Epstein-Barr Virus (EBV) Induced Polyclonal and Monoclonal B-Cell Lymphoproliferative Diseases Occurring after Renal Transplantation

Clinical, Pathologic, and Virologic Findings and Implications for Therapy

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Nineteen renal allograft recipients developed B-cell lymphoproliferative diseases. Clinically there were two groups: a) young patients (mean age, 23 years) who presented soon (mean, 9 months) after transplantation or antirejection therapy with fever, pharyngitis, and lymphadenopathy resembling infectious mononucleosis, and b) older patients (mean age, 48 years) who presented later (mean, 6 years) after transplantation with localized tumor masses. Histologically, the diseases were classified as polymorphic diffuse B-cell hyperplasia (PDBH) or polymorphic B-cell lymphoma (PBL). Immunologic cell typing revealed either polyclonal or monoclonal B-cell proliferations. Malignant transformation of polyclonal proliferations in two patients was suggested by the finding of clonal cytogenetic abnormalities. Epstein-Barr virus (EBV) specific serology, staining of biopsy specimens for the Epstein-Barr nuclear antigen, and EBV DNA molecular hybridization studies implicated EBV as the cause of both PDBH and PBL. Acyclovir, an antiviral agent that blocks EBV replication *in vitro,* inhibited oropharyngeal shedding of EBV and caused complete remission in four patients with polyclonal B-cell proliferations. The monoclonal tumors were acyclovir resistant. We suggest that surgical treatment, radiotherapy, or chemotherapy may be more appropriate therapy in selected patients with acyclovir resistant tumors. Therapeutic decisions require not only documentation of the viral etiology of these tumors, but also immunologic and cytogenetic analysis to determine the stage of tumor evolution in individual patients.

IN 1969, McKHANN[1] reported an increased age-adjusted incidence of cancer in renal allograft recipients, and Penn[2] independently reported five cases of malignant lymphoma. Since these reports were published, it has been well established that immunosuppression predisposes to the development of *de novo* cancers. The reported incidence of cancer in renal transplant patients is now 6%, 100 times greater than that expected in the general population matched for age.[3] Furthermore, the risk increases with the duration of immunosuppression.[4] Malignant lymphoma, the second most frequent cancer after squamous cell carcinoma of the skin and lips, makes up 20% of the total. The risk of a transplant recipient developing malignant lymphoma is increased as much as 350 times. Because these lymphoproliferative disorders occur infrequently in any one series (incidence 1% to 2%), the systematic investigation of the clinical, histologic, immunologic, and etiologic features, has been delayed.

Studies performed in 19 renal transplant recipients at our institution have now demonstrated that the Epstein-Barr virus causes a spectrum of lymphoproliferative diseases ranging from an infectious mononucleosis-like illness to solid malignant lymphoma. Histologic, immunologic, and cytogenetic findings have been shown to be critical in selecting appropriate therapy for these patients.

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Methods

Patients

From January 7, 1968, through October 1982, 1655 renal transplants were performed on 1593 patients at the

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TABLE 1. *Diagnostic Protocol for Evaluation of Patients with Lymphoproliferative Diseases after Organ Transplantation*

1. Clinical history; physical examination; routine laboratory studies including CBC, peripheral smear, renal and liver function tests; chest x-ray; CT scan of head and abdomen; bone marrow aspirate and bilateral trephine biopsies; serum quantitative immunoglobulins; other diagnostic studies as indicated (including staging laparotomy).
2. Heterophil antibody determination; Epstein-Barr virus serology including anti-VCA (IgG, IgM, IgA), anti-EA (D and R), and anti-EBNA; serial antibody titers including acute and convalescent phase.
3. Throat gargle for umbilical cord lymphocyte transformation (EBV oropharyngeal shedding).
4. Tissue biopsy specimens are studied as follows:
   a) Routine histologic examination and classification.
   b) Electron microscopy.
   c) Immunologic cell typing (Surface and cytoplasmic immunoglobulin (alpha, gamma, mu, delta, heavy and kappa and lambda light chains), HLA-DR, OKT-4, OKT-8, OKT-3, BA-1) on frozen tissue sections and cell suspensions.
   d) Cytogenetic analysis of metaphase preparations.
   e) EBNA staining of touch imprints.
   f) EBV DNA hybridization studies on frozen tissue.

University of Minnesota Hospital. The standard immunosuppressive regimen has been described.[5] Briefly, all but 150 recipients of kidneys from cadaver or HLA mismatched related donors received antilymphoblast globulin (ALG) (30 mg/kg/day) I.V. for 2 weeks, methoprednisolone (20 mg/kg/day) I.V. for 3 days, azathioprine (5 mg/kg/day), tapered to a maintenance dose of 2 to 2.5 mg/kg/day by 1 week, prednisone (2 mg/kg/day), tapered to 0.5 mg/kg by 2 months and 0.25 mg/kg by 1 year, and local graft irradiation (150 rad every other day for 3 doses). Rejection episodes were treated with local irradiation to the graft, an increase in oral prednisone dosage to 2 mg/kg/day, and in some instances administration of methylprednisolone (20 mg/kg) i.v. for 3 days and/or ALG (20 mg/kg/day) I.V. for 10 days. Since 1980, about 150 patients have received cyclosporine and prednisone[6] without ALG. The protocol consists of cyclosporine, 14 mg/kg, immediately prior to transplantation and daily for 1 week after operation, and then $\leq$12 mg/kg/day thereafter with adjustments made depending on renal function and clinical course. In addition, prednisone (2 mg/kg/day) is started on the day of operation and tapered at 0.5 mg/kg/day increments until a maintenance dose of 0.25 mg/kg/day is reached by 4 weeks.

Two patients in this series (Nos. 2 and 3) received total lymphoid irradiation (TLI) prior to a second transplant. Patient No. 2 received 4050 rad over 5 months and patient No. 3 received 3250 rad over 2 months.

During this period, 19 patients developed lymphoproliferative diseases following renal transplantation, an incidence of 1.2%. Six of these patients (Nos. 1, 4, 5, 9, 10, and 16) were studied retrospectively, and the remaining thirteen patients were part of a prospective study. All 19 patients are included in this review. The diagnostic protocol for evaluation of patients with lymphoproliferative diseases after organ transplantation is outlined in Table 1.

Histology

Biopsy and autopsy material were prepared as previously described.[7] Biopsy specimens were fixed in B_5. Sections were stained with hematoxylin-eosin and periodic acid-Schiff. Biopsy specimens were classified according to the morphologic criteria described by Frizzera et al.[7]

Immunologic Cell Typing

Immunologic cell typing was performed on cell suspensions and frozen tissue sections, as previously described.[8] In brief, surface immunoglobulin and cytoplasmic immunoglobulin were studied by direct immunofluorescence in suspensions of viable cells and in frozen tissue sections with fluorescein-conjugated monospecific antibodies against heavy or light chains. Fc receptors were studied with fluoresceinated aggregated human IgG, and complement receptors were studied with bovine erythrocytes sensitized with IgM and mouse complement. Binding of unsensitized sheep erythrocytes was studied in cell suspension only. HLA-DR, BA-1, and T-cell antigens (identified by OKT-4, OKT-8, OKT-11, and TA-1 antibodies) were studied with mouse monoclonal antibodies by indirect immunofluorescence techniques. Cell suspensions and tissue sections were studied with the fluorescent microscope equipped with Ploem epifluorescence and phase contrast, which allows evaluation of the size and morphologic features of positive and negative cells. The cell markers described were present on cytologically malignant cells and could be differentiated from those on normal cells.

