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Post-Transplantation Lymphoproliferative Disorders Arising in Solid Organ Transplant Recipients Are Usually of Recipient Origin

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Recent clinical, pathological, and molecular studies have increased our understanding of posttransplantation lymphoproliferative disorders (PT-LPDs). Studies have shown that the majority of PT-LPDs arising in bone marrow transplant recipients are of donor origin; however, the source (host or donor) of the lymphoid cells that make up PT-LPDs arising in solid organ transplant recipients has not been systemically investigated. In this study, 18 PT-LPDs occurring in 16 organ transplant recipients (13 heart, 2 kidney, 1 lung), 9 donor tissues (for 10 recipients), and 14 uninvolved recipient tissues (from 12 patients) were examined employing restriction fragment length polymorphism analysis to determine their host or donor origin. The PstI-digested DNAs were analyzed by Southern blot hybridization using two highly informative polymorphic probes that map to chromosome 21 (CRI-PAT-pL427–4) and chromosome 7 (CRI-PAT-pS194). All solid organ PT-LPDs with corresponding uninvolved recipient DNA showed identical hybridization patterns; none of the PT-LPDs exhibited a hybridization pattern that matched donor DNA. These findings suggest that the vast majority of PT-LPDs arising in solid organ transplant recipients, in contrast to those arising in bone marrow transplant recipients, are of recipient origin. (Am J Pathol 1995, 147:1862–1870)

Posttransplantation lymphoproliferative disorders (PT-LPDs) are a complication of immunosuppression associated with organ transplantation. The incidence of these lymphoid proliferations, ~90% of which in both solid organ and bone marrow transplant (BMT) recipients contain Epstein-Barr virus (EBV), varies based on the type of organ or tissue transplanted as well as on the type and degree of immunosuppression employed.[1–12] PT-LPDs occur only rarely in BMT recipients where the reported incidence varies from 0.6 to 1.6%,[5–7] whereas the incidence in solid organ transplant recipients is more variable, ranging from ~1% in renal to nearly 10% in heart-lung transplant recipients.[2,3,13,14] In addition, solid organ transplant recipients who have received cyclosporin A and/or OKT3 as part of their immunosuppression are at higher risk for developing PT-LPDs[11,12] whereas BMT recipients are more likely to develop these lesions if they have received mismatched marrow or undergone T cell depletion in the course of transplantation or, have developed severe graft-*versus*-host disease treated with anti-T cell immunotherapy after transplantation.[10,15–17]

PT-LPDs frequently occur in extranodal sites, including occasionally the transplanted organ, suggesting that passenger donor lymphocytes obtained during transplantation may be related to the development of these lesions.[1,2,6,14,15,17–29] In addition, studies employing restriction fragment length polymorphism (RFLP) analysis have identified donor lymphocytes in solid organ transplant recipients more than 2 months after transplantation.[19] Because PT-LPDs are associated with EBV, it has also been suggested that passenger donor lymphocytes are the source of EBV infection[19] and that these EBV-infected donor

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lymphocytes are allowed to proliferate unchecked because of the lack of sufficient cytotoxic T cell activity in the iatrogenically immunosuppressed transplant recipient.[30–32]

Previous studies have shown that PT-LPDs may be of either donor or recipient origin; however, the majority of the large published studies examining the origin of these lesions have been of PT-LPDs occurring in BMT recipients.[5–8,10,15–17,19–29,33–38] In these patients ~80% of these lymphoid proliferations have been of donor origin.[5–8,10,15–17,27,28,37,38] However, for the most part, there are only scattered case reports showing that approximately equal numbers of PT-LPDs occurring in solid organ transplant recipients are of donor and host origin.[19–26,29,33–36,39] In addition, because of the small number of patients in each of these studies, whether donor or recipient origin correlates with EBV status, type of tissue transplanted, the primary site of PT-LPD (ie, transplanted or native tissue), histological features, the molecular-genetic makeup and/or clinical aggressiveness of the lesions has not been ascertained. The only other relatively large study examining the donor or recipient origin of PT-LPDs in solid organ transplant recipients analyzed a relatively homogenous group of lesions, all of which were classified as high grade lymphomas.[39] Therefore, we studied a heterogeneous group of 18 PT-LPDs, from the three major categories as defined by Knowles et al,[1] occurring in 16 solid organ transplant recipients for evidence of donor or recipient origin at the molecular level. This was accomplished by RFLP analysis by comparing hybridization patterns of donor, uninvolved recipient, and PT-LPD tissue using probes to highly polymorphic regions of human DNA.

