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The Pathology of Posttransplant Lymphoproliferative Disorders Occurring in the Setting of Cyclosporine A-Prednisone Immunosuppression

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Posttransplant lymphoproliferative disorders (PTLDs) were diagnosed in 43 patients from the Pittsburgh–Denver series between June 1980 and March 1987. This constitutes a detection rate of 1.7%. Major categories of clinical presentation included a mononucleosislike syndrome, gastrointestinal/abdominal disease, and solid organ disease. The median time of onset in patients initially immunosuppressed with cyclosporine-A (CsA)-containing regimens was 4.4 months after transplant, regardless of tumor clonality. A strong association of PTLD with Epstein–Barr virus (EBV) was observed. A histologic spectrum of lesions from polymorphic to monomorphic was observed. Whereas polymorphic lesions could be either clonal or nonclonal, monomorphic lesions appeared to be clonal in composition. The presence of large atypical cells (atypical immunoblasts) or necrosis did not appreciably worsen the prognosis. Twelve patients had clonal, 13 had nonclonal, and five had both clonal and nonclonal tumors. Clonality was indeterminate in 13 cases. Most patients were treated with a regimen based on reduced immunosuppression and supportive surgery. Almost all nonclonal and about half of the clonal lesions respond to this conservative therapy, indicating that it is an appropriate first line of treatment. This behavior suggests that a spectrum of lesions ranging from infectious mononucleosis to malignant lymphoma constitutes the entity known as PTLD. Some monoclonal tumors can undergo regression, however, apparently in response to host immune control mechanisms. Because of its short latency and strong association with EBV, PTLD is an important model for the study of virus-associated tumor progression in humans. (Am J Pathol 1988, 133:173–192)

AN INCREASED INCIDENCE of lymphoproliferative disorders in immunosuppressed organ transplant recipients is well established.[1–6] These tumors most commonly are of B cell origin and are associated with active infection by the Epstein–Barr virus (EBV).[7–15] The lesions may be clonal (monoclonal or multiclonal) or nonclonal (polyclonal) and in many instances they will undergo regression if immunosuppression is reduced.[8,12,16–18]

This report analyzes clinicopathologic features of posttransplant lymphoproliferative disorders (PTLDs) arising in the patient series from the University of Pittsburgh. The population is composed of liver, kidney, heart, and heart/lung recipients who received immunosuppressive regimens that included cyclosporine-A (CsA). The present study allows a more comprehensive picture of CsA-associated lymphoproliferations than can be afforded by reports limited to tumors arising in association with specific organ allografts (*ie,* heart)[5,19,20] or in patients receiving non-CsA-containing immunosuppression.[7,9,11,12,21]

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Materials and Methods

Patient Population

Organ transplants were performed at the University of Pittsburgh (1981–1987) or at the University of Colorado (1981 and earlier). The study population consists of all patients from this transplant series who received a pathologic diagnosis of either lymphoma or posttransplant lymphoproliferative disorder (PTLD) between June 1980 and March 1987. Microscopic features of the latter are discussed below.

A total of 43 patients received these diagnoses during this period. One patient who was originally included in this category (No. 8) was subsequently determined to have Hodgkin's disease and is not included in this series. In six cases (No. 19, 38, 39, 41–43), adequate tissue for further analysis was unavailable. The inclusion of these latter six cases in this series is based on the descriptions provided in the original pathology reports. Although these cases are included in population statistics they are excluded from pathologic studies.

