0041-1337/88/4504-0719$02.00/0
TRANSPLANTATION

#811 CV 719-727

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Vol. 45, 719–727, No. 4, April 1988
Printed in U.S.A.

THE FREQUENCY OF EPSTEIN-BARR VIRUS INFECTION AND ASSOCIATED LYMPHOPROLIFERATIVE SYNDROME AFTER TRANSPLANTATION AND ITS MANIFESTATIONS IN CHILDREN[1]

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Twenty cases of Epstein-Barr virus (EBV)-associated lymphoproliferative syndrome (LPS), defined by the presence of EBV nuclear antigen and/or EBV DNA in tissues, were diagnosed in 1467 transplant recipients in Pittsburgh from 1981–1985. The frequency of occurrence in pediatric transplant recipients was 4% (10/253), while in adults it was 0.8% (10/1214) ($P < .0005$). The frequency of LPS in adults declined after 1983 coincidental with the introduction of cyclosporine monitoring. However there was no apparent decline of LPS in children. We describe these ten pediatric cases and one additional case of LPS in a child who received her transplant before 1981.

The frequency of EBV infection in 92 pediatric liver recipients was 63%. Of these subjects, 49% were seronegative and 77% of those acquired primary infection. Of 11 cases of pediatric EBV-associated LPS, 10 were in children who had primary infection shortly before or after transplantation. These results reinforce the importance of primary EBV infection in producing LPS, which was previously shown in adults. Children are at greater risk because they are more likely to be seronegative for EBV and to acquire primary infection.

Three clinical types of LPS were recognized in children. The first (5 cases) was a self-limited mononucleosislike syndrome. The second syndrome (4 cases) began similarly, but then progressed over the next two months to widespread lymphoproliferation in internal organs and death. The third type (2 cases) was an extranodal intestinal monoclonal B cell lymphoma, occurring late after primary infection.

Previously we have studied Epstein-Barr virus (EBV)* infection and EBV-associated lymphoproliferative syndromes (LPS) in adults who had undergone kidney, liver, heart, or heart-lung transplantations in Pittsburgh (*1*, *2*). Patients who underwent primary EBV infections after transplantation were at greater risk of developing this complication (*2*). However the absolute number of adults who developed primary infection was low because only 8% of our adult patients were seronegative before transplantation and at risk for such infection (*3*). It was apparent that the situation might be quite different in children, who are more likely to be seronegative for EBV.

In this article we compare the frequency of LPS in adults and children who received transplants at the University of Pittsburgh Health Center between 1981 and 1985. Then we present data on the incidence of primary and reactivation EBV infections in the pediatric transplant population, as well as the association of these types of infection with clinical lymphoproliferative disease, and we describe the heterogeneous clinical features of this entity.

MATERIALS AND METHODS

Patient population. From 1981 to 1985, 1214 adults and 253 children received organ transplants in Pittsburgh. In adults, there were 725 kidney, 284 liver, and 205 heart and heart-lung transplantations. In children, there were 45 kidney, 193 liver, and 15 heart and heart-lung transplantations. To determine the frequency of EBV infection in children, 164 liver recipients were studied serologically.

Diagnosis of EBV infection. Serum specimens were routinely collected on all transplant patients for serologic diagnosis. One specimen was collected before or at time of transplant so that the pretransplant serologic status could be determined. Samples were collected twice monthly for the first three months and then once at 6 months and once again at 12 months after transplantation.

Patients were diagnosed as having a primary EBV infection on the basis of de novo development of IgG antibodies against viral capsid antigen (VCA). In the absence of documentation of conversion of the IgG anti-VCA titer, other serologic changes were taken into account to diagnose primary infections. The following are some that helped make such a diagnosis: The presence of IgM anti-VCA titer, the absence of anti-Epstein-Barr nuclear antigen (EBNA), and the presence of a heterophil agglutination titer (*4*). Reactivation infection occurring after transplantation was based on a fourfold or greater rise in IgG antibody titer against VCA in a patient who was seropositive before transplantation. Tests for IgM antibodies against VCA, IgG antibodies against early antigen (EA), IgG antibodies against EBNA, and heterophil antibodies were determined as previously described (*2*).

EBNA and EBV-DNA in tissues. EBV-associated LPS was defined by the detection of EBNA or EBV-DNA in tissue. The presence of EBNA was determined by anticomplement immunofluorescence (*2*) on cryostat tissue sections or cell smears fixed in acetone. DNA hybridization tests by Southern blot analysis were performed in the laboratory of one of us (G. Miller) at Yale University. Tissue samples obtained at biopsy or autopsy were shipped frozen to New Haven, where the total cellular DNA was extracted by the method of Wahl (*5*). Then 10 μg of

[1] This work was supported in part by NIH Grant 1 R01 AI 19377.

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* Abbreviations used: ABC, avidin-biotin complex; CMV, cytomegalovirus; EA, early antigen; EBNA, Epstein-Barr nuclear antigen; EBV, Epstein-Barr virus; LPS, lymphoproliferative syndrome; VCA, viral capsid antigen.

cellular DNA, as estimated by optical density, was digested with 40 units of *Bam* HI for 3 hr at 37°C and electrophoresed in a 0.8% agarose gel. The DNA was transferred by Southern method. The blot was probed with a chimeric plasmid pACYC184 containing the *Eco*RI B fragment of EBV strain FF41 (*6*). The probe was radiolabelled with P32 by nick translation.