Materials and Methods

Patients

The clinical features of the 16 patients in this study are summarized in Table 1. The patients in this study included 1 lung, 2 kidney, and 13 heart transplant recipients who ranged in age from 18 months to 72 years (median of 44 years) at the time of PT-LPD diagnosis. Of the 16 patients, 14 were maintained on triple drug immunosuppressive regimens consisting of cyclosporin A (CSA), azathioprine, and prednisone. One patient had received only azothioprine and prednisone during the 3 years before PT-LPD diagnosis; his immunosuppressive regimen before that time is unknown. One patient was maintained on FK506, azothioprine, and prednisone because of CSA-induced thrombocytopenia. Eight patients had received OKT3 or anti-thymocyte globulin as either part of induction immunosuppression or to treat episodes of rejection. The time from transplantation to the development of PT-LPD ranged from 6 weeks to almost 10 years (116 months) with a median time of 11 months. The clinical course of eight of these patients has been previously reported.[40,41]

Table 1. *Clinical Features of the 16 Patients with PT-LPDs Analyzed Donor* versus *Recipient Origin*

Organ Transplanted	Heart = 13; kidney = 2; lung = 1
Sex	Male = 8; female = 8
Age	Range 1.5 to 72 years; median = 44 years
Immunosuppression	14 cyclosporine A + azothioprine + prednisone 1 cyclosporine A + prednisone 1 FK506 + azothioprine + prednisone 8 OKT3/ATG therapy for induction or rejection Rejections treated with steroids, OKT3 and/or ATG
Time from transplant to PT-LPD diagnosis	Range 1.5 to 240 months; median = 11 months

Specimens

Eighteen PT-LPD specimens were obtained from the 16 solid organ transplant recipients using standard diagnostic procedures during the course of clinical evaluation. These specimens included eight lymph node, four lung, two tonsil/adenoid, two bowel, one soft tissue, and one liver, each containing PT-LPD lesions. Two temporally and anatomically separate PT-LPDs were examined in two individuals. Donor tissue, consisting of cryopreserved spleen cells, was available for examination in 10 cases (nine patients). Peripheral blood (seven specimens), solid tissue (four specimens) or bone marrow aspirate (one specimen) morphologically, immunophenotypically and/or genotypically uninvolved by PT-LPD was available from 12 transplant recipient patients with 14 PT-LPD lesions. In eight instances the uninvolved specimen was obtained within 1 month of PT-LPD diagnosis; in the remaining four cases the specimens were obtained seven to 39 months after PT-LPD diagnosis.

The PT-LPDs were classified on the basis of previously described criteria[1] as either plasmacytic hyperplasia (PH), polymorphic PT-LPD (polymorphic), or malignant lymphoma/multiple myeloma (ML/MM). The polymorphic lesions had been previously classified as either polymorphic B cell hyperplasia (PBCH) or polymorphic B cell lymphoma (PBCL), based on criteria described by Frizzera et al.[42] Each

Table 2. *Patients and Specimens Used to Determine Donor* versus *Recipient Origin of PT-LPDs*

Histological category	No. of patients	No. of PT-LPDs	No. of donor tissues	No. of recipient tissues
PH	5*	5	4	3
Polymorphic	10†	11	5	9
PBCH		4	2	4
PBCL		7	3	5
MM/ML	2‡	2	1	2
Total	16	18	10	14

*Includes one patient who developed a monomorphic PT-LPD.
†Includes one patient with two polymorphic specimens (PBCH and PBCL).
‡Includes one patient with a previous PH specimen.

lesion had been analyzed for clonal immunoglobulin heavy and light chain and T cell receptor β chain gene rearrangements, for the presence and clonality of EBV, and for the presence of structural alterations of various oncogenes and tumor suppressor genes (including H,K,N-*ras*, c-c-*myc*, *bcl*-1, *bcl*-2, and *p53*). The results concerning 12 specimens obtained from 11 patients have been published previously.[1] The histological and molecular genetic criteria defining each category have been previously described.[1,43]