Histologic Evaluation

Formalin-fixed, paraffin-embedded histologic sections from 37 patients were available for review. Sections were evaluated for degree of polymorphism, necrosis, plasmacytic differentiation, and number of large atypical cells ("atypical immunoblasts").[7] Polymorphism refers to the presence of lymphoid cells in various stages of differentiation and not to the presence or absence of nonlymphoid cell types. A grade of 0 was given for the absence of polymorphism. Some cases showed minimal polymorphism in association with plasmacellular differentiation. These cases were graded as 1. Other cases showed a wide range of lymphocyte forms. These cases received a polymorphism grade of 2. Necrosis was graded as 0 for absent, 1 if involving <10% of tissue, 2 for 10–25%, and 3 for >25% involvement. Plasmacytic differentiation was graded as 0 for minimal to absent, 1 for mild, and 2 for moderate to marked. The predominant cell type in this latter category was classified as plasmacytic or plasmacytoid. A plasmacytic cell, with mature eccentric nucleus, is distinguishable from a plasmacytoid cell, which has an eccentric but less mature nucleus. The term atypical immunoblast refers to large mononuclear cells with vesicular nuclei, irregular nuclear membranes, and prominent nucleoli. These cells have been described in both PTLDs and infectious mononucleosis.[7,22] Atypical immunoblasts were graded as 0 for absent, 1 for rare, and 2 for occasional, and 3 for frequent.

Immunocytochemical Studies

Analysis of cytoplasmic immunoglobulins was performed on paraffin-embedded tissues in 32 cases using the avidin-biotin peroxidase technique of Hsu with or without pronase digestion,[23] or with the peroxidase-antiperoxidase technique. Color was developed with 0.05% diaminobenzidine (DAB) using light microscopy to titrate the stain. Slides were counterstained with hematoxylin. Anti-kappa and anti-lambda antisera were purchased from Dako Inc. Cytoplasmic kappa:lambda light chain ratios were calculated on the basis of a count of 200 mononuclear cells per slide. A kappa:lambda ratio of 5:1 or greater was considered indicative of a monoclonal kappa component. Conversely, a lambda:kappa ratio of 3:1 or greater was interpreted as evidence for a monoclonal lambda proliferation.[8]

In specimens from nine patients frozen sections were stained using the immunoperoxidase technique without digestion and with 3-amino-9-ethylcarbazole or DAB as the visualizing reagent. Frozen tissue immunofluorescence staining for immunoglobulin light chains was performed in selected specimens from six cases and used goat $F(ab')_2$ fragments (Tago) followed by rhodamine-conjugated rabbit $F(ab')_2$ anti-goat IgG. Reagents were ultracentrifuged before use to remove immune aggregates. Slides were read in a Leitz Laborlux microscope with epifluorescence attachment. Primary antibody was replaced by diluent to serve as a negative control and appropriate positive biologic controls (plasmacytomas, tonsils) were included in each batch of stains. Similar techniques were employed for those cases stained at St. Mary's Hospital, London (KP).

Immunoglobulin Gene Rearrangement Studies

Recombinant DNAs containing segments of the immunoglobulin heavy chain joining region (J_h), a heavy chain constant region ($C\mu$), and light chain constant regions (Ckappa and Clambda), were obtained from Dr. Philip Leder, Harvard University.[24] Purification of high molecular weight DNA, restriction enzyme analyses, and Southern blot analyses were performed on specimens from 20 patients according to protocols described previously.[25] Frozen tissue sections were pulverized in dry ice, freeze-dried, and solubilized in standard lysing solution before regular purification procedures. This protocol is modified from

that described by Graham et al.[26] All tumors were studied with JH (BamHI) and Clambda (EcoRI) probes, and most were also studied with Ckappa (BamHI) and Cμ (BamHI) probes. Many were studied with additional enzymes. In all of the tumors where a gene rearrangement was detected with any probe, JH analysis using BamHI was positive and provided a clear, sensitive detection.

Some specimens were also analyzed for rearrangements of immunoglobulin gene regions by Drs. M. Cleary and J. Sklar, as described previously.[17]

EBV Studies

The determination of primary, reactivation, or remote EBV infection was based on specific EBV indirect immunofluorescence serologic tests for titers of IgM anti-VCA, IgG anti-VCA, IgG anti-EA (D and R), and anticomplement anti-EBNA in pretransplantation and posttransplantation samples as previously reported.[13] Primary infections were those in which a negative pretransplant IgG anti-VCA converted to positive after allograft. Reactivation or secondary infections were diagnosed when preexistent IgG anti-VCA titers rose more than four-fold after transplantation.