Pathology of biopsy and autopsy specimens. Snap-frozen tissues were stained using the avidin-biotin complex (ABC) technique and commercially available monoclonal antibodies to immunoglobulins, and T cell, B cell, and monocyte lineages. Histological tissues were fixed in neutral-buffered formalin, postfixed in alcohol-zinc-formalin, and stained with the routine diagnostic and immunochemical stains when appropriate. Positive and negative controls were included in each batch.

Statistics. Observed proportions were compared by using the chi-square test.

RESULTS

Frequency of EBV infection after transplantation. Previously we reported on the prevalence of antibody to EBV in 134 adult kidney, liver, heart, and heart-lung transplant recipients (*3*). To summarize, 92% of transplant recipients were seropositive before transplant and 8% were seronegative. After transplantation, the infection rate among the seropositive recipients (reactivation infection) was 33%, and the infection rate among seronegative recipients (primary infection) was 82% (*3*). The frequency of primary and reactivated EBV infection in pediatric transplant recipients from 1983 to 1986 is shown in Table 1. Of 164 patients who received transplants during this period, pretransplant serologic status was determined for 148; no pretransplant sera were available for 16. A group of 92 liver recipients had serologic and virologic follow-up for greater than 60 days and could be evaluated for EBV infection. The mean onset of primary and reactivation infection in pediatric liver recipients is about 60 days after transplantation (*7*). Sera collected within the first 30 days were frequently difficult to evaluate because of passive transfer of antibodies from blood or blood products transfused at or shortly after the operation.

It is apparent from Table 1 that 63% of the evaluable patients became infected. This frequency is significantly higher than in adults because there were more children who had primary infection ($P<0.05$). The frequency of primary infection in seronegative patients was 77%. The frequency of reactivation infection of seropositive recipients was 48%. These rates are similar to those found in the initial study of the first 51 pediatric recipients seen in this center in 1983–1984 (*7*).

Frequency of lymphoproliferative syndrome (LPS). From 1981 to 1985, 30 cases of transplant-associated lymphoproliferative syndrome were diagnosed in Pittsburgh on the basis of histologic criteria. Of these, 18 cases were in adults and 12 cases were in children ≤16 years of age. Ten cases have been excluded from this study because their tissues were negative for EBNA or EBV-DNA (3 adult patients) or because appropriate tissue was not submitted for viral studies (5 adults, 2 children). This includes 3 from our own previous report (*2*). It is noteworthy that only 3 of 23 tissue samples submitted for viral studies were actually negative. However since a clear definition of LPS is lacking in the absence of virologic data, it is methodologically more rigorous to analyze only cases in which tissues show evidence of EBV infection. Thus, our present analysis concerns 20 patients (10 adults and 10 children) whose tissues had EBNA and/or EBV-DNA by nucleic acid hybridization. It should be noted that submission of tissue for EBV examination was based on clinical indication and not serologic evidence of infection.

The virological and clinical data of the 10 adults have been previously reported and will not be reviewed here (*1, 2*). One child (L.S.) was previously described (*1, 2*), and is also included in this study. Another pediatric recipient (K.E.) who received a liver transplant in 1972 is not included in the epidemiologic assessment (Table 2) but is included in the assessment of clinical, laboratory, and pathological data (Table 3).

Table 2 shows the frequency of EBV-associated LPS in 1467 transplant recipients from 1981 to 1985. When comparing the frequencies of LPS in adults in 1981–1983 and 1984–1985, there was a significant decrease from 1.5% to 0.3%. The reason for this is unknown, but it may be related to the better control of immunosuppression after the practice of administering cyclosporine by monitoring blood levels was introduced in 1983. A comparable decrease was not seen in children. The total number of LPS cases was significantly higher in children (4.0%) than adults (0.8%) ($P<.0005$). A preponderance of all cases 14/20, 70%) was associated with serologic evidence of primary EBV infection. The remainder, with one exception, had serologic evidence of reactivation (*2*).

In order to estimate the risk of developing LPS after either primary or reactivation infection, Table 4 was compiled. The proportion of adult recipients who were seronegative or seropositive for EBV and became infected was obtained from the study of 134 adult patients cited above (*3*) and applied to the total adult population of 1981–1985. Similarly, the two respective proportions for children were obtained from Table 1. The probability or risk of a seronegative or seropositive patient, as well as one with acquired primary or reactivation infection, developing LPS is illustrated in the last column. For example, 8% of 124 seronegative children, and 10.5% of children with

TABLE 1. Frequency of primary and reactivation EBV infection in pediatric liver transplant patients

Pretransplant serologic status	Total	No. evaluated	No. infected	% Infected
Seronegative	73 (49%)	48	37	77%
Seropositive	75 (51%)	44	21	48%
Total	148 (100%)	92	58	63%

TABLE 2. Incidence of EBV-associated lymphoproliferative syndrome in adults and children who received transplants in Pittsburgh

Type of patient	1981	1982	1983	1984	1985	Total
Adults	1/127[a]	4/171	3/228	2/324	0/364	10/1214[b]
	0.8%	2.3%	1.3%	0.6%	0 %	0.8%
	8/526 (1.5%)[c]			2/688 (0.3%)[c]		
Children	0/17	2/35	0/42	4/74	4/85	10/253[b]
	0%	5.7%	0%	5.4%	4.7%	4.0%
	2/94 (2.1%)			8/159 (5.0%)		
Both	1/144	6/206	3/270	6/398	4/449	20/1467
	0.7%	2.9%	1.1%	1.5%	0.9%	1.4%
Primary EBV Infection	1/1[d]	2/6	2/3	5/6	4/4	14/20
	100%	33%	67%	83%	100%	70%

[a] No. recipients with LPS/total No. recipients.
[b] $P<0.0005$.
[c] $P<0.05$.
[d] No. recipients with LPS and primary EBV infection/No. recipients with LPS.