Cytogenetics

Fresh tissue was obtained from biopsy specimens and processed within 1 hour of biopsy. Using surgical blades, the tissue was minced finely into an even cell suspension. Metaphase chromosomes were harvested from direct preparations and short-term unstimulated cultures using previously described methods.[9] Chromosomes were G-banded using the Wrights' technique of Sanchez et al.[10] Metaphases were photographed on high contrast S0115 film, and multiple photokaryotypes were constructed in each case. Karyotypes have been designated according to the International System for Human Cytogenetic Nomenclature (ISCN) (1981), and chromosome abnormalities called clonal according to the definition set forth by the Second International Workshop on Chromosomes in Leukemia.[11]

Viral and Serologic Studies

Heterophil agglutinins of the Paul-Bunnell-Davidsohn type were tested for in each patient. EBV antibody titers to the viral capsid antigen (VCA) (IgG and IgM) and to the early antigen (EA) diffuse (D) and restricted (R) were determined by indirect immunofluorescence.[12] Antibody titers to the Epstein-Barr nuclear antigen (EBNA) were measured by the method by Reedman and Klein.[13] Oropharyngeal shedding of EBV was assessed as previously described.[14] Briefly, deep throat gargles with 35 ml of Roswell Park Memorial Institute (RPMI) tissue culture medium 1640 and 10% fetal calf serum were obtained and filtered, and the filtrate was tested for the ability to transform umbilical cord lymphocytes. Each sample was set up in triplicate. All negative results were reported at least once on separate cord blood lymphocytes. All cultures were kept two months before being called negative. Positivity was judged by growth transformation and establishment of permanent cell lines. All cell lines so established were EBNA positive.

The presence of EBV-specific sequences in frozen tumor specimens was demonstrated by cRNA/DNA filter hybridization, vDNA/DNA reassociation kinetic analysis as previously described by Saemundsen et al.[15] and results expressed as genome equivalents per cell. More recently, the Southern blot method[16] has been utilized because smaller amounts of tumor tissue are required. This method uses cellular DNA from frozen biopsy specimens isolated by Protease K digestion of lysed cells followed by deproteinization with chloroform-phenol as described by Collette.[17] Digestion of 10 μg cell DNA with BAM HI (Boerhinger) was done with 30 U of enzyme at 37 C for 6 hours. The fragments were separated by electrophoresis through a 0.8% agarose gel and transferred to nitrous cellulose by modification of Southern's method.[16] The plasmid pDK10 containing the 3kb internal reiterated (IR) BAM HI-V sequence of EBV DNA was kindly supplied by Tim Dambaugh and Elliot Kieff. Plasmid was transferred into *Escherichia coli* LE 392 under NIH P2 biohazard guidelines. Plasmid DNA was purified and the IR segment isolated from plasmid DNA. EBV DNA was labelled with ^{32}P dGTP to a specific activity of 10^8 CPM/μg. Hybridization to patient DNA was carried out under stringent conditions. Autoradiography was done with Kodak XRP5 film using a Pickard Lightning Plus intensifying screen.

Results

Clinical

Nineteen patients, 11 males and 8 females, developed lymphoproliferative diseases following renal allograft transplantation. The clinical data on these patients is tabulated in detail in Tables 2 and 3. The age at the time of diagnosis in these patients ranged from 11 to 68 years, with a mean age of 37 years. Eight patients had received kidneys from living related donors and 11 from cadaver donors. Six patients had received two transplants. The interval from transplantation to diagnosis of the lymphoproliferative disease ranged from 2 weeks to 11 years, with a mean of 3.2 years. Sixteen patients received standard immunosuppression including azathioprine, prednisone, local graft irradiation, and ALG. One patient (No. 2) received total lymphoid irradiation in addition to azathioprine, prednisone, local graft irradiation, and ALG, and an additional patient (No. 3) received azathioprine, prednisone and total lymphoid irradiation. We have had only one patient (No. 6) treated with cyclosporine and prednisone who developed an abnormal lymphoproliferative disease. Only six patients had been treated for prior rejection episodes. These patients had a history of a variety of opportunistic infections, both prior to and concomitant with the lymphoproliferative disorders. The cause of the chronic renal failure was variable.

These 19 patients have been subdivided into two groups based on several distinguishing characteristics illustrated in Table 4.

Group I

Eight of 19 patients (48%) were categorized as having a lymphoproliferative disease resembling an infectious mononucleosis-like illness. Five of the eight patients presented with fever, sore throat, and lymphadenopathy (Nos. 1–3, 6, and 8), while one patient (No. 7) presented with a skin rash and widespread lymphadenopathy. One patient (No. 4) presented with fever, malaise and a rising creatinine and liver function tests initially thought to represent acute rejection and viral hepatitis. One patient (No. 5) presented with fever, malaise, rash, and pulmonary nodules also suggestive of an infectious process.

The patients in this group were usually young, with a mean age at the time of diagnosis of 23 years (range, 11–46 years). The mean interval from transplantation to diagnosis was 9 months (range, 2 weeks to 2.8 years) and was less than 1 year in six of eight patients. Three of the eight patients has received two transplants and five patients had received transplants from cadaver donors. Two patients received total lymphoid irradiation (TLI) prior to a second transplant. Three patients developed the lymphoproliferative disease within six months after therapy for rejection. Concomitant cytomegalovirus and herpes simplex virus infections occurred in five of eight and three of eight patients, respectively.