Some specimens have been analyzed previously for EBV DNA by either J. Pagano, MD (University of North Carolina), G. Miller, MD (Yale University), or J. Sklar, MD, PhD (Stanford University).[13]

Evaluation of Tumor Response to Therapy

A variety of therapeutic interventions were applied to patients with PTLDs over the time span of this retrospective study. In addition, the courses of many patients were complicated by opportunistic infections, delayed diagnoses, and/or anecdotal changes in therapy.

The categorization system employed in this report takes into account these confounding variables so as to provide, in as unbiased a manner as possible, an accurate statement of tumor behavior.

The term "regression" is applied to those cases that demonstrated clinical or pathologic reduction and eventual disappearance of tumor mass in response to reduced immunosuppression, with or without acyclovir. Surgical intervention was limited to biopsy procedures of the tumor under investigation. Surgical resection of all grossly evident tumor precluded inclusion into this category. Patients who received chemotherapy or radiation therapy were also excluded from this category.

The term "resolution" is used to represent cases in which tumor disappeared in the face of more aggressive intervention. Because the possibility exists that this intervention (eg, total surgical resection, chemotherapy, radiotherapy) effectively eradicated the tumor, a case for spontaneous regression cannot be made. It must be emphasized that placement of a case into this category does not imply that the lesion was incapable of regression. Rather, it serves to recognize the limitations of retrospective analysis.

Cases categorized as having had "no response" demonstrated clinical progression in spite of all measures taken. In contrast, cases with "no evaluable response" had an ambiguous clinical course in which, for a variety of reasons, no evaluation of tumor behavior was possible.

Rare cases had "partial resolution," implying that an ongoing response of tumor to therapy was apparent. Death occurred from unrelated causes, prohibiting the further expression of tumor behavior in this group of patients.

Patients with "recurrence" showed evidence of recurrent tumor occurring some time after primary therapy.

Finally, patients in the "autopsy" category had the diagnosis of PTLD first made at autopsy.

Tables 1–5 are arranged according to these categories, in order to present a clearer exposition of data.

Results

Patient Population and Tumor Incidence (Table 1)

The patient population includes 29 men and 14 women (M:F ratio, 2.1:1). Ages at transplant range from 1–62 years with a mean age of 23.1 ± 2.7 years. Eighteen patients were 18 years of age or less at transplant, and the remaining 25 were over 18 years old. The M:F ratio in the pediatric group (<18) was 1.6:1, and in the adult group, 2.7:1.

There are a total of 23 liver, 12 kidney, 5 heart, and 3 heart/lung patients in this study. This corresponds to a relative frequency of 1.0% for kidney, 2.2% for liver, 1.8% for heart, and 4.6% for heart/lung transplants. The mean age of the liver allograft subpopulation is 15.2 ± 3.2 years, which is lower than that of the other organ transplant groups. This statistic reflects the large number of pediatric liver patients treated at our institution.

The yearly incidence of transplant patients who went on to develop lymphoproliferative disorders ranged from 1.4% in 1984 to 3.6% in 1982. The overall recognized frequency of PTLD development is