DNA Extraction

Genomic DNA was extracted from fresh or cryopreserved mononuclear cell suspensions or from cryopreserved tissue blocks using a salting-out procedure.[44]

Southern Blot Hybridization Analysis

Five or 10 μg aliquots of genomic DNA were digested with the *Pst*I restriction endonuclease according to the manufacturer's instructions (Boehringer Mannheim, Indianapolis, IN), electrophoresed in 0.8% agarose gels, denatured with alkali, neutralized, and transferred to nitrocellulose filters as previously described by Southern.[45] The filters were hybridized in 50% formamide/3X standard sodium citrate (SSC) at 37°C to probes that had been ^{32}P-labeled by the random primer extension technique.[46] The filters were then washed in 0.2X SSC/0.5% sodium dodecyl sulfate at 60°C for 2 hours and autoradiographed at −70°C for 16 to 72 hours, as previously described.[47]

DNA Probes

The DNAs from the donor tissues, PT-LPD specimens, and uninvolved recipient tissues were studied by hybridization of *Pst*I digested DNAs with individual identification probes, which hybridize to highly polymorphic regions of the human genome. The probes chosen for this study, α-P^{32} CRI-PAT-pL427–4 (heterozygosity of 0.94) and α-P^{32} CRI-PAT-pS194 (heterozygosity of 0.85; Oncor, Gaithersburg, MD), hybridize to highly polymorphic regions of human chromosomes 21 and 7, respectively.[48,49] These commercially available probes, which are used in forensic and paternity DNA fingerprinting applications for identification of an individual, are in combination informative in >99% of cases.

Results

Histopathology

The 18 PT-LPDs examined in this study were classified as follows; PH, five cases; polymorphic PT-LPD, 11 cases (four PBCH and seven PBCL) and ML/MM, two cases (one pleomorphic immunoblastic lymphoma and one MM; Table 2). Two patients had temporally separate PT-LPDs. One adult patient, 2 months after heart transplantation, developed PT-LPD lesions classified as PH, which regressed after a reduction in immunosuppression. This patient developed MM 13 months later. The other patient, also an adult heart transplant recipient, was diagnosed with two separate polymorphic lesions. The first lesion, which arose in the lung, was classified as PBCH at the time of diagnosis; the second lesion, which occurred in the skin 4 months later, was classified as PBCL.

Clonality

Southern blot hybridization analysis for clonal rearrangements of the immunoglobulin heavy chain (IgH) gene had been previously performed in all cases, either as part of published studies (12 specimens, 11 patients) or as part of diagnostic evalua-