TABLE 2. *Clinical Features of Renal Transplant Recipients with Lymphoproliferative Diseases**

Patient	Age at Diagnosis of LPD (yr) Sex	Donor Source (Transplant #)	Time Post-transplant	Immunosuppression	Rejection(s) (No.)	Infection		
						Prior to LPD	Concomitant to LPD	Renal Disease
Group I								
1. (J.U.)	19 M	LRD (1)	2 wk	AZ, PR, LGI, ALG	No	Exposed to IM	CMV, HSV	Congenital bladder neck obstruction and hydronephrosis
2. (T.E.)	15 M	CAD (2)	3.5 mos	AZ, PR, LGI, ALG, TLI	Yes (3)	CMV	CMV, HSV	Henoch-Schönlein purpura
3. (J.A.)	15 M	CAD (2)	5 mos	AZ, PR, TLI	No	CMV	CMV	Focal sclerosing GN
4. (L.F.)	13 F	CAD (2)	2.8 yr	AZ, PR, LGI, ALG	Yes (3)	—	—	Lt renal agenesis, rt hydronephrosis
5. (G.L.)	11 F	LRD (1)	12 mos	AZ, PR, LGI, ALG	Yes (2)	—	CMV	Chronic GN
6. (P.S.)	46 M	LRD (1)	16 mos	CsA, PR	No	—	Oral *C. albicans*	Diabetic nephropathy
7. (D.V.)	26 M	CAD (1)	4 wk	AZ, PR, LGI, ALG	No	—	CMV	SLE
8. (H.T.)	41 M	CAD (1)	6 wk	AZ, PR, LGI, ALG	No	HSV	HSV	Polycystic kidneys
Group II								
9. (D.A.)	47 F	CAD (2)	2.3 yr	AZ, PR, LGI, ALG	Yes (3)	Miliary tuberculosis	—	Polycystic kidneys
10. (E.W.)	52 F	CAD (1)	13 mos	AZ, PR, LGI, ALG	No	Varicella zoster	—	Chronic GN
11. (B.A.)	51 F	LRD (1)	4.2 yr	AZ, PR, LGI, ALG	No	—	—	Diabetic nephropathy
12. (D.M.)	22 F	LRD (2)	3.5 yr	AZ, PR, LGI, ALG, CYTX	Yes (2)	Viral hepatitis, *C. Neoformans*	Varicella zoster	Polycystic kidneys
13. (M.R.)	62 F	CAD (1)	5 yr	AZ, PR, LGI, ALG	No	Varicella zoster, *C. neoformans*	—	Polycystic kidneys
14. (M.S.)	68 M	LRD (1)	8 yr	AZ, PR, LGI, ALG	No	Viral hepatitis, Varicella Zoster, Listeria monocytogenes	Salmonella Group B; CMV	Chronic pyelonephritis
15. (C.S.)	60 M	CAD (1)	2.8 yr	AZ, PR, LGI, ALG	Yes (1)	—	*C. albicans*, HSV	Prune belly syndrome
16. (G.B.L.)	28 M	CAD (2)	13 mos	AZ, PR, LGI, ALG	No	—	Pneumocystis carinii	Chronic GN
17. (G.R.)	68 M	LRD (1)	10 yr	AZ, PR, LGI, ALG	No	Viral hepatitis	—	Hypertension
18. (R.B.)	32 M	LRD (1)	11 yr	AZ, PR, LGI, ALG	No	—	—	Chronic GN
19. (J.B.)	36 F	CAD (1)	6 yr	AZ, PR, LGI, ALG	No	—	—	Unknown

* LPD, lymphoproliferative disease; AZ, azathioprine; PR, prednisone, LGI, local graft irradiation; TLI, total lymphoid irradiation; CsA, cyclosporine; CAD, cadaver; LRD, living related donor; GN, glomerulonephritis.

TABLE 3. *Clinical Presentation, Therapy, and Course of Lymphoproliferative Diseases**

	Symptoms/Signs	Treatment	Clinical Course	Outcome (time after diagnosis)	Organ Involvement
Group I					
1. (J.U.)	Fever, sore throat, generalized lymphadenopathy, malaise	AZ DC, PR↓, Chemotherapy	Disseminated LPD, liver failure, acidosis, sepsis	Death (10 days)	Disseminated
2. (T.E.)	Fever, sore throat, cervical lymphadenopathy, ↑ tonsil, night sweats, malaise	AZ DC, PR↓, Acyclovir	Remission ×2 with acyclovir therapy; transition from polyclonal to monoclonal tumor associated with resistance to acyclovir; widespread dissemination of LPD	Death (8 mos)	LN, tonsil, brain, BM, forearm
3. (J.A.)	Fever, sore throat, cervical lymphadenopathy, ↑ parotid	AZ DC, PR↓	Headache, intracerebral hemorrhage, uncal herniation 2° CNS tumor	Death (2 wk)	LN, oropharynx, colon, TK, brain, thyroid, lungs, heart, parotid
4. (L.F.)	Fever, malaise, ↑ creatinine, ↑ liver function tests	AZ DC, PR DC, transplant nephrectomy	Resolution of liver lesion and ↑ LFT (2 mos.)	Alive without recurrence on dialysis (6 yr)	TK, liver
5. (G.L.)	Fever, malaise, rash, lung nodules	AZ CD, PR↓	Complete heart block, respiratory failure, CMV, seizures	Death (2 mos)	LN, liver, BM, TK, brain, heart, lungs
6. (P.S.)	Fever, weight loss, axillary, cervical and inguinal lymph-adenopathy	CsA DC, PR DC Acyclovir	Resolution with acyclovir therapy (3 wk course); no recurrence; accelerated rejection requiring dialysis 3 mos after immunosuppression discontinued; transplant nephrectomy	Alive without recurrence on dialysis (14 mos)	LN, BM
7. (D.V.)	Rash, cervical, inguinal, and axillary lymphadenopathy	AZ DC, PR↓ Acyclovir	Resolution with acyclovir therapy (3 wk course); chronic rejection requiring dialysis 6 mos after ↓ immunosuppression; no recurrence	Alive without recurrence on dialysis (12 mos)	LN
8. (H.T.)	Fever, sore throat, malaise, generalized lymphadenopathy	Acyclovir	Resolution with acyclovir therapy (3 wk course); no recurrence. Normal renal function on immunosuppression	Alive without recurrence (6 mos)	LN
Group II					
9. (D.A.)	Fever, malaise, lung nodules	AZ DC, PR↓	Renal and liver failure; disseminated LPD	Death (3 mos)	LN, TK, lung
10. (E.W.)	Headache	Whole brain irradiation (5300 rads)	Complete response (14 mos); CNS recurrence	Death (15 mos)	Brain
11. (B.A.)	Headache, confusion, blurred vision	Whole brain irradiation (5600 rads)	Complete response (7 mos); CNS recurrence	Death (7 mos)	Brain
12. (D.M.)	Headache, blurred vision, vertigo	Whole brain irradiation (4725 rads), Acyclovir	Unresponsive to therapy, progressive blindness, ↑ intracranial pressure, death	Death (2 mos)	Brain
13. (M.R.)	Incidental hard palate ulcer (varicella zoster, *C. neoformans*)	AZ↓	Resolution over 3 mos, chronic rejection requiring dialysis 12 mos after AZ↓	Alive without recurrence on dialysis (5 yr)	Hard palate
14. (M.S.)	Sore throat, dysphagia, exophytic tumor at base of tongue	AZ DC, PR↓, chemotherapy†	Developed lymph node and liver metastases; progressive liver involvement acidosis, sepsis	Death (15 mos)	LN, oropharynx, liver, BM, TK

TABLE 3. (*Continued*)