TABLE 3. Clinical, laboratory, and pathology findings of EBV-associated lymphoproliferative syndrome[a]

Patient	Sex	Age at tx (years)	Laboratory Findings: Event	Months post-transplant	VCA IgG	VCA IgM	EBNA	HA/MS	EBV Isolation	Clinical findings and treatment	Pathological findings	Tissue evidence of EBV: EBNA	Tissue evidence of EBV: DNA hybridization	Outcome
KH	F	2.2	EBV inf.	Pre-Tx	5	20	<5	ND	ND	Fever, abdominal pain, tonsillitis, WBC=3300, ATL=10% Rx: acyclovir and reduced cyclosporine	Tonsils, lymph node: polymorphous nonmalignant proliferation	Inguinal node (+)	Tonsil (+)	Recovered
			Clin. onset	2	10	10	10	−	+					
CC	M	7.9		Pre-Tx	<5	<5	<5	ND	ND	Fever, lymphadenopathy, splenomegaly, tonsillitis, WBC=3000, ATL=30% Rx: acyclovir and reduced cyclosporine, 2 years later, episodes of fever and adenopathy	Tonsils, inguinal node: polymorphous nonmalignant proliferation	Tonsil (−)	Tonsil (+)	Recovered possible chronic infection
			EBV inf.	1.5	10	5	<5	ND	+					
			Clin. onset	7	1280	10	<5	−	−					
KL	M	3.1		Pre-Tx	<5	<5	<5	ND	ND	Fever, abdominal pain, cervical lymphadenopathy, WBC=6700, ATL=3% Rx: reduced cyclosporine	Cervical lymph node: polymorphous nonmalignant proliferation	Cervical node (+)	Cervical node (+)	Recovered
			EBV inf./ clin. onset	11	320	5	5	−	−					
DW	M	14.6		Pre-Tx	<5	10	<5	ND	ND	Fever, sore throat, tonsillar enlargement, cervical adenopathy Rx: reduced cyclosporine and prednisone	Tonsils: polymorphous nonmalignant proliferation	Tonsil (+)	ND	Recovered
			EBV inf./ clin. onset	6	80	<5	5	ND	−					
RO	M	1.2		Pre-Tx	NA	NA	NA	ND	ND	Purulent rhinorrhea, difficulty breathing, recurrent otitis media, WBC=2900, ATL=rare Rx: reduced cyclosporine and prednisone, Imuran discontinued	Nasopharyngeal mass: focal infiltration of plasma cells (nonmalignant)	Nose mass (+)	ND	Recovered
			EBV inf./ clin. onset	11	160	<5	<5	ND	+					
JC	M	5.8		Pre-Tx	<5	<5	<5	ND	ND	Fever, diarrhea, jaundice, enlarged tonsils and spleen. liver bx=immunoblasts, GI and cerebral hemorrhages from coagulopathy, WBC=14,500, ATL=80% Rx: cyclosporine and steroids reduced	Kidney, liver, marrow, lymphoid system: diffuse lymphoproliferative process; monomorphous large cell lymphoma, B cells by immunoperoxidase	Mesenteric node (+)	Mesenteric node (+)	Died 2 months post-transplant
			EBV inf./ clin. onset	2	20	5	<5	−	−					
KE	F	1.7		Pre-Tx	<5	<5	<5	ND	ND	Fever, sore throat, jaundice, tonsillar and cervical node enlargement ATL=55%	Tonsils: polyclonal polymorphous lymphoid hyperplasia; at autopsy, polymorphous infiltrates of liver, kidneys, lungs, intestinal wall, brain, etc.	Tonsil (+)	Tonsil (+)	Died 3 weeks after onset of syndrome
			EBV inf./ clin. onset	154	<5	<5	<5	+	ND					

TABLE 3. Clinical, laboratory, and pathology findings of EBV-associated lymphoproliferative syndrome[a]

Patient	Sex	Age at tx (years)	Laboratory Findings: Event	Months post-transplant	VCA IgG	VCA IgM	EBNA	HA/MS	EBV Isolation	Clinical findings and treatment	Pathological findings	Tissue evidence of EBV: EBNA	Tissue evidence of EBV: DNA hybridization	Outcome
MM	F	8.6		Pre-Tx	NA	NA	NA	ND	ND	Typical "mono;" 5 months later, tonsillitis with polyclonal hyperplasia and remission. 12 months later, anemia, axillary adenitis, acute renal failure, hypogammaglobulinemia, respiratory distress. Rx: acyclovir, cessation of cyclosporine.	Tonsils: polymorphous polyclonal lymphoid hyperplasia; at autopsy diffuse monomorphous infiltrates in liver, pericardium, and most organs; large cell lymphoma	Mesenteric node (−)	Mesenteric node (+)	Died 1 month after onset of second syndrome
			EBV inf.	20	1280	40	<2	ND	ND					
			Clin. onset	25–37	320	80	<5	ND	−					
CB	F	11.8		Pre-Tx	20	5	<5	ND	ND	Fever, deterioration of liver function, coagulopathy and intracerebral hemorrhage, WBC=600, ATL=10%	Liver bx: polymorphous lymphoproliferation; lung bx: CMV pneumonia; autopsy: lymphoproliferative syndrome involved lymph nodes, GI tract, liver	Liver (+)	Liver (−)	Died 2 months post-transplant
			EBV inf./ clin. onset	1	20	40	5	ND	−					
JM	M	4.2		Pre-Tx	<5	<5	<5	ND	ND	Exudative pharyngitis, WBC=8100, ATL=occ, 23 months later, rectal bleeding, abdominal pain and tenderness, suspected appendicitis. Laparotomy revealed ileal and mesenteric masses. Readmitted twice for resection of recurrent tumors Rx: excision of masses; cyclosporine reduced	Perforated ileal tumor; monomorphous monoclonal IgM lambda lymphoma	Ileal tumor (+)	Ileal tumor (+)	Relapses
			EBV inf.	1	10	5	<5	ND	+					
			Clin. onset	24	640	20	<5	ND	ND					
LS	M	16.5		Pre-Tx	<5	ND	ND	ND	ND	Fever, diarrhea, peritonitis, laparotomy revealed multiple tumors and perforation. WBC=3800 Rx: excision of tumor; acyclovir; withdrawal of immunosuppression (allograft lost)	Intestinal tumor: large cell immunoblastic lymphoma, monoclonal lambda, IgG predominant	Intestinal tumor (−)	Intestinal tumor (+)	Recovered
			EBV inf./ clin. onset	3	20	<5	<5	−	ND					