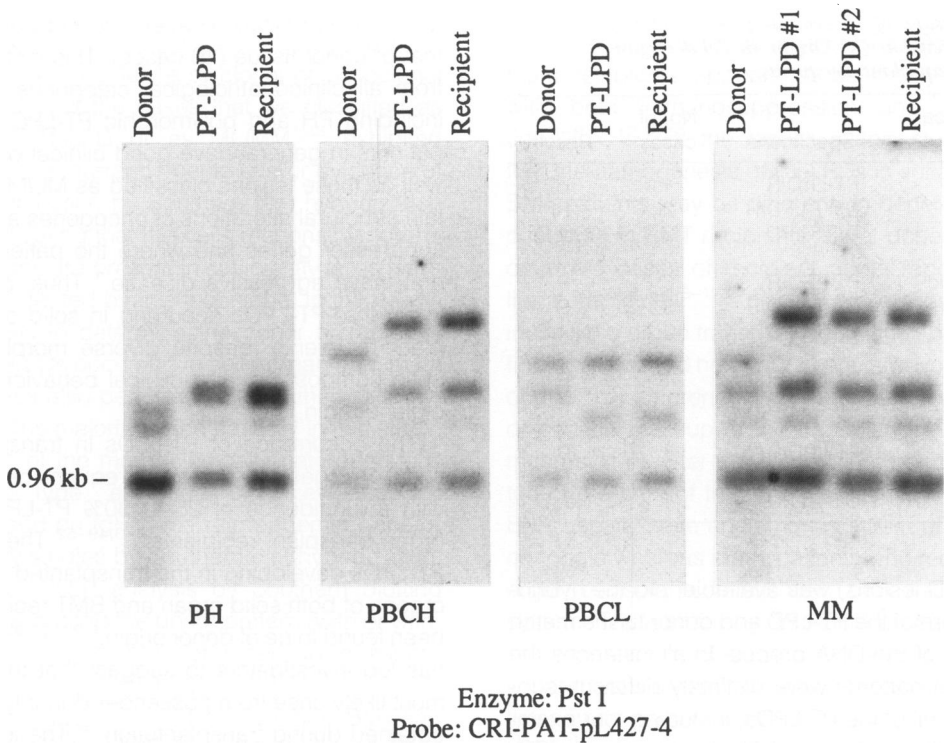

Figure 1. *Representative examples of donor, PT-LPD, and recipient tissues from each of the clinicopathological categories examined with the CRI-PAT-pL427–4 probe after digestion with the restriction endonuclease,* PstI, *to determine donor or recipient origin of PT-LPD lesions. The hybridization patterns of the PT-LPDs and recipient tissues are identical, whereas those of the donor tissues and the PT-LPDs are distinctly different. Identical results were obtained with the CRI-PAT-pS194 probe. (PT-LPD #1, PH; PT-LPD #2, MM).*

tion. Three of the five cases of PH exhibited the germline configuration; the DNA in the remaining two cases of PH was degraded. In contrast, 10 of 11 polymorphic PT-LPDs showed clonal IgH gene rearrangement; the DNA was degraded in the remaining case. Two bands were identified in four specimens and a single rearrangement band was present in six specimens. Both cases of ML/MM exhibited clonal IgH gene rearrangements.

Epstein-Barr Virus

Fifteen of the 18 PT-LPDs exhibited evidence of EBV infection. The EBV infection was polyclonal in four lesions (all PH), oligoclonal in one case (a polymorphic lesion) and monoclonal in 10 cases (eight polymorphic, including the one polymorphic case in which Southern blotting for detection of clonal IgH gene rearrangement was not successful, one ML, and one MM). The three EBV^- cases included a PH in a pediatric heart transplant recipient and two polymorphic lesions in an adult heart transplant recipient and an adult kidney transplant recipient, respectively.

Primary Sites of Disease

The PT-LPD lesions occurred in sites other than the transplanted organ in 17 instances; the PT-LPD occurred in the transplanted lung in one patient.

DNA Hybridization Studies for Donor or Recipient Origin

The number of recipient specimens, donor tissues, and PT-LPD lesions available for study from each histological category is listed in Table 2. In the 14 instances where recipient tissue was available, the hybridization patterns of the PT-LPD and the recipient tissue were identical when employing either the CRI-PAT-pL427–4 or the CRI-PAT-pS194 probe (12 recipient specimens corresponding to 14 PT-LPDs; Figure 1, Table 3). These results include PT-LPD lesions from all three clinicopathological categories including three cases of PH, nine cases of polymorphic PT-LPD, and two cases of ML/MM (one pleomorphic immunoblastic lymphoma and one MM). In contrast, in no instance, in any of the 10 cases where donor tissue (nine donor specimens corresponding

Table 3. *Results of Determining Donor (D)* versus *Recipient (R) Origin in Solid Organ Transplant Recipients*

Histological type	Specimens	No. of cases	Result
PH (5)	PT-LPD/R	1	R
	D/PT-LPD/R	2	R
	D/PT-LPD	2	Not D
Polymorphic PT-LPD (11)	PT-LPD/R	6	R
	D/PT-LPD/R	3	R
	D/PT-LPD	2	Not D
MM/ML PT-LPD (2)	PT-LPD/R	1	R
	D/PT-LPD/R	1	R
Total (18)	PT-LPD/R	8	R
	D/PT-LPD/R	6	R
	D/PT-LPD	4	Not D