	Symptoms/Signs	Treatment	Clinical Course	Outcome (time after diagnosis)	Organ Involvement
15. (C.S.)	Fever, hematochezia, liver scan defect	AZ DC, PR↓, rt hemicolectomy, rt hepatic lobectomy	Progressive liver involvement, acidosis, sepsis	Death (4 mos)	Liver, colon
16. (G.B.L.)	Fever, abdominal pain, hematochezia	Resection of localized small bowel tumor	Died of Pneumocystis carinii pneumonia without evidence of tumor	Death (9 mos)	No residual tumor
17. (G.R.)	Malaise, rt flank pain, palpable right lower quadrant mass	Resection of tumor mass involving transplanted kidney, jejunum, and retroperitoneum	Postoperative small bowel perforation, peritonitis, sepsis, liver failure. No residual tumor at autopsy.	Death (2 mos)	TK, jejunum, retroperitoneum
18. (R.B.)	Fever, weight loss, malaise, sigmoid colon compression by extrinsic mass on BE	Sigmoid colectomy, small bowel resection of tumor arising in mesentery/ retroperitoneum	Progressive sepsis	Death (2 wk)	Retroperitoneum, rt adrenal, liver, lung, BM, brain, colon, small bowel
19. (J.B.)	Exophytic cervical tumor noted on routine exam. Staging laparotomy negative	Intracavitary radiation, chemotherapy‡	Developed widespread metastases unresponsive to chemotherapy. Root of mesentery tumor mass resulting in superior mesenteric artery thrombosis and small bowel infarction	Death (8 mos)	Mesentery, lung, cervix, liver, thyroid, LN, TK

* AZ, azathioprine; PR, prednisone; LPD, lymphoproliferative diseases; CsA, cyclosporine; LN, lymph node; BM, bone marrow; TK, transplanted kidney; DC, discontinued.

† Cyclophosphamide, vincristine sulfate, bleomycin sulfate, adriamycin, prednisone.

‡ Cyclophosphamide, vincristine sulfate, adriamycin, prednisone.

Prior to the introduction of antiviral therapy (see below), the clinical course in these patients was that of a rapidly progressive, widely disseminated lymphoproliferative disease resembling fatal infectious mononucleosis, with a mortality rate of 75%. For example, patient No. 1 died 4 weeks after transplantation of a rapidly progressive disseminated lymphoproliferative process complicated by liver failure and bacterial sepsis. In retrospect, the patient had been exposed to infectious mononucleosis 1 month prior to transplantation. This patient developed heterophil antibodies, and a polyclonal rise in serum immunoglobulins and serial anti-VCA IgM and IgG antibody titers were diagnostic of a primary EBV infection (Table 5). Two patients (Nos.

TABLE 4. *Summary of Patient Groups with Post-transplantation Lymphoproliferative Diseases*

Group I: Infectious Mononucleosis-like Illness (42%)	Group II: Localized Solid Tumor Masses (58%)
Young patients Mean age, 23 ± 13 yr Range, 11 yr to 46 yr	Older patients Mean age, 48 ± 16 yr Range, 22 yr to 68 yr
Short interval from transplantation to diagnosis Mean interval, 9 ± 11 mos Range, 2 wk to 2.8 yr	Long interval from transplantation to diagnosis Mean interval 6 ± 3.9 yr Range 1.1 yr to 11 yr
Symptoms of a viral illness (fever, pharyngitis, lymphadenopathy)	Symptoms related to solid tumor masses (confined to CNS in 27%)
Widespread disease	More localized, primarily extranodal disease
Mortality rate (50%)	Mortality rate (91%)
Short clinical course when fatal Mean survival, 2.7 ± 3.6 mos Range, 10 days to 8 mos	Longer clinical course when fatal Mean survival, 6.6 ± 0.5 mos Range, 2 wk to 15 mos
Responds to Acyclovir	No response to Acyclovir

3 and 5) also had a widespread lymphoproliferative disease and died of CNS involvement 2 weeks and 8 months after initial diagnosis. One patient (No. 4) developed fever, malaise, rising creatinine, and elevated liver function tests after treatment for three rejection episodes in the preceding 8 months. The involved transplanted kidney was removed, immunosuppression discontinued, and biopsy proved lesions (polymorphic B-cell lymphoma) in the liver regressed over the subsequent two months. This patient has no evidence of disease 6 years later on hemodialysis. One patient (No. 5) presented with fever, malaise, an urticarial skin rash, and bilateral nodular lung infiltrates initially believed to be a CMV infection. Signs of a viral myocarditis developed, and she died 2 months after admission with a widely disseminated lymphoproliferative disease. Our four most recent patients (Nos. 2, 6–8) were treated with the synthetic antiviral agent, acyclovir, which has been shown to block EBV replication *in vitro*. Three patients are still alive without evidence of disease (14 months, 12 months, and 6 months follow-up).

The clinical course in the group I patients who died (Nos. 1, 2, 3, and 5) was short, the mean interval from diagnosis to death being 2.7 ± 3.6 months (range, 10 days to 8 months). The lymphoproliferative disease was widespread in all patients and most frequently involved lymph nodes (seven patients), transplanted kidney, bone marrow, brain (each in four patients), liver, lung, heart (each in three patients), thyroid, parotid, tonsil, soft tissue, oropharynx, colon (each in two patients), and skin (one patient).

Group II

Eleven of 19 patients (52%) presented with localized solid tumor masses. In contrast to group I, group II patients never had typical symptoms or signs of infection. Three patients presented with headache and were found to have primary CNS lymphoma. Other patients presented with symptoms related to solid tumors involving the lung (No. 9), oropharynx (Nos. 13, 14), small or large bowel (Nos. 15–18), or cervix (No. 19).

The patients in this group were older (mean age at diagnosis, 48 years; range, 22–68 years) compared to group I. The mean interval from transplantation to diagnosis was longer (mean, 6 years; range, 1.1–11 years) and was greater than 2 years in 9 of 11 patients. Three of the 11 patients had received two transplants and six patients had received transplants from cadaver donors. All of these patients had received standard immunosuppressive therapy including azathioprine, prednisone, local graft irradiation, and ALG. One patient had been treated for rejection 7 months before the onset of disease.