[a] Abbreviations used: NA: not available; ND: not done; EBV: EBV infection, as defined by first serologic evidence; WBC = white blood cells; ATL = atypical lymphocytes; HA/MS: heterophil antibodies, monospot test.

primary EBV infection may develop LPS. It is clear that the probability that LPS will occur in a seronegative patient or a patient who undergoes primary infection is higher in both adults and children. The difference is significant for both adults ($P<0.005$) and children ($P<0.005$).

The Spectrum of LPS in children. Table 3 summarizes the clinical, laboratory, and pathological data of 11 pediatric cases of EBV-associated LPS. Figure 1 shows the EBV nucleic acid hybridization by Southern blot.

One of the difficulties in diagnosing EBV infections in these patients is that often the "pretransplant" serum was obtained during the operation and the sample was often contaminated by transfused blood (7).

Of the eleven patients, 7 were clearly seronegative before transplantation. Six of these converted their anti-VCA titer after transplantation and clearly underwent primary infection. One patient (K.E.), whose pretransplant serum was negative for anti-VCA, died three weeks after transplantation, before her EBV serology titers could convert. Evidence that she had primary infection was the presence of a positive heterophil

TABLE 4. Estimated risk of developing EBV-associated lymphoproliferative syndrome after EBV infection after transplantation

Patient type	EBV serology Pre-Tx	No.	% Infected	No. Infected	No. with LPS	Estimated risk: No. LPS/ patient group	Estimated risk: No. LPS/ No. infected
Adults	Negative	100	82%	82	4	4.0%	4.9%
Adults	Positive	1114	33%	368	6	0.5%	1.6%
Children	Negative	124	77%	95	10	8.0%	10.5%
Children	Positive	129	48%	62	0	0%	0%
		1467	41%	607	20		3.3%

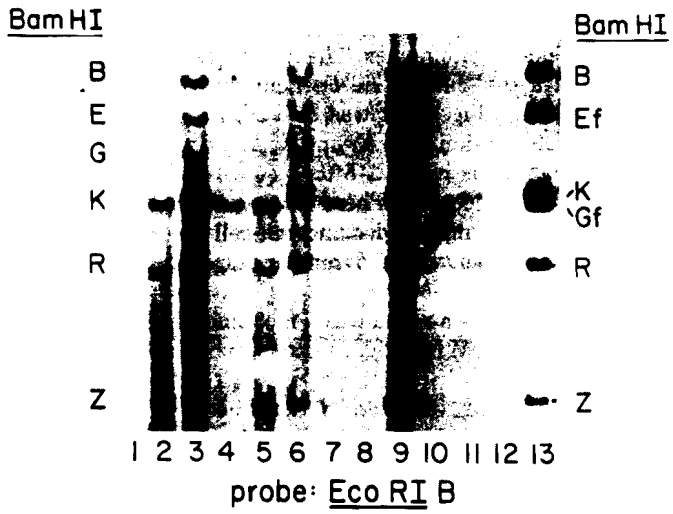

FIGURE 1. Southern blot showing the presence of EBV DNA in tissue samples from 7 children with lymphoproliferative syndromes following liver transplantation. Cellular DNA was digested with *Bam* HI and probed with the *Eco*RI B fragment of EBV DNA cloned in pACYC184. The *Bam* HI subfragments found in *Eco*RI B are shown at the left and right margins. The terminal *Bam* HI fragments (*Bam* HI Ef and *Bam* HI Gf) are different size in the probe (lane 13) since they contain *Eco*RI sites. Tissue samples from patients in group 1 (nonfatal lymphoproliferative disease) are found in lane 4 (K.H. tonsil), lanes 6 and 8 (C.C. tonsil and axillary node), and lane 7 (K.L. cervical node). Samples from patients with fatal lymphoproliferative disease, group 2, are seen in lane 2 (M.M. mesenteric node) lane 5 (J.C. mesenteric node), and lane 11 (K.E. tonsil). The sample in lane 9 is the gut lymphoma from J.M. The signal in lane 13 with plasmid DNA is equivalent to about 18 copies of the EBV genome per cell.

agglutination (monospot test) and the evidence of EBV infection in tissues. Our serologic interpretation of the remaining 4 patients is as follows.

The "pretransplant" sera from K.H. and C.B. were positive, probably because the serum samples were obtained at the time of operation while the patients were being transfused. However both had positive IgM titers and no anti-EBNA, suggesting that they were undergoing a primary infection around the time of the operation or just before it. K.H. also had EBV isolated de novo from the mouth.