to 10 PT-LPD lesions) was available, did the hybridization pattern of the PT-LPD and donor tissue match using either of the DNA probes. In all instances the hybridization patterns were distinctly different, suggesting that all of the PT-LPDs, including four examples of PH, five polymorphic lesions, and one MM, were of recipient origin. Furthermore, clonality based on IgH gene rearrangement analysis did not appear to correlate with donor or recipient origin, because all 12 clonal PT-LPDs (10 polymorphic and two ML/MM lesions) and the three PT-LPDs that exhibited the germline configuration (all PH) were of recipient origin. In addition, all 13 EBV^+ and all three EBV^- PT-LPDs exhibited evidence of recipient origin, suggesting that donor or recipient origin of PT-LPDs in solid organ transplant recipients does not appear to be related to the EBV status of an individual PT-LPD lesion. Furthermore, the one PT-LPD in this study that arose in transplanted tissue appears to be of recipient origin, because the hybridization pattern of the PT-LPD (a lung lesion) was identical to that of uninvolved tissue obtained from the bowel 14 months after this lung transplant recipient's PT-LPD diagnosis.

Discussion

The findings of this study expand previously published results and clearly demonstrate that the vast majority of PT-LPDs occurring in solid organ transplant recipients are of recipient origin. Using molecular analysis, employing probes to highly polymorphic regions of the human genome, all 18 PT-LPDs developing in the 16 solid organ transplant recipients exhibited either a hybridization pattern identical to that of uninvolved recipient tissue (14 cases) and/or a hybridization pattern distinctly different from that of donor tissue (10 cases). This includes cases from all clinicopathological categories of PT-LPD, including PH and polymorphic PT-LPD, where the patients in general have good clinical outcomes as well as those lesions classified as ML/MM that contain structural alterations of oncogenes and/or tumor suppressor genes and where the patients, in general, have aggressive disease.[1] Thus, our findings imply that PT-LPDs occurring in solid organ transplant recipients, despite diverse morphology, genetic composition, and clinical behavior, are of recipient origin.

The occurrence of PT-LPDs in transplanted tissues or organs is not an infrequent phenomenon, with an incidence of 25 to 30% PT-LPDs in solid organ transplant recipients.[1–3,49–52] The majority of PT-LPDs developing in the transplanted tissues and organs of both solid organ and BMT recipients have been found to be of donor origin,[6,20–27,33,34,38] which has led investigators to suggest that these lesions most likely arise from passenger donor lymphocytes obtained during transplantation.[19] The identification of passenger donor lymphocytes within host lymph node tissue[19] also suggests that these lymphoid cells may give rise to PT-LPDs outside the transplanted organ. However, in this study, the one PT-LPD (a polymorphic PT-LPD) that developed primarily in a transplanted organ (lung) was clearly of recipient origin, with both probes exhibiting a hybridization pattern identical to that of the uninvolved recipient small bowel tissue obtained more than a year following the diagnosis of PT-LPD. Thus, our findings indicate that PT-LPDs developing in transplanted tissues are not necessarily of donor origin. However, since *in situ* hybridization studies have documented that infiltrating lymphocytes and macrophages in transplanted tissues are frequently of recipient origin,[53] the lymphoid cell population that developed into the PT-LPD in this lung transplant recipient was most likely obtained in this manner. In addition, because all the other PT-LPDs in the current study are also of recipient origin, it is unlikely that mobile passenger donor lymphocytes migrating from the transplanted organ to host tissues give rise to a significant number of PT-LPDs in the solid organ transplant patient population.

It could be argued that RFLP analysis by Southern blotting may not be sensitive enough to detect a small number of donor-derived PT-LPD cells present within PT-LPD lesions, particularly those that are composed of morphologically heterogeneous cell populations, such as PH and polymorphic PT-LPD. While it is true that many of the cases that we clas-

sified as PH do not exhibit clonal IgH gene rearrangement(s) nor evidence of clonal or oligoclonal EBV infection,[1] all the cases that we classified as polymorphic PT-LPD or as ML/MM[1,43] exhibited clonal IgH gene rearrangements and/or evidence of clonal EBV infection by Southern blot hybridization analysis, indicating that the cells responsible for the PT-LPD lesions are present in sufficiently large numbers to be detected by this technique. In this study, RFLP analysis to determine the donor or recipient origin of PT-LPDs occurring in solid organ transplant recipients was also performed by Southern blot hybridization. The majority of the cases[13] in this study, including 11 of the morphologically heterogeneous polymorphic type, exhibited clear evidence of clonality based on IgH gene rearrangement and/or EBV clonality studies by Southern blot hybridization, implying that RFLP analysis by Southern blotting would have exhibited the donor pattern (with or without the recipient pattern) if donor cells were responsible for the PT-LPD lesions.