TABLE 5. *EBV-Specific Antibodies in Renal Transplant Recipients with Lymphoproliferative Diseases*

Patient	Anti-VCA IgG	Anti-VCA IgM	Anti-EA D	Anti-EA R	Anti-EBNA	Interpretation of Serologic Results
Group I						
1. (J.U.)	<10	<10	<10	<10	<2	Primary EBV infection
	80	80	<10	<10	<2	
2. (T.E.)	40	<2	<5	<5	5	Reactivation EBV infection
	320	<2	40	40	<2	
3. (J.A.)	10	<2	<5	—	20	Long-past EBV infection
	<5	<2	<5	—	<2	
4. (L.F.)	320	<10	5	—	20	Long-past EBV infection
	160	<10	<10	<10	20	
5. (G.L.)	—	—	—	—	—	—
6. (P.S.)	<10	<10	<10	<10	<2	Primary EBV infection
	2560	80	160	—	<2	
7. (D.V.)	640	—	<10	20	80	Reactivation EBV infection
	2560	—	<10	160	80	
8. (H.T.)	160	<10	<10	10	40	Reactivation EBV infection
	640	<10	<10	10	40	
Group II						
9. (D.A.)	640	<10	5	—	<2	?Long-past EBV infection
	160	<10	5	—	10	
10. (E.W.)	—	—	—	—	—	—
11. (B.A.)	2560	<2	80	80	10	?Reactivation EBV infection
	5120	<2	80	160	10	
12. (D.M.)	80	—	<10	10	<10	Reactivation EBV infection
	320	—	<10	20	<10	
13. (M.R.)	320	—	320	—	320	Reactivation EBV infection
	2560	—	—	—	—	
14. (M.S.)	160	—	40	—	80	Reactivation EBV infection
	5120	—	<5	—	160	
15. (C.S.)	640	<2	10	—	40	Long-past EBV infection
	640	<2	5	—	40	
16. (G.B.L.)	—	—	—	—	—	—
17. (G.R.)	640	—	<10	320	160	?Long-past EBV infection
18. (R.B.)	—	—	—	—	—	—
19. (J.B.)	640	—	<10	<10	20	?Reactivation EBV infection
	1280	—	<10	40	40	

* VCA, viral capsid antigen; EA, early antigen; D, diffuse, R, restricted; EBNA, Epstein-Barr nuclear antigen; —, study not performed.

These patients had a spectrum of opportunistic infections prior to the onset of the lymphoproliferative disease, but concomitant CMV or HSV infections were each seen in only one patient.

The clinical course in these patients was that of an aggressive, lethal lymphoproliferative disease in ten of 11 patients. Three patients (Nos. 10–12) had disease confined to the brain. Two patients (Nos. 10 and 11)

TABLE 6. *Morphologic Features of Lymphoproliferative Diseases**

Classification	Follicular Center Cells	Plasmacytic Differentiation	Large Lymphoid Cells	Atypical Immunoblasts	Invasiveness	Necrosis
"Nonspecific" reactive lymphoid hyperplasia	++ (GC)†	++	+/++	−	−	−
Polymorphic diffuse B-cell hyperplasia (PDBH)	++ (D)	++	++/+++	−	+	−
Polymorphic diffuse B-cell lymphoma (PBL)	++ (D)	+	++/+++	+/+++	+	+++
Immunoblastic B-cell sarcoma (IS)	—	+	+	+/+++	+	+

* Adapted from Frizzera, et al.[7]

† GC, germinal centers; D, diffuse.

initially responded to irradiation therapy, but eventually died 15 and 7 months later of CNS recurrence. One patient (No. 12) did not respond to a combination of irradiation and acyclovir and died of progressive CNS disease. One patient (No. 9), admitted with fever and lung nodules, developed progressive renal and hepatic dysfunction and died 3 months after admission with kidney, lung, and lymph node involvement. One patient (No. 14) had had a tumor at the base of the tongue excised, developed a solitary cervical lymph node metastasis 6 months later, and died 15 months after initial diagnosis of progressive liver involvement in spite of chemotherapy and reduced immunosuppression. At autopsy, massive tumor nodules were found in both hepatic lobes. One patient (No. 15) with a liver mass eroding into the colon died 6 weeks after a hepatic resection with tumor involvement of the remaining lobe. A small bowel tumor was resected in one patient (No. 16) who died 9 months later of Pneumocystis carinii pneumonia without evidence of tumor. Two patients (Nos. 17 and 18) with retroperitoneal and bowel involvement died after operation with progressive sepsis. One patient (No. 19) with initially localized cervical lymphoma treated by intracavitary radiation, later developed widespread metastases that were unresponsive to chemotherapy. This patient died of massive small bowel infarction secondary to occlusion of the superior mesenteric artery by a tumor mass infiltrating the root of the mesentery.

The clinical course in the ten patients who died was slightly longer than in group I with a mean survival of 6.6 ± 0.5 months (range, 2 weeks to 15 months). The disease was usually initially localized and extranodal most frequently involving the brain (four patients), liver transplanted kidney (each in four patients), lymph node, lung (each in three patients), bone marrow, colon, oropharynx (each in two patients), and the retroperitoneum, small bowel, adrenal, thyroid and cervix (each in one patient).

Histology

The histologic classification of these lymphoproliferative diseases has now been standardized by Frizzera et al.[7] Table 6 lists their morphologic features as compared to those of related lymphoid processes from which they can now be differentiated. The lymphoid proliferations in all 19 patients had morphologic features of B-cell proliferations and were classified as polymorphic diffuse B-cell hyperplasia (PDBH) (five patients) or polymorphic B-cell lymphoma (PBL) (14 patients). One patient (No. 14) initially had PDBH, but a subsequent metastatic lesion had histologic features of PBL. One patient (No. 2) initially had PBL, but later developed metastatic lesions most consistent with immunoblastic B-cell sarcoma (IS). PDBH is characterized by a polymorphic diffuse, invasive, B-cell proliferation involving follicular center cells (small cleaved and large noncleaved) and "post-follicular" lymphocytes with varying degrees of plasmacytic differentiation (lymphoplasmacytoid lymphocytes, plasma cells, and immunoblasts). The large cells do not show nuclear atypia. PBL is distinguished from PDBH primarily by the presence of large immunoblasts with marked nuclear atypia and extensive necrosis. The presence of follicular center cells with diffuse distribution differentiates PDBH and PBL from other reactive lymphoid hyperplasias and from immunoblastic B-cell sarcoma.

Immunologic Cell Typing and Cytogenetic Studies

Immunologic cell typing results in eight patients (Table 7) demonstrated that these lymphoproliferations may be either polyclonal or monoclonal B-cell, and they may evolve from polyclonal to monoclonal proliferations in association with the appearance of cytogenetic abnormalities. A proportion of the malignant cells from the biopsy samples in seven patients (Nos. 1, 2, 6–8, 13, and 14) contained surface immunoglobulin or cytoplasmic immunoglobulin (or both) of the IgM, IgG, or IgA class and the kappa or lambda specificity, *i.e.*, polyclonal. In one patient (No. 2), a biopsy taken 6 months after the initial polyclonal diagnosis demonstrated the presence of only IgG-kappa surface immunoglobulin and cytoplasmic immunoglobulin on malignant cells, *i.e.*, monotypic (monoclonal). We concluded at this point that the

TABLE 7. *Histologic Classification, Immunologic Cell Typing of Cytologically Malignant Cells, and Cytogenetic Abnormalities*