Table 1—Posttransplant Lymphoproliferative Disorder: Patient Population

Patient no.	Response of tumor to therapy*	Sex	Age	Organ	Transplant date	Months to tumor	Months to recurrence	Follow-up (months)
1	Regression	M	44	Heart	1/20/83	2.9	-	17.9
2	Regression	F	28	Heart/lung	11/3/86	2.2	-	13.3
3	Regression	F	62	Kidney	3/14/83	4.5	-	55.4
4	Regression	M	15	Kidney	1/6/85	6.4	-	31.3
5	Regression	M	3	Liver	8/7/84	11.2	-	31.6
6	Regression	M	7	Liver	8/6/84	7.8	11	35.0
7	Regression	M	1	Liver	4/4/85	11.7	-	23.1
8	Regression	M	2	Liver	4/10/86	2.3	-	20.1
9	Regression	M	43	Liver	2/25/86	4.4	-	19.5
10	Regression/resolution†	M	16	Kidney	12/12/82	3.5	-	59.4
11	Resolution	M	30	Heart	10/22/84	3.3	-	36.9
12	Resolution	M	56	Heart	1/19/85	3.1	-	34.2
13	Resolution	M	22	Heart/lung	5/25/83	2.1	-	17.0
14	Resolution	F	25	Kidney	1/31/80	5.3	-	92.4
15	Resolution	M	20	Kidney	4/15/81	6.1	-	77.0
16	Resolution	M	52	Kidney	8/23/81	12.2	-	66.6
17	Resolution	M	56	Kidney	2/10/82	6.2	-	66.9
18	Resolution	M	30	Kidney	7/10/82	4.5	-	63.5
19	Resolution	M	11	Kidney	5/3/85	13.7	-	20.1
20	Resolution	M	23	Kidney	8/15/86	2.2	-	16.0
21	Resolution	M	17	Liver	5/9/82	6.1	-	64.0
22	Resolution	F	21	Liver	3/20/83	6.7	-	52.9
23	Resolution	F	3	Liver	5/10/84	2.6	-	43.2
24	Resolution	M	9	Liver	9/3/85	2.9	-	26.8
25	Resolution	F	44	Liver	10/4/86	1.1	-	2.9
26	Resolution	F	3	Liver	5/5/85	22.7	-	11.0
27	Partial resolution	M	22	Heart	11/11/81	6.1	-	5.6
28	Partial resolution	F	13	Liver	8/31/77	68.3	-	4.8
29	Resolution	M	4	Liver	1/14/84	25.1	3	24.5
30	No evaluable response	M	34	Kidney	2/27/86	1.5	-	0.5
31	No evaluable response	M	20	Liver	1/12/82	7.5	-	15.9
32	No evaluable response	F	1.5	Liver	2/20/72	162.0	-	0.1
33	No response	M	20	Heart/lung	1/31/83	3.8	-	1.0
34	No response	F	8	Liver	11/1/82	26.2	24	13.3
35	No response	M	5	Liver	12/17/85	0.7	-	1.2
36	No response	M	54	Liver	8/18/86	1.2	-	0.3
37	No response	F	27	Liver	2/26/86	12.1	-	0.2
38	Autopsy diagnosis only	M	51	Heart	9/27/82	6.6	-	-
39	Autopsy diagnosis only	M	28	Kidney	2/9/80	4.0	-	-
40	Autopsy diagnosis only	F	11	Liver	9/19/85	1.9	-	-
41	Autopsy diagnosis only	F	4	Liver	8/22/85	4.0	-	-
42	Autopsy diagnosis only	F	26	Liver	9/1/83	4.3	-	-
43	Autopsy diagnosis only	M	23	Liver	10/30/83	1.8	-	-

* See Materials and Methods section.
† Some tumors resected, others allowed to regress.

1.7% in this patient series. This figure may underestimate the true incidence of the disorder since some cases are first recognized at autopsy, and the autopsy rate at the authors' institution is about 50%. No pattern of increasing or decreasing rate of PTLD development has been apparent to date. The years 1980–1982 were marked by a predominance of tumor development in renal transplant recipients. From 1983 to date, the majority of lesions have occurred within hepatic allograft recipients. This relative rise appears directly related to the increasing number of liver transplants performed at this Center.

Table 2—Immunosuppressive Regimens of 43 PTLD Patients

Drug regimen	Number
CsA-prednisone	21
CsA-prednisone + OKT3	9
CsA-prednisone + azathioprine	4
CsA-prednisone + ALG	3
CsA-prednisone + azathioprine + OKT3	3
CsA-prednisone + azathioprine + ALG	2
CsA-prednisone + TDD	1

ALG, antilymphocyte globulin; TDD, thoracic duct drainage.

