.

Analysis of risk factors for PTLD has generally sought to provide support for, and enhance our understanding of, the EBV hypothesis. Thus, primary versus reactivation EBV infection and high levels of immunosuppression have been shown to predispose to lymphoma development [10–14]. Cytomegalovirus infection has also been considered a risk factor for PTLD, attributed at least in part to its association with EBV reactivation [15]. Reports of other risk factors for PTLD are sparse. In an early study, Birkeland [16] proposed an association between
the degree of HLA mismatch and (lymphoid and nonlymphoid) posttransplant tumors. This theory hearkens to the concept of "allograft antigenic stimulation," which has served as a frequently invoked, but to date unproven, leitmotif in the discussion of risk factors for PTLD. In contrast, a recent report identified good HLA-DR matching as a potential risk factor for this disease [11]. A later study from the same center did not confirm this but did show a predominance of HLA-A2, -Bw57, or -DR7 in donors for patients who developed PTLD [17•]. This finding was interpreted in the context of EBV immune reactivity.

Specific underlying diseases have occasionally been put forth as risk factors for PTLD development. Most such claims derive from early reports. However, one recent study [13] showed a statistically significant correlation between Langerhans cell histiocytosis and later PTLD. Most accepted or speculative factors associated with development of PTLD contain elements of either immunostimulation or immunologic hyporesponsiveness. One phenomenon that incorporates both of these features is acquired immunologic tolerance. We can define tolerance broadly as "...a physiologic state in which the immune system does not react destructively against the organism that harbors it" [18].

In order to develop our premise, namely that there is a point of intersection between tolerance and PTLD, we first present a view of tolerance as one potential end state of donor–host immunologic interactions [19••]. Animal models of tolerance induction are described and neoplastic complications of these models are shown. The potential significance of these findings to the clinical situation is then discussed.

Transplantation rejection and tolerance

Acute rejection is mediated primarily by direct recognition pathways, in which host leukocytes, predominantly T lymphocytes, recognize alloantigens displayed on professional, donor-derived antigen-presenting cells (APCs) [18]. Such APCs are largely synonymous with the "passenger leukocyte" population. Indirect recognition, in which host lymphocytes recognize alloantigens presented by host-derived APCs, also contributes to rejection [20•,21], and such T cells can be seen in blood and in the allograft during rejection episodes. Analysis of indirect presentation has shown that one dominant target emerges during the initial response [22]. In subsequent responses, additional determinants may also be targeted. Thus rejection is not only dynamic but also shows cumulative immunologic "learning."

The importance of APCs in initiating rejection was first suggested by experiments showing that donor-specific tolerance could be produced by transplantation of an F1 (RxS) organ into an (R) recipient, followed by removal of the organ and retransplantation into a second (R) recipient [23]. This effect was attributed to emigration of passenger leukocytes out of the graft during the primary transplant. The tolerant state could be broken with an infusion of (RxS) dendritic cell–enriched preparations but not by (RxS) B- or T-cell-enriched preparations. This implied that the original donor dendritic cell population was responsible for the initiation of organ rejection. In this view, the organ parenchyma is a silent partner that is subject to injury by a rejection reaction first directed against or initiated by the donor dendritic cells.

If this model is a sine qua non of tolerance, then tolerant individuals should be devoid of donor dendritic cells. This clearly is not the case, as evidenced by persistence of these cells in microchimeric patients [24,25]. We therefore must concede that survival of donor dendritic cells is compatible with tolerance and conclude that tolerance represents one steady-state condition of microchimerism. This is not the only possible steady state, and in other cases microchimerism may exist in a host who is devoid of self-sustained donor-specific tolerance [26,27]. Persistence of a clinically stable state in this circumstance is dependent on continued immunosuppression. In the absence of this outside aid, the host–donor relationship may proceed to a dysregulated state reflected by either allograft rejection or graft-versus-host disease (GVHD). In this viewpoint, the post-transplant state is viewed as a series of negotiations between two immune systems. If successful, coexistence is maintained and tolerance achieved. If negotiations are unsuccessful, conflict breaks out and the more powerful immune system prevails, pillaging the organs defended by the vanquished adversary.

The individual mechanisms that may contribute to acquired tolerance induction are reviewed elsewhere [28]. These include, eg, veto cells, anergy, clonal deletion, suppressor activity, and costimulatory deficient dendritic cells [29]. Regardless of the specific pathways, tolerogenesis is necessarily an active process in light of the continued generation of new immune cells by the host (estimated at $2 \times 10^6$ T cells and $2 \times 10^6$ B cells daily [30]). There is also evidence that hematopoietic stem cells from the allograft may establish residence in the host and generate progeny cells [31]. The relevant question then becomes: How do the donor and host immune systems achieve a mutual tolerance in some patients but not in others, and what complications can arise from this state of affairs?