Patient	Biopsy	Diagnosis*	Immunologic Cell Typing Surface/ Cytoplasmic Ig†	Cytogenetic Analysis: No. of Metaphases Total	Normal	Abnormal	Karyotypes
1. (J.U.)	Lymph node	PDBH	Polyclonal B-cell		—		
2. (T.E.)	Lymph node‡	PBL	Polyclonal B-cell		—		
	Lymph node	PBL	Polyclonal B-cell		—		
	Lymph node	IS	Monoclonal (IgG-kappa)		—		
3. (J.A.)	Brain	PBL	—		—		
4. (L.F.)	Kidney	PBL	—		—		
5. (G.L.)	Lung	PBL	—		—		
6. (P.S.)	Lymph node	PBL	Polyclonal B-cell	18	14	4	46, XY/47, XY, +3
7. (D.V.)	Lymph node	PDBH	Polyclonal B-cell		—		
8. (H.T.)	Lymph node	PDBH	Polyclonal B-cell	20	20	0	46, XY
9. (D.A.)	Lung	PBL	—		—		
10. (E.W.)	Brain	PBL	—		—		
11. (B.A.)	Brain	PBL	—		—		
12. (D.M.)	Brain	PBL	—		—		
13. (M.R.)	Hard palate	PDBH	Polyclonal B-cell		—		—
14. (M.S.)	Oropharynx	PDBH	—				
	Lymph node	PBL	Polyclonal B-cell	12	5	7	46, XY/47, XY, +14
	Liver	PBL	Polyclonal B-cell		—		
15. (C.S.)	Liver	PBL	—		—		
16. (G.B.L.)	Small bowel	PBL	—		—		
17. (G.R.)	Abdominal mass	PBL	—		—		
18. (R.B.)	Abdominal mass	PBL	—		—		
19. (J.B.)	Cervix	PBL	Monoclonal IgG-kappa	6	0	5	46, XX, ?t(2; 19)(p23; p13)
						1	47, XX, +X, ?t(2; 19)(p23; p13)
	Soft tissue§	PBL	Monoclonal IgG-kappa	15	10	5	46, XX/46, XX, ?t(2; 19) (p23; p13), t(6; 16) (q27; q13)

* PDBH, polymorphic diffuse B-cell hyperplasia; PBL, polymorphic B-cell lymphoma; IS, immunoblastic B-cell sarcoma; Ig, immunoglobulin; — denotes studies not performed or unsatisfactory for interpretation. In some cases, there were insufficient numbers of viable cells for immunologic cell typing and cytogenetic analysis.

† Polyclonal refers to the presence of cytologically malignant cells with polytypic surface and/or cytoplasmic immunoglobulin, *i.e.*, IgG, IgM, or IgA (kappa or lambda). Specimens were all positive for IgM, IgG, IgA. Patient 2 (T.E.) and 19 (J.B.) had monoclonal B-cell proliferations as defined by the presence of only gamma-kappa surface and/or cytoplasmic immunoglobulin.

‡ Biopsies obtained at 16 wks, 31 wks, and 39 wks after transplantation.

§ Biopsy specimen obtained 4 mos after initial diagnosis of cervical PBL.

polyclonal B-cell proliferation had been replaced by a monotypic (monoclonal) proliferation that is characteristic of other B-cell lymphomas. One additional patient (No. 19) presented with a cervical polymorphic B-cell lymphoma which later metastasized to soft tissue and contained only IgG-Kappa surface and cytoplasmic immunoglobulin on the malignant cells.

Clonal cytogenetic abnormalities were present in two patients (Nos. 6 and 14) that had polyclonal B-cell proliferations according to immunologic cell typing data. This suggests that although the B-cell proliferation was polyclonal in origin, a small clone of cytogenetically abnormal cells had developed within the proliferation. This may be associated with enhanced malignant growth potential. In a third patient (No. 19), there was clonal evolution from a t(2;19) translocation in the initial biopsy to the appearance of a second translocation t(6;16) in the metastatic lesion 4 months later. The proliferations in these three patients were all classified as PBL. In the one patient with PDBH in whom adequate cytogenetic data were available (No. 8), no chromosomal abnormalities were found.

Virologic Studies

Heterophil agglutinins of the Paul-Bunnell-Davidsohn type were detected in only one patient (No. 1) (1:1792 after guinea pig absorption). This patient had no prior antibodies against EBV and developed serologic evidence of a primary EBV infection with a rise in the anti-VCA IgG and IgM titers (Table 5). One additional patient (No. 6) also demonstrated serologic evidence of a primary EBV

TABLE 8. *Detection of EBV-Specific Sequences in Renal Transplant Recipients with Lymphoproliferative Diseases*

Patient	Biopsy Specimen	No. of EBV Genome equivalents/cell: cRNA/DNA filter Hybridization	No. of EBV Genome equivalents/cell: vDNA/DNA Reassociation	Southern Blot
2. (T.E.)	Lymph node	—*	3	—
3. (J.A.)	Brain	9	—	—
	Parotid	9	14	—
	Heart	4	—	—
6. (P.S.)	Lymph node	—	—	+
7. (D.V.)	Lymph node	—	—	Neg.
8. (H.T.)	Lymph node	—	—	+
12. (D.M.)	Brain	—	—	Neg.
13. (M.R.)	Palate	8	—	—
14. (M.S.)	Lymph node	<1	2	—
	Liver	13	7	—
15. (C.S.)	Liver	3	5	—
17. (G.R.)	Kidney	—	—	+
18. (R.B.)	Colon	—	—	+
19. (J.B.)	Cervix	—	—	+

* —Denotes study not performed.

infection. This patient did not have detectable heterophil agglutinins in the serum at any time. Six patients had evidence of a reactivation EBV infection with significant rises in the anti-VCA IgG titers. Anti-VCA IgM was not measured in 4 patients, but the presence of anti-EBNA excluded a primary infection. Rises in the anti-VCA IgG titers were suggestive, but not diagnostic, of reactivation of EBV infection in two patients (Nos. 11 and 19). An additional five patients had serologic evidence of prior exposure to EBV without evidence of an active infection. These patients often had persistently elevated anti-VCA IgG titers.

EBV cRNA/DNA filter hybridization, vDNA/DNA reassociation kinetic analysis, and the Southern blot test were used to detect the presence of EBV-specific DNA sequences in biopsy specimens from 12 of the 19 patients (Table 8). In all patients except Nos. 7 and 12, EBV specific DNA sequences were present, as determined by one or more of these methods. Touch imprints of the lymph-node biopsy specimen obtained 2 months apart in patient No. 2 were stained for EBNA with the anti-complement immunofluorescence technique.[13] Over 80% of the large atypical cells were strongly EBNA-positive.[14] Oropharyngeal shedding of EBV was demonstrable in four (Nos. 2, 7, 14, and 19) of five patients tested (patient No. 6 was negative).

Therapy

The mortality rate of patients in group II is 91% and emphasizes the lack of effective therapy in these patients. Three patients (Nos. 10, 11, and 12) with primary lymphoma confined to the brain received whole brain irradiation. Short-term palliation was achieved, but there were no long-term survivors. Localized gastrointestinal lymphomas may be excised successfully, as demonstrated in one patient (No. 16) and in the series by Calne et al.[18] Other therapeutic approaches, including surgery, radiation, chemotherapy and discontinuation of immunosuppression, have met with little success. Only two patients (Nos. 4 and 13) in our early series, prior to beginning acyclovir therapy, had survived, and tumor regression in both was associated with allograft loss and discontinuation of immunosuppression.