Immunosuppressive protocols (Table 2)

Forty-one of the 43 patients received initial immunosuppression based on the use of CsA-prednisone

and the remaining two (No. 28 and 32) received CsA later in their course. One patient who underwent liver transplant in 1977 received azathioprine and prednisone as initial immunosuppression. This was later switched to CsA-prednisone. A second patient who was transplanted in 1972 likewise received azathioprine and prednisone and was switched to CsA-prednisone in mid 1984.

Twenty-one patients received CsA-prednisone as the sole immunosuppressive agents. The remainder received various combinations of OKT3, azathioprine, anti-lymphocyte globulin or thoracic duct drainage as summarized in Table 2.

Time to Tumor Onset (Table 1)

The time interval between organ allograft and tumor diagnosis ranged from 0.7 to 162 months with a mean of 11.5 and a median of 4.5 months. Of note, the two patients who were initially immunosuppressed with azathioprine-containing regimens had tumor diagnosed at 68.3 and 162.0 months after transplant. If these two patients are excluded from the analysis, the range of time intervals from transplant to tumor diagnosis in individuals initially immunosuppressed with CsA-prednisone regimens is 0.7–26.2 months with a mean time interval of 6.4 ± 1.0 and a median of 4.4 months. No difference in time interval was seen in those patients initially immunosuppressed with CsA who later received supplemental azathioprine. Likewise, no difference in onset time could be documented for those subsets of patients receiving either supplemental OKT3 or ALG.

Clinical Presentation (Table 3)

The clinical presentation of PTLD reflected a combination of constitutional symptoms, effects related to the sites of lymphocyte proliferation, and the presence or absence of concomitant infectious disease. The clinical presentations and their categorizations are presented in Table 3.

The 43 patients were categorized according to the predominant site of symptoms. Thirteen patients had signs and symptoms referable to the head and neck region. The most common form of presentation within this category was a mononucleosislike syndrome with fever, adenopathy, tonsillitis, and sore throat. A 14th patient presented with rectal bleeding in addition to a cervical mass. Especially in children, adenotonsillitis could lead to acute and life-threatening airway obstruction that requires urgent intervention for relief.

Twelve patients had abdominal disease, with signs and symptoms of gastrointestinal involvement in 11 of 12 cases. Fever and pain together with bowel perforation were the most common symptoms in this patient subset. One of these patients (No. 17) had, in addition to bowel perforation, prostatic obstruction that was also due to tumor. Another patient had a cervical mass in addition to rectal bleeding. The 12th patient (No. 5) had fever, anorexia, and vague abdominal pain suggestive of a mononucleosislike syndrome.

Ten patients had signs or symptoms suggestive of organ dysfunction. In five patients the presentation suggested liver involvement (No. 24, 25, 32, 36, 37). One of these patients (No. 32) had jaundice in association with a mononucleosislike syndrome. It is noteworthy that all of these patients were liver transplant recipients. Three additional patients (No. 20, 28, 30) had a clinical presentation of renal dysfunction. Two other patients (No. 2, 9) presented with evidence of pulmonary disorders. In one case this manifested as pneumothorax; in the other, as nonresolving nodular lung infiltrates after successful treatment of Pneumocystis pneumonia.

Two patients presented with other signs and symptoms. In one (No. 1), inguinal adenopathy was the initial finding. In the other (No. 34), weight loss, fever, and anorexia were noted.

The diagnosis of PTLD was first made at autopsy in six patients (No. 38–43). The final clinical presentations of these patients are tabulated in Table 3.

Associated infections (Table 4)

EBV

Specific studies regarding the virologic aspects of this disorder have been presented previously.[13] Based on serologic analysis, forty of the 43 patients had evidence of EBV infection. Twenty-eight patients had primary EBV infection, 12 had secondary infection, and 3 had past infection at the time that PTLD was diagnosed.

Not surprisingly, primary infection was more common in the younger age category. Ninety-four percent (17 of 18 cases) of pediatric cases demonstrated evidence of primary infection. In contrast, primary infections were seen in 42% of patients aged 18 years or older. Only 1 of 12 cases of reactivation infection occurred in the pediatric population.

Each of the patients with initial immunosuppressive therapy consisting of azathioprine regimens and tumor onset after an extended time interval also had evidence of primary EBV infection.