A "pretransplant" serum was not available on M.M. However the elevated IgG and IgM anti-VCA titers and absence of anti-EBNA are indicative of a primary infection.

A "pretransplant" serum was not available for R.O. His only available serum posttransplant was positive for anti-VCA, but negative for IgM and anti-EBNA. It was not possible to diagnose primary EBV infection in this patient. In summary we believe that 10 of 11 patients had evidence of primary infection around the time of, or after, transplantation.

The IgM titers converted in 4 patients. They were elevated in 4 others but not elevated in 3. IgM antibody to VCA is frequently low in our immunosuppressed populations who develop de novo EBV infection and may even be absent (*2*)—and in our opinion it is not as useful in diagnosing primary infection as in the immunocompetent (*4*). The anti-EA titers (not shown in Table 3) were positive in 6 patients and negative in 5. We have found that these determinations are neither entirely specific nor sensitive for de novo infection. They are also frequently positive in the absence of other signs of infection (*2*). Notably, antibody against EBNA failed to develop in 7 of 11 patients, and titers of only 1:5 developed in 3. The absence of an anti-EBNA response, or a low response, is also characteristic of immunosuppressed patients, and is quite useful in the diagnosis of primary EBV infection in this group of patients (*2, 8*).

Only 1 of 6 patients tested developed heterophil antibodies as determined by monospot tests. While it is well known that such antibodies may not develop in children undergoing primary EBV infection (*9*), this too may be an expression of immunosuppression, as adult transplant recipients undergoing primary infection also rarely developed heterophil antibodies (*2*).

Nine of the 11 patients developed serologic evidence of EBV infection shortly before or in the first year after transplantation, mostly within the first 6 months. One patient became infected at 2 years (J.M.) and one (K.E.) at 12.8 years after transplantation. In 9 cases clinical symptoms attributable to EBV infection occurred around the time of infection. However in C.C., they occurred 6 months later; and in J.M., 2 years later. In the latter case, a well defined intestinal lymphoma was involved.

Clinically, these 11 cases present 3 distinguishable syndromes. These are a self-limited form of mononucleosis syndrome with localized LPS, a similar syndrome progressing to widespread lymphoproliferation and death, and isolated extranodal lymphoma.

Four patients (K.H., C.C., K.L., and D.W.) had moderately severe attacks of EBV mononucleosis. All these patients are still living 2–3 years after onset of their illness, although one patient (C.C.) had subsequent episodes of fever and lymphadenopathy. A fifth patient (R.O.) developed an isolated nasopharyngeal mass associated with the adenoids that had EBNA. This was excised without further problems. Case histories of two illustrative cases are given here.

K.H. developed a clinical syndrome consistent with EBV mononucleosis two months after transplantation (Table 3). She underwent a tonsillectomy for airway obstruction and an inguinal node biopsy. The tonsillar tissue contained EBV-DNA and a right inguinal lymph node biopsy was positive for EBNA. Both tissues showed nonmalignant polymorphous B cell hyperplasia. The course of the illness was about one month. The patient has remained well except for a subsequent attack of varicella from which she recovered.

C.C. developed primary EBV infection 1.5 months after transplantation based on conversion of IgG anti-VCA titer. He remained asymptomatic until 5½ months later when he developed tonsillar and lymph node enlargement after a course of treatment for rejection that included OKT3 antiserum. His anti-VCA titer rose to 1:5120, and IgM titers remained positive.

The tonsil and inguinal node both showed polymorphous nonmalignant lymphoid proliferation. The tonsil was negative for EBNA but positive for EBV-DNA (Fig. 1). The patient was treated with acyclovir and reduction of cyclosporine. Fever resolved and lymph nodes regressed, but 10 months later the patient was readmitted for fever and lymphadenopathy. A left axillary node showed granulomatous, necrotizing adenitis and was positive for EBV-DNA. The patient may have had chronic relapsing EBV infection.

In four other patients (J.C., K.E., M.M., and C.B.), the illness began very much like those above but it had a fulminating course with fatal outcome. J.C. and M.M. had what appeared to be large cell lymphomas, but K.E. and C.B. had diffuse polymorphous lymphoproliferation. In the case of C.B., CMV pneumonia was also present terminally and may have contributed to her demise. Two of these cases are described briefly.

Two months after transplantation, J.C. developed fever, diarrhea, jaundice, tonsillitis, splenomegaly, leukocytosis, and a striking atypical lymphocytosis (80%) concomitant with serologic evidence of primary EBV infection. His death was due to gastrointestinal and cerebral hemorrhages as a result of hepatogenic coagulopathy. At postmortem, in addition to a generalized aspergillus infection, there was a widespread lymphomatous involvement of the kidneys, liver, gut, bone marrow, and lymphoid system. Histologically, this process was a monomorphous large cell lymphoma with many mitoses. Plasmacytoid features were minimal. Areas of necrosis were prominent. A mesenteric lymph node was positive for EBNA and EBV-DNA (Fig. 1).

K.E. developed fever, sore throat, jaundice, and a positive monospot test 154 months after transplantation. A right tonsillar biopsy revealed the presence of EBNA and EBV-DNA (Fig. 1). All immunosuppression was discontinued but this did not affect the clinical course. She died of asphyxiation during an episode of hematemesis. At autopsy, a diffuse polyclonal lymphoproliferation was found that had infiltrated the liver, kidneys, pancreas, salivary glands, lungs, adrenal glands, ovaries, uterine wall, bone marrow, brain, meninges, and peripheral nerves. The mucosa and submucosa of the entire gastrointestinal tract, and the serosa of the large intestine were also involved. The lymphoproliferation was pleomorphic, not monomorphous as in J.C., and had pronounced plasmacytoid features. By immunoperoxidase staining the cells had B lymphocyte masses, with immunoglobulin production, and both light chains equally represented.