In group I, three of our first four patients died of a rapidly progressive disseminated lymphoproliferative process in spite of reduction in immunosuppression and, in one case (No. 1), chemotherapy. The four most recent patients who presented with EBV-induced polyclonal B-cell proliferations (Nos. 2, 6, 7, and 8) have been treated with intravenous acyclovir (500 mg per square meter every 8 hours for 3 weeks). The first patient has previously been described in detail.[19] A dramatic resolution of fever and symptoms occurred concomitantly with acyclovir therapy, and there was objective regression of the lymphoproliferative process involving lymph nodes, tonsils, and bone marrow. In addition, acyclovir suppressed oropharyngeal shedding of EBV. Unfortunately, treatment for an episode of rejection 2 months after the initial course of acyclovir was associated with recurrence of the lymphoproliferative disease 1 month later. A second and third clinical and objective response of the tumor to acyclovir provided strong evidence of the therapeutic efficacy during the polyclonal growth phase of the tumor, especially when combined with a reduction in immunosuppressive agents. Acyclovir was not associated with resolution of lymphoproliferation during the last recurrence, and aggressive tumor growth continued. Simultaneously, there was a histologic change of the lesion from a polymorphic B-cell lymphoma to a classic B-cell immunoblastic sarcoma. In addition, the tumor evolved from a polyclonal to a monoclonal B-cell proliferation that expressed only IgG kappa.

We have subsequently administered acyclovir to three patients with either polymorphic diffuse B-cell hyperplasia (Nos. 7 and 8) or polymorphic B-cell lymphoma (No. 6), all of which were polyclonal B-cell proliferations. We have successfully induced remission in all of these patients and they remain alive and well 14 months, 12 months, and 6 months after therapy. An additional patient (No. 19) with a monoclonal B-cell proliferation did not respond to acyclovir.[20]

Discussion

The data in these 19 patients demonstrate that a spectrum of lymphoproliferative diseases exists after renal transplantation, ranging from an infectious mononucleosis-like polyclonal B-cell proliferation to a monoclonal B-cell lymphoma.[7,14,19–22] In one group, young patients presented soon after transplantation or antirejection therapy with an infectious mononucleosis-like illness characterized by fever, pharyngitis, and lymphadenopathy. The clinical course in the untreated patients was that of a disseminated, rapidly progressive, and lethal lymphoproliferative disease. In the patients with adequate immunologic cell typing, a polyclonal B-cell proliferation was demonstrated. Patients in this group, however, may be subdivided into those with a) polymorphic B-cell hyperplasia, in which no cytogenetic abnormalities were detected, or b) polymorphic B-cell lymphoma in which the identification of clonal cytogenetic abnormalities in the polyclonal proliferation is consistent with malignant transformation. PDBH, therefore, appears to represent a benign EBV-induced polyclonal B-cell proliferation without the usual characteristics of malignant transformation (morphologic criteria, monoclonality, cytogenetic abnormalities), but which can be fatal in a setting of impaired host immunity. At some point, an unknown event occurs that results in malignant transformation and the emergence of a monoclonal B-cell lymphoma with enhanced growth potential. We have evidence that concomitant with this event, there is a transition of the histology from PDBH to PBL associated with the development of clonal cytogenetic abnormalities. The simultaneous presence of a polyclonal B-cell proliferation and clonal cytogenetic abnormalities that were present in all patients with PBL suggests an evolutionary phase in which a subpopulation of cells have undergone malignant transformation. There is experimental evidence that cytogenetic abnormalities in EBV-transformed cells are associated with altered cellular growth patterns. Human diploid lymphoblastoid cell lines, transformed by EBV, will grow in brains of nude mice but do not survive if injected subcutaneously.[23] In contrast, aneuploid Burkitt's lymphoma cell lines will grow both subcutaneously and intracerebrally in nude mice. The final stage is the monoclonal PBL with clonal cytogenetic abnormalities. These tumors may occur in older patients who present later after transplantation with localized tumor masses.

EBV has been implicated as the cause of these lymphoproliferative diseases on the basis of serologic evidence, EBNA staining, and molecular hybridization, as we have reported previously.[14,19–22] Findings in an additional seven patients that were included in this review support our previous conclusions. Eight of 14 patients with serial measurements of EBV-specific antibodies sufficient for interpretation has serologic evidence of a primary (two) or reactivation (six) EBV infection temporally related to the diagnosis of the lymphoproliferative disease. The absence of increasing antibody to EBV in the other patients may reflect impaired humoral immunity. Some individuals with inherited immune deficiency, for example, have impaired antibody responses to EBV.[12] Antibody responses, however, were not prognostically valuable in our patients.

Three techniques, cRNA/DNA filter hybridization, vDNA/DNA reassociation analysis, and the Southern blot test, have been utilized to identify EBV-specific DNA sequences in tumor biopsy specimens. Similar studies have provided strong evidence for the role of EBV in the pathogenesis of Burkitt's lymphoma and nasopharyngeal carcinoma[24,25] and, more recently, in the lymphoproliferative diseases seen in patients with ataxia telangiectasia and the X-linked lymphoproliferative syndrome.[15] Since EBV causes a polyclonal activation of B cells bearing EBV receptors[26] *in vitro*[27] and *in vivo*,[28] the combined immunologic and virologic evidence strongly implicates EBV as the causative agents in these patients.

Therapeutic approaches in the past, including chemotherapy, have met with little success. Only two patients in our earlier review have survived, and tumor regression in both was associated with allograft loss and discontinuation of immunosuppression. Similar management in other patients has been unsuccessful. Since most patients had EBV-carrying polyclonal B-cell proliferations, it seemed reasonable to believe that antiviral therapy might be effective in these diseases. Acyclovir (9-[(2-hydroxyethoxy)-methyl] guanine) is a new synthetic antiviral agent that blocks replication of EBV DNA by inhibiting the EBV-associated DNA polymerase.[29,30] Acyclovir inhibits EBV DNA replication only in virus-producing cell lines such of P3HR-1, resulting in a reduction in the viral capsid antigen-positive cells and a decrease in the number of viral genomes per cell. Acyclovir does not alter the synthesis of the latent EBV genome in lymphoblastoid cell lines.

We have demonstrated that acyclovir inhibited oropharyngeal shedding of EBV and successfully induced remission in four patients with polyclonal B-cell proliferations. Acyclovir was ineffective, however, in two patients with EBV-associated monoclonal B-cell proliferations. We hypothesize that acyclovir was effective during the polyclonal growth phase because it interrupted the lytic cycle of EBV replication, which was responsible

for the polyclonal B-cell proliferation. Acyclovir was ineffective once the tumor was monoclonal and presumably made up of latently infected (and, therefore, acyclovir resistant) malignantly transformed B cells. The evidence is consistent with the idea that the polyclonal B-cell proliferation is dependent on viral replication for the continued infection, transformed, and proliferation of additional B lymphocytes. Once malignant transformation has occurred, however, the proliferation is autonomous and no longer dependent on viral replication.