That the development of PT-LPDs in both BMT and solid organ transplant recipients is highly associated with EBV has been clearly documented. *In situ* hybridization and immunofluorescence studies have localized EBV viral proteins and transcripts to the PT-LPD tumor cells, and EBV DNA has been identified by polymerase chain reaction and Southern blot hybridization analysis.[1,4–10] In addition, by using a probe to the terminal repeat region, EBV has been found to be clonal in many cases,[1,8,52] implying that the EBV may be directly involved in the pathogenesis of PT-LPDs. In addition, several studies indicate that primary EBV infection or recent reactivation of EBV, as well as an increase in the circulating EBV viral load, often occurs in temporal proximity to the diagnosis of PT-LPD.[2,3,36] The origin of EBV, particularly in those cases of primary infection, is often difficult to identify; however, it has been suggested that passenger donor lymphocytes are the source of EBV[19] and that these EBV-infected donor lymphocytes are allowed to proliferate unchecked because of the lack of sufficient cytotoxic T cell activity in the iatrogenically immunosuppressed transplant recipient.[30–32] However, we found that all PT-LPDs in solid organ transplant recipients, whether EBV^+ or EBV^-, were of recipient origin, indicating that proliferating EBV-infected donor lymphocytes are not necessary for the development of the lesions. Alternatively, passenger donor lymphocytes may be the vehicles of EBV transmission with the virus subsequently infecting host B cells that escape immune surveillance because of a lack of T-cell function in the setting of immunosuppression.[30–32]

Whereas the pathogenesis of PT-LPDs in all transplant recipients appears to be highly associated with both immunosuppression and EBV infection,[1–6,11,12,16,51] the results of this study suggest that the pathogenesis of PT-LPDs in solid organ and BMT patients may be different. In general, PT-LPDs occurring in BMT recipients are of donor origin with only rare cases of recipient origin reported in the literature,[5–8,10,15–17,27,28,37,38] while those developing in solid organ transplant recipients, based on the findings reported here, are nearly always of recipient origin. This difference may be related to the cellular or genetic makeup of the transplant recipient's immune system after transplantation, because, by virtue of the type of transplant, patients who received BMT usually have an immune system of foreign donor origin whereas those patients who received solid organ transplants retain their own native immune system. Thus, either by statistical probability based on the sheer number of donor *versus* recipient B lymphocytes present or by the unique properties of the human immune system the cell of origin of PT-LPDs appears to be different on the basis of the type of transplant. In addition, other factors may influence the development of PT-LPDs in these two transplant populations. Specifically, the incidence of PT-LPD in solid organ transplant recipients appears to be increased in those patients who receive CSA, an "event" following transplantation,[2,11] while in BMT recipients the occurrence of PT-LPDs appears to be more influenced by events occurring at or near the time of transplantation, such as in cases of mismatch or where T-cell depletion has been performed as part of marrow purging.[16,17,28] In addition, episodes of severe graft-*versus*-host disease also appear to correlate with an increased incidence of PT-LPD in BMT recipients,[10,15] whereas episodes of rejection or graft-*versus*-host disease do not appear to correlate at all with the development of these lesions in solid organ transplant recipients.[40] Thus, it appears that the pathogenesis of PT-LPDs in these two broad categories of transplant recipients differs, although how they differ has yet to be elucidated.

In summary, we found that PT-LPDs occurring in solid organ transplant recipients are of recipient origin, regardless of clinicopathological category of PT-LPD or EBV status of the tumor, implying that PT-LPDs of donor origin arising in solid organ transplant recipients are rare. These findings suggest that the pathogenesis of PT-LPDs occurring in solid organ transplant recipients differs from those occurring in BMT recipients, because the vast majority of PT-LPDs occurring in the latter patients are of donor origin.

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