The third clinical form of LPS is that of an isolated lymphoma (J.M., L.S.) typified by the case described below. One has had relapses (J.M.) and one (L.S.) has recovered with withdrawal of immunosuppression (Table 3).

J.M. developed serologic evidence of primary EBV infection one month after transplantation but he was asymptomatic. He did well until two years later when he had an episode of hematochezia. His hematocrit remained stable, and the bleeding was thought to be self-limited. Two weeks later, he was admitted with abdominal pain, rebound abdominal tenderness, and leukocytosis. A laparotomy was performed for suspected appendicitis. A perforated ileal mass and a mesenteric mass were found and resected. A diffuse lymphoproliferative lesion consistent with a lymphoma was found infiltrating all layers of the small bowel. Ulceration into the gut lumen and large areas of necrosis were present. The cells were intermediate in size (different from J.C. and M.M., in whom they were large cell or immunoblastic) with minor or moderate plasmacytoid features. IgM lambda immunoglobulin was readily demonstrable and the tumor was monomorphous. EBNA was found in the tumor, and EBV-DNA was also present by hybridization studies (Fig. 1). Postoperatively immunosuppression was withheld for one week, and then cyclosporine was given at one-half the previous dosages (50 mg twice daily). Prednisone was maintained at 5 mg daily. The patient has been readmitted twice for progressive tumors and a tumor resection was performed 6 months after the first resection. Cell markers were identical on this second tumor.

The pathology of lymphoproliferative syndrome. Two broad pathologic patterns were discerned that were similar to what Frizzera et al. (*10*) classified as polymorphous diffuse B cell hyperplasias and lymphomas. The first pattern was seen in the lymph nodes and tonsils and adenoids of all patients of the first group (e.g., Fig. 2); it was also seen in C.B. and in the initial tonsillar lesion of K.E. and M.M. in the second group. This polymorphous reaction often contained vascular thromboses and tissue necrosis. There was usually more necrosis in the tonsils than lymph nodes and the cells were a mixture of plasma cells, plasmacytoid lymphocytes, and histiocytes of varying morphology.

The second pattern noted was a monomorphous lymphoma-like proliferation (M.M., Fig. 3). It was seen in some patients with the second and in both patients with the third clinical pattern (J.C., M.M., J.M., and L.S.). These tumors also contained necrotic areas. However these were not confined to nodes but involved internal organs and formed sheets of cells. Tumors from J.M. and L.S. contained monotypic IgMλ by immunoglobulin analysis; one had B cells but no immunoglobulins (J.C.), and the third had only a few cells with B cell markers.

DISCUSSION

EBV infects most of the population and causes little significant disease. In Pittsburgh, 92% of the adult transplant population have serologic evidence of previous infection (*3*), and half the pediatric transplant population is seropositive (*7*) before operation.

However when EBV infects immunologically compromised individuals, an ordinarily innocuous infection may develop into any of a number of dangerous diseases. Immunosuppression may be congenital or acquired. In one type of poorly understood congenital immunodeficiency, an X-linked as well as a non–X-linked lymphoproliferative syndrome has been described, in which the patient is unable to handle EBV mononucleosis, and a fatal disease with lymphoproliferations in major organs may develop (*11*, *12*). In ataxic telangiectasia, patients have a high incidence of lymphoreticular malignancies that may show evidence of EBV-DNA (*13*). In acquired immune deficiency syndrome, B cell lymphomas with evidence of latent EBV infection and chromosomal translocation typical of Burkitt's lymphomas, as well as other types of non-Hodgkin's lymphomas have been described (*14*, *15*).

The posttransplantation state represents one form of acquired immunodeficiency produced iatrogenically. Under azathioprine as well as cyclosporine immunosuppression, specific immune mechanisms against EBV, particularly cytotoxic T cell immunity, appear to be depressed. This is manifested by the absence of effective regression of EBV-infected B cells in vitro, and increased oral virus shedding by transplant recipients (*16*, *17*). As with cytomegalovirus (CMV), there is intense

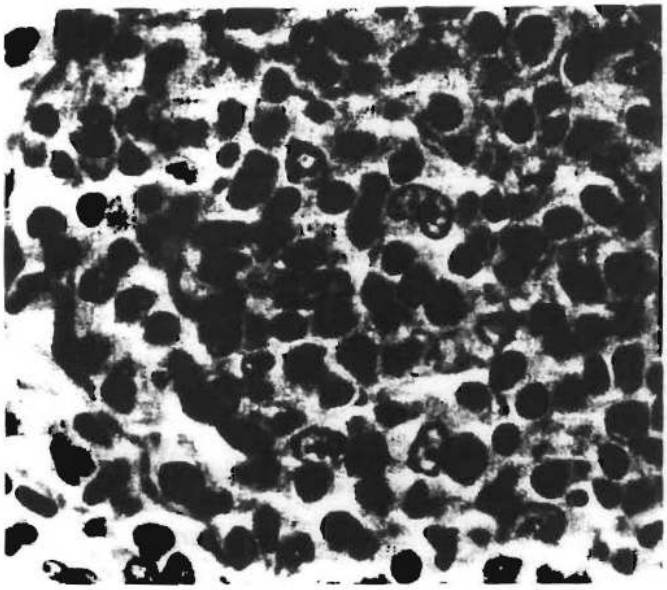

FIGURE 2. Tonsil from K.H. The polymorphous nature of the proliferation is readily apparent. Small lymphocytes, plasma cells, and histiocytes are among the cell types seen. Mitoses are present.