Our present therapeutic strategy based on these data is as follows. 1) Patients who have an infectious mononucleosis-like illness and an EBV-associated polyclonal PDBH without cytogenetic abnormalities are treated with acyclovir alone. The dosages of immunosuppressive drugs may be reduced, depending on the clinical severity of the disease and response to therapy. 2) Patients who have polyclonal PBL with cytogenetic abnormalities consistent with malignant transformation are also treated with acyclovir. Immunosuppression is also reduced or discontinued and transplant nephrectomy performed if allograft rejection results. Patients are followed closely and serial biopsies are taken to document transition to a monoclonal B-cell proliferation. 3) Patients with monoclonal B-cell tumors are treated with conventional radiotherapy or chemotherapy. Immunosuppression is discontinued and transplant nephrectomy carried out if rejection occurs.

It is obvious that the data presented require confirmation by ourselves and others in larger numbers of patients. The results discussed provide a framework for further work.

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Discussion

Dr. Richard E. Wilson (Boston, Massachusetts): Dr. Hanto and his colleagues at Minnesota have clearly defined these two categories of patients; (1.) the extremely young recipient, who develops a polyclonal disease, almost certainly an EB virus-induced lesion, with extremely short survival, but for whom Acyclovir may be curative, and (2.) the older patient, who has genetically transformed cells with a monoclonal B-cell lymphoma that occurs long after transplantation, is very lethal, and does not respond to Acyclovir.

We all agree that the specter of malignancy induction in transplant recipients has haunted the transplant surgeon for many years. We showed that the patient with a functioning transplant had a seven times greater risk of dying of malignancy than those patients who had rejected their kidneys. The extremely high risk for lymphoma of B-cell origin with predilection for the CNS system has been well documented in the past, and the recent hue and cry in the press about the AIDS problem, with similar relationships to promotion and induction of lymphoid malignancy by viral mechanism, possibly CMV and a leukogenic virus, has made it necessary for all of us to understand this problem and the concepts.

I have always felt that the most rapidly proliferating cell population in the immunosuppressed patient is the lymphoid system. With the highest likelihood for mutagenesis and a less effective immune surveillance system, presumably a T-cell function, lymphoma and leukemia induction would be the greatest risk. The CNS, being naturally devoid of lymphoid cells, might be the site where these transformed cells tend to lodge and grow.

The EB virus, as Dr. Hanto points out, drives the B-cell population even harder, making malignant tumor induction even more prevalent, like in the graft v host animals that Dr. Robert Schwartz described many years ago. The use of modern technology to reveal the causative agent, and the ability to block the action with Acyclovir is exciting, but the major question remains: Why only 19 out of over 1500 patients in their series developed these malignancies? How many of the total population were shedding EB virus, and did not get malignancy? What about these patients was different? We have no evidence of pre-existing cell transformation in the patients who developed malignancy. We must assume, however, that there was a latent effect of the EB virus in the delayed monoclonal group; yet there was no apparent clue that these patients were going to develop the B-cell lymphoma 6 years later.

I would like to ask Dr. Hanto his thoughts about special studies that might be used in this extremely high-risk group of patients to predict which of those patients who are shedding the virus will develop lymphoma. Is another agent required? Is EB virus just a promoter, and is there another virus that is an inducer? Or is it primarily related to the intensity of the immune suppressive therapy? We do know that at high therapeutic drug levels, when they were rejecting or getting their initial transplant—this is when the surveillance system seemed to break down even more.

Professor Peter J. Morris (Oxford, England): This study has clearly distinguished two types of lymphoma occurring after transplantation. I wanted to ask two questions which relate to cyclosporin A in particular, for this drug is going to be widely used in the next few years in transplant units, and it is expected to be available generally in the USA later this year.

Theoretically, there is a reason that we might expect more lymphomas in patients treated with cyclosporin A, particularly of the lymphoid type that Dr. Hanto described, namely the one that occurs in the young patient, and is really quite aggressive. The reason for this is that, at least *in vitro*, cyclosporin A does prevent the generation of killer T-cells, with specificity for the EBV infected lymphoid cells. It is these cells which provide protection against the on-going proliferation of the EBV infected cells. Furthermore, in the absence of the killer cells, cyclosporin A allows the generation of suppressor cells and, hence, the uninhibited proliferation of the infected cells, giving rise to a polyclonal lymphoma. This is only an *in vitro* experiment, but it does provide a theoretical basis for what is possibly an increased risk of this lymphoproliferative type of lymphoma in the young patient treated with cyclosporin A.

Now, if this is so, then I'd like to ask the Minneapolis group two questions. First, would it be possible to follow these patients and their seriological conversion status, so that when they become seroconverted (presuming they are seronegative to start with) they could be treated prophylactically with Acyclovir? Or, secondly, is there a place for vaccination of the seronegative recipients before they receive a transplant?

Dr. Douglas W. Hanto (Closing discussion): First, before answering these questions, I would re-emphasize the three groups of patients which are important from the standpoint of determining appropriate therapy. A spectrum of disease exists, but the evidence implicates EBV as the cause in each group. First, patients may develop an EBV-induced polyclonal B-cell proliferation, classified as PDBH, and which contains no cytogenetic abnormalities. These patients usually (not always) are young, develop the disease soon after transplantation, and present with a disease resembling infectious mononucleosus. These patients respond to acyclovir, but, depending on the severity of the disease, may require a reduction in immunosuppression as well.

Second, patients may develop an EBV-induced B-cell proliferation, which morphologically appears malignant and is classified as PBL, but is polyclonal, as in the first group. However, clonal cytogenetic abnormalities are present in some cells and suggest early malignant transformation. The early stage of this disease also resembles infectious mononucleosus. The polyclonal component of this disease can be treated using acyclovir. A reduction or discontinuation of immunosuppression and transplant nephrectomy is probably required, since these tumors may evolve into monoclonal, acyclovir-resistant tumors with enhanced malignant growth potential. This occurred in one patient.

The third group is made up of patients with EBV-related monoclonal B-cell proliferations, classified as PBL or IS. These tumors are resistant to acyclovir and require standard surgical therapy, radiotherapy, and/or chemotherapy. Immunosuppression is discontinued, and transplant nephrectomy is performed if rejection occurs.

Dr. Wilson, we cannot at this time explain the fact that in a transplant population in which over 75% of patients shed EBV only a small percentage go on to develop EBV-induced lymphoproliferative diseases. There are, of course, probably many factors which lead to reactivation of EBV and other viruses after transplantation. There is increasing evidence that EBV-specific immune responses are impaired in renal transplant patients. These include humoral, T-cell, and NK cell responses. It would seem logical to assume that the degree of impairment of these responses varies among patients. Why then some patients would have