Murine models of tolerance

Inoculation of major histocompatibility complex–disparate hematopoietic cells into neonatal mice leads to donor-specific immunologic hyporesponsiveness and to tolerance for grafts from the donor [32]. Such tolerance was originally considered a unique property of the nascent immune
system with minimal relevance to either the adult animal or to areas such as clinical transplantation. Matzinger [30] questioned this assumption in the context of her "danger hypothesis" for explaining the raison d'être of the immune system. In her interpretation, the primary purpose of the immune system is not to distinguish self from non-self but rather to distinguish dangerous from nondangerous environments. The hypothesis draws from the two-signal theory of Cohn [33], which states that inexperienced lymphocytes require two signals for activation and that, in the presence of the first signal without the second, they will either become tolerant or die. Matzinger suggested that the small number of normal, inexperienced T cells that comprised the newly developed immune system was easily saturated with donor B cells in the neonatal tolerance experiments. The B cells supplied signal 1 only, thus tolerizing the animal to the donor antigen. As the number of recipient immune cells increased with age, an identical inoculum would no longer be saturating and some recipient T cells could receive both signals by interaction with the less common donor dendritic cells. This would result in sensitization instead of tolerance. The model predicted that proper manipulation of donor cell number and composition could induce tolerance in the adult animal (or sensitization in the neonates). This prediction was verified by Ridge et al. [34*], thereby demonstrating that neonatal tolerance follows the same rules as does acquired tolerance in the adult and that the neonatal tolerant state is contingent on the interaction between two immune systems. Thus, tolerance-generating models of hematopoietic cell transfer in neonatal and adult rodents may serve as guides to host–donor immune interactions that occur in the microchimeric posttransplant setting. Likewise, breakdown of tolerance and complications of host–donor immune interactions in the murine model may provide signposts to alternative pathways available to the hybrid immune system in the transplant patient.

**Lymphomas following transfer of allogeneic hematopoietic cells in mice**

Janossy et al. [35–37] reported an increased frequency of lymphomas in mice following induction of neonatal tolerance. Tumors were of recipient origin, and analysis of two separate lymphomas revealed a T-cell origin in both [36]. One tumor induced donor-specific tolerance when implanted into syngeneic animals, suggesting that it arose from a subset of cells involved in mediating donor-specific tolerance in the progenitor animal. An increase in the absolute number of cells comprising the donor inoculum was associated with an increase in the frequency of recipient lymphomas in this model. Infusion of disrupted donor cells did not lead to lymphoma development, excluding transfer of virus as the cause of tumors. Inhibition of donor cell proliferation prevented the onset of lymphomas, and from this finding the authors inferred that long-term chimerism was a necessary prereq-

uisite to tumor development. Removal of Thy1+ T cells from the donor inoculum moderately reduced but did not eliminate tumor development. In these animals, donorspecific allografts were maintained for variable periods, dependent on the particular strain combination. The frequency of lymphomas was also related to the individual strain combinations used, but this did not parallel the degree of tolerance induction and the authors concluded that these were independent events [38]. Autoantibody formation was observed in a number of animals, and this was considered to reflect a component of GVHD activity. However, clinical GVHD was not seen.

These studies expanded the earlier studies of Schwartz and Beldotti [39], Schwartz and Andre-Schwartz [40], Datta and Schwartz [41], and Andre-Schwartz et al. [42], who showed lymphoma development following murine splenocyte transfer. In their model of parental (A)→F, (AxB) cell inoculation, GVHD was a frequent cause of early mortality. This could be alleviated by using older mice and by treating recipients with a short course of immunosuppression. However, mice that survived clinical GVHD without immunosuppression also developed lymphomas, indicating that drug therapy was not necessary for tumor development. Tumors in this model were also of recipient origin [42]. The authors suggested that low-grade chronic GVHD, possibly interacting with a weakly oncogenic virus, was a precipitating factor for tumor development [41]. In separate studies, Walford [43] showed an increased incidence of lymphomas in the absence of clinical GVHD following transfer of splenocytes across a weak histocompatibility locus into neonatal mice. In this model, bilateral immunologic interactions were also considered to be the likely instigator of the tumors.

**Posttransplant lymphoproliferative disorders**

An increased frequency of lymphoid tumors in transplant patients has long been documented. Most tumors arise within the first 1 or 2 years following transplantation, and they may be single or multiple. The latter may arise from individual, unique clones [44]. According to Penn [45], approximately 87% of PTLDs are of B-cell and 13% are of T-cell origin.

The role of EBV in PTLD is well established [46]. In vitro, the latently EBV-infected B cell has an "activated" phenotype and expresses a number of EBV-latency-associated proteins [47]. However, the in vivo phenotype of the latently infected lymphocyte may be that of a resting B cell, with little or no expression of viral antigens [48]. The exact in vivo stimulus that first activates this latently infected cell is unknown.