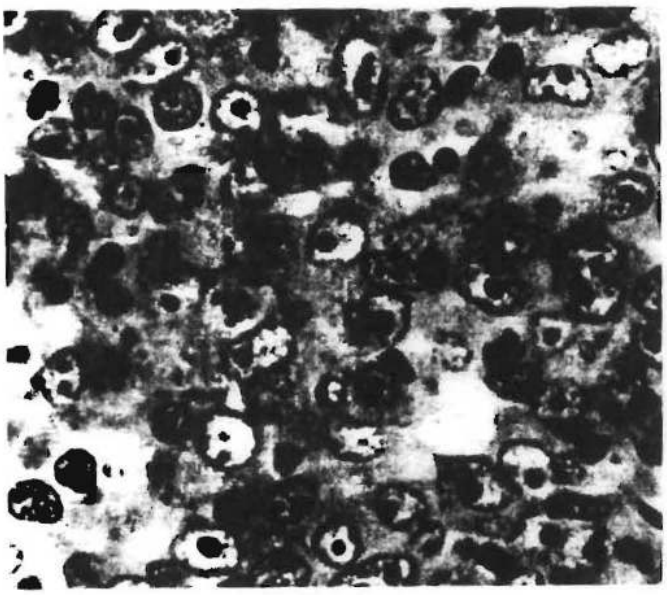

FIGURE 3. Abdominal lymph node from autopsy of M.M. The monomorphous large cell population, with prominent nucleoli, most closely resembles an immunoblastic sarcoma. In this instance only some B cell markers, but not immunoglobulins, were demonstrable on the cells.

infective activity by EBV during the immediate posttransplant period. The rate of infection with EBV, comprising both primary and reactivation infection, is about 41% of all transplant recipients (Table 4).

Even in immunosuppressed subjects, most EBV infections are clinically silent. A small but significant proportion of patients, however, develop apparent disease (*18*). One life-threatening entity is the spectrum of clinical syndromes that may be called EBV-associated lymphoproliferative syndromes (LPS) first defined in transplant patients by Hanto et al. (*19*). We recently described the importance of primary EBV infection in adult transplant recipients who developed LPS (*3*). In this report, the association is even more strikingly demonstrated in children. In 11 cases of EBV-associated LPS in pediatric transplant recipients, 10 had serologic evidence of primary EBV infection. It is interesting that while EBV-associated LPS in adults appeared to decrease in frequency after 1983, its incidence in children has not changed appreciably despite introduction of monitoring of immunosuppressants by measuring blood levels of cyclosporine. The fact that previously uninfected recipients are those at greatest risk for the development of LPS suggests the need to investigate the source of EBV infection. In the case of CMV, the source has been traced in most cases to the donor organ or blood products used during the perioperative period (*20*). However so far we have not been able to incriminate the donor organ or blood products as source of EBV infection (*7*).

On the basis of experience at the University of Minnesota, Hanto et al. (*21*) divided EBV-associated LPS into three categories. Group 1 represents "polymorphic diffuse B cell hyperplasia," without evidence of malignancy. Clinically the illness resembles EBV mononucleosis. Group 2 represents "polymorphic B cell lymphoma." This illness is also mononucleosis-like, but tissues may show morphologic features of malignancy. There may be a "risk of progression from a polyclonal to a monoclonal B cell proliferation" (*21*). Group 3 includes "older" patients who develop extranodal tumors that are monoclonal and malignant.

The three syndromes described in our children, correspond well clinically and pathologically to Hanto's three groups. Our first group consists of mononucleosis syndrome with only benign polymorphous hyperplasia. Our group 2 showed progressive fatal lymphoproliferation. Pathologically one patient showed the same type of benign hyperplasia as in our group 1, but the other two showed a malignant transformation similar to Hanto's group 2. It is quite possible that groups 1 and 2 represent a continuum ranging from benign mononucleosis to widespread immunoblastic or lymphomatous proliferation in vital organs. Our group 3 consists of a 4-year-old boy and a 16-year-old boy who developed monomorphous gastrointestinal lymphomas. Cells cultured from the tumor of the first patient have shown a chromosomal translocation (8 to 14) such as is found in Burkitt's lymphoma (unpublished data [22]). This patient has relapsed twice despite resection of the tumor and decrease in immunosuppression.

The three syndromes we describe are also consistent with what has been observed in the X-linked lymphoproliferative syndrome. An analysis of 161 patients and 44 kindreds by Grierson et al. (*23*) revealed 3 major patterns: fatal EBV mononucleosis, acquired hypogammaglobulinemia, and malignant lymphoma. The fatal mononucleosis syndrome is similar to that in our second group. It is interesting that severe hypogammaglobulinemia was seen in one of our patients who had the fatal mononucleosis syndrome (M.M.). The precise immune deficiency in X-linked, as well as non-X-linked, lymphoproliferative syndromes is unclear. In the former a number of B cell abnormalities, including low IgA and IgM levels and inability to respond to ϕX 174 antigen by specific IgG production have been described (*23*). A deficient natural killer cell response has been suggested in the latter syndrome (*12*). In the X-linked syndrome, an excess rather than a deficient cytotoxicity response has also been postulated as the cause of the severe hepatitis seen in fatal disease (*24*). Infiltration in the liver was a prominant feature in all 4 of our patients who died, 2 of whom had bleeding coagulopathies. These interesting observations

suggest that the precise nature of the immune defect accounting for the abnormal response to EBV in transplant recipients remains to be further defined.