The phenotype of most EBV-positive PTLDs resembles that of the activated in vitro EBV-infected B cell [49]. The cytokine profile of PTLDs appears to be a Th2
and this pattern may be reflected systemically by elevated serum interleukin-4 and IgE levels, as shown in some cases [51]. Remission can often be induced by restoring host immunologic controls, using such means as withdrawal of immunosuppression [2] or administration of EBV-specific effector cells [3-5]. This antitumor activity often occurs in parallel with mild to severe allograft rejection. However, in some cases significant rejection does not occur and it is possible to maintain the patient on minimal or no immunosuppression following tumor resolution.

The nature of EBV-negative PTLDs has not been as well characterized. These tumors tend to arise later than EBV-positive PTLD and they often resemble non-Hodgkin's lymphomas. B- and T-cell forms have been described. Some tumors have also been found to be negative for the Kaposi sarcoma virus (human herpesvirus 8) (M. A. N., unpublished observations). We have observed occasional remission of these tumors, but the prognosis is generally considered to be less favorable than that of EBV-positive tumors.

**Insights from animal models**

Simple transfer of immune cells with establishment of long-term chimerism is associated with an increased rate of lymphoma development in small-animal models. This risk is related neither to EBV (which does not infect rodent cells) nor to iatrogenic immunosuppression. It may occur in the presence or absence of GVHD. The same type of immune cell transfer may also lead to tolerance, but the two phenomena appear to be either partially or wholly independent in the experimental setting.

How might this situation relate to the clinical setting? One practical example of immune cell transfer to generate tolerance lies in the use of allogeneic blood transfusions to enhance the survival of subsequent allografts. The mechanism and full extent of the effect of this maneuver are still debated [52-57], but there is evidence that the leukocyte component mediates changes that lead to enhanced graft acceptance [58]. At this time there is no evidence that blood transfusions predispose to subsequent lymphoid tumor. Nevertheless, in one recent study [56], four cases of PTLD occurred in 55 recipients who received pretransplant infusion of HLA-DR-mismatched blood. This represented a statistically significant difference from the group of 45 patients with DR-matched transfusions, none of whom developed PTLD. Interpretation of the data is clouded by the fact that two of the four patients with PTLD also received OKT3, which is also a risk factor for PTLD.

This finding contrasts to the earlier-cited study showing that good HLA matching was a potential risk factor for PTLD [11]. If the murine studies are to serve as a guide, they indicate that both the donor immunocyte inoculum size and the specific donor-host HLA types may disclose high- and low-risk subpopulations. This also recalls a previously mentioned study concluding that HLA-A2 and -DR7 were overrepresented among donors of patients who developed PTLD [17*].

The animal model suggests that a constant interaction between the two immune systems, possibly associated with a low-grade proliferation of the relevant cells, may set up a substrate for neoplasia. In the human situation, we may speculate that the omnipresent EBV becomes activated due to B-cell involvement in this process, and the virus quickly becomes the central figure as it establishes an uncontrolled proliferation aided by crippled downstream immune controls (i.e., depressed anti-EBV cytotoxic T-cell activity). This would lead to typical PTLD. In other cases, we suggest that chronic stimulation of cells may also provoke neoplastic changes in the absence of this virus. These tumors may require a longer period to develop, because they would not be driven by the manic proliferative forces unleashed by EBV. Nevertheless, most would originate from the numerically dominant immune system, would be more evenly distributed among the B- and T-cell subsets, and could possibly include tumors of other cells, such as dendritic cells, participating in the two-way immune interaction. The tumors would preferentially occur at sites of host-donor interaction, i.e., nodal and extranodal sites, as well as the allograft. Because these tumors are related to a chronic two-way immune interaction, no clear relationship will be found between tumor development and either the level of immunosuppression or the degree of donor-specific tolerance. Likewise, no consistent relationship with a single virus will be found. The tumors would show changes characteristic of malignancy, such as oncogene or tumor suppressor alterations, but it is unlikely that a single consistent abnormality will be uncovered. If the tumor cells are studied in detail, they may on occasion be found to arise from cells with specificity directed toward the companion immune system.

**Conclusions**

At present, the EBV paradigm of PTLD reigns supreme, and this concept has led to great strides in both our understanding of these tumors and our ability to treat this disorder. The success of this model may eclipse the contribution of other, unrelated cofactors. We have attempted to make the case that nonviral factors that exist within the posttransplant patient may also be primary contributors to the lymphomagenic environment. This speculation is presented in order to provoke investigations to confirm or deny this possibility. We suggest that there is a crossroad between the development of tolerance and lymphomagenesis, and this crossroad is identified as the bilateral immunologic activity that occurs in the microchimeric transplant patient.
References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- Of special interest
- Of outstanding interest


This paper summarizes the current pathologic approach to PTLD.


This paper presents a good general discussion of the known risk factors for PTLD.


This paper presents the arguments for microchimerism as a critical determinant of the posttransplant fate of graft and host.


This paper summarizes the current emphasis on indirect antigen presentation as a potentially important mediator of rejection.


The studies of this report align the mechanism of neonatal tolerance with those of acquired tolerance in the adult.


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