Our pediatric patients with LPS appear to be different from adult patients in many respects. First, EBV-associated LPS does not appear to be decreasing as it is in adult recipients despite better cyclosporine monitoring (Table 2, [25]). It is possible that children are less well able to cope with primary EBV infection. We may also be seeing more LPS in children because proportionately more have primary infection, and liver transplantation in children has increased since 1984. Second, the presentation of LPS is different in children. Half the 14 LPS cases described in adults from Pittsburgh were intestinal tumors (*1, 2*). Only 2 of 11 children had such an extranodal presentation. So far we have not seen the late central nervous system lymphomas reported from Minnesota and Stanford in our adult or pediatric transplant recipients (*21, 26*).

The outcome varied in the three clinical groups described. Patients of the first group were first seen with a typical mononucleosis syndrome. Involved tissues, which showed a polymorphous reactive proliferation, were limited to lymph nodes and tonsils. These patients had a benign clinical course.

The second group had the worst prognosis. Lymphoproliferation extended to extranodal organs, particularly the liver. Even when proliferation remains histologically a polymorphous reactive type (K.E.), hemorrhages caused by liver failure (J.C., C.B., K.E.) may lead to a fatal outcome. Two patients in this group (J.C. and M.M.) showed a monomorphous large cell lymphoma that terminally invaded multiple organs. Hanto et al. (*21*) believe that a monoclonal lymphomatous proliferation may result from an initially polymorphous process. Indeed our patient M.M. initially had a mononucleosis syndrome with polymorphous polyclonal lymphoid hyperplasia in the tonsils before she developed monomorphous generalized lymphoproliferation that led to her death. We do not yet know, in this case, the clonality of the infiltration—and we do not know how one syndrome pathogenetically leads to the other.

The third type represents extranodal lymphomas in which the role of EBV is unclear. It is unlikely that the presence of EBV genomes or their expression in tumor tissue is adventitious, as they have not been found in the usual non-Hodgkin's lymphomas (*27*). To what extent independent viral replication and infection takes place, and to what extent there is just clonal expansion of latently infected tumor cells, is also unknown. The finding that some posttransplant LPS are oligoclonal by immunoglobulin gene rearrangement (*28*) has not clarified the picture. Our two patients (J.M. and L.S.) had isolated extranodal intestinal lymphomas that were monomorphous in appearance and monoclonal in immunoglobulin synthesis. One patient in particular also has an 8-to-14 chromosomal translocation (J.M., unpublished data). Klein postulates that transformation of B cells by EBV alone is inadequate for oncogenesis and that, in addition, one or more chromosomal translocations that may facilitate oncogene expression are necessary. We are still studying the immunoglobulin clonality of our specimens, but more direct experimental studies to test this hypothesis would be desirable.

The appropriate therapy for EBV-associated LPS is at best empirical and remains a challenge. Hanto et al. (*21, 29*) believe that acyclovir may be useful, particularly in patients in their groups 1 and 2, in whom active EBV replication may play a role in the pathogenesis. Acyclovir has been used extensively in Pittsburgh. Of the 11 patients described here, 8 received acyclovir and also had their immunosuppression decreased; 4 have recovered without apparent clinical relapse, after a maximum of 6 years of follow-up. However no firm conclusions can be drawn from such uncontrolled observations, and controlled trials are needed. During acyclovir administration, EBV oral shedding is suppressed, but it always reappears after the drug is stopped (*30*). Better anti-EBV agents are clearly needed.

In a previous report, we reported that both monoclonal and polyclonal tumors responded to decrease of immunosuppression. In a number of cases, as pointed out by Hanto et al. (*21*), the tumor was also surgically excised. The case of J.M. shows that recurrences may also occur with decrease of immunosuppression. Further studies are needed to correlate type of LPS—in terms of histology, clonality, immunoglobulin gene arrangement, and EBV expression—with response to therapy and prognosis.

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Received 10 November 1986.
Accepted 3 August 1987.

0041-1337/88/4504-0727$02.00/0
TRANSPLANTATION

Vol. 45, 727-729, No. 4, April 1988
Printed in U.S.A.

USE OF OKT3 MONOCLONAL ANTIBODY IN THE TREATMENT OF ACUTE CARDIAC ALLOGRAFT REJECTION

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OKT3 is a murine monoclonal antibody that recognizes the T3 surface antigen present on mature T cells, and it has been used to successfully treat renal allograft rejection. We report our experience with OKT3 in the treatment of cardiac allograft rejection. Eight patients with endomyocardial biopsy evidence of moderate or severe rejection were given fourteen daily intravenous treatments of OKT3. Six of the eight patients had complete recovery following OKT3 therapy; one required additional steroid therapy for recurrence and one patient failed to respond. Five of the six patients with a complete response have experienced no further rejection (mean follow-up 437 days). Adverse reactions to OKT3 were common early in the treatment course, but were well tolerated. We concluded that OKT3 is a safe and effective treatment of cardiac allograft rejection and that a majority of patients experience long-term rejection-free periods.

The survival of cardiac transplant patients has increased significantly in the past five years (*1*). Contributing factors include the introduction of cyclosporine, stringent patient selection criteria, and the early diagnosis of graft rejection by endomyocardial biopsy (*2, 3*). Despite these advances, acute rejection of the allograft remains a major source of morbidity in the cardiac transplant patient. Conventional therapy has relied upon high-dose steroids and antilymphocyte globulins (ALG)* (*4, 5*). High-dose steroids are associated with an in-

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* Abbreviation used: ALG, antilymphocyte globulin.