Table 3—Clinical Features of PTLDs

Patient no.	Clinical presentation	Category	Tumor sites	Surgical treatment	Change in immunosuppression: CsA (mg/kg/d)	Change in immunosuppression: Pred (mg/d)	Acyclovir	Other treatment	Outcome	Follow-up (months)
Regression										
1	Inguinal adenopathy	Other	Inguinal node	Biopsy	R21-13	R30-25	No	None	NED; expired of other causes	17.9
2	Pneumothorax	Lung	Allograft (Lung)	Open lung biopsy	R2.9–1.4	NC	No	None	NED	9.2
3	GI bleeding	GI/abd.	Stomach	Biopsy	R8-1	NC	No	None	NED	51.3
4	Sore throat	H/N	Tonsils, nasopharynx	Tonsillectomy	R6-0	R17.5-15	No	None	NED	27.2
5	Fever, anorexia vague abdominal pain	GI/abd.	Cervical nodes, retroperitoneal nodes, tonsils	Lymph node biopsy Tonsillectomy, biopsy	R19-12	R7.5-5	No	None	NED	27.5
6	Fever, tonsillitis	H/N	Tonsils, nodes		R22-11	R15-10	Yes	None	NED	30.9
7	Otitis, upper respiratory infection	H/N	Adenoids	Adenoidectomy Adenoidectomy	R35-12	R7.5-5	No	None	NED	19.0
8	Hoarseness	H/N	Adenoids		R50-30	R10-5	No	None	NED	16.0
9	Pneumocystis pneumonia	Lung	Lung	Open lung biopsy	R5.5-0	R7.5-5	No	None	NED	15.4
10	Ileum perforation	GI/abd.	Small bowel	Small bowel resection	R8-0	NC	Yes	None	NED	55.3
Resolution										
11	Cervical adenopathy	H/N	Cervical node	Biopsy	R6.6-3.3	NC	No	None	NED	32.8
12	Fever, cervical adenopathy	H/N	Cervical node	Biopsy	R5.8-2	NC	No	None	NED	30.1
13	Tonsillitis	H/N	Tonsils	Tonsillectomy	R12-3	NC	Yes	None	NED; expired of other causes	17.0
14	Ileum perforation	GI/abd.	Small bowel	Small bowel resection	R16-6	NC	No	None	NED	88.3
15	Ileum perforation	GI/abd.	Small bowel, mesentery	Small bowel resection	R18-9	R20-10	No	None	NED	72.9
16	Enlarged salivary gland	H/N	Submandibular gland	Local resection	R11-2	R20-15	No	5400 rad cervical area	NED	62.5
17	Ileum Perforation, prostate obstruction	GI/abd.	Small bowel, mesentery and prostate	TURP, small bowel resection	R8-2	NC	No	Dxrbn, Cp, Vcr	NED	62.8
18	Ileum perforation	GI/abd.	Small bowel	Small bowel resection	R10-0	NC	No	Dxrbn, Cp, Vcr	NED	59.4
19	Upper respiratory infection	H/N	Adenoid hyperplasia	Adenoidectomy	R5.5-3.6	NC	No	None	NED	16.0
20	Fever, elevated kidney function tests	Kidney	Allograft	Allograft nephrectomy	R7.1-0	R20-0	Yes	None	NED	11.9
21	Intestinal obstruction, fever	GI/abd.	Small bowel	Small bowel resection	R20-3	R25-10	No	None	NED	59.9
22	Fever, abdominal complaints	GI/abd.	Small bowel, large bowel	Small bowel resection	R13-5	R10-7.5	No	None	NED	48.8
23	Fever, upper airway obstruction	H/N	Cervical nodes, tonsils	Tonsillectomy, biopsy	R15.5-7.8	R10-5	Yes	None	NED	39.1
24	Retransplant for hepatic artery thrombosis	Liver	Hepatic hilar node	Resection	NC	NC	No	None	NED	22.7
25	Elevated LFT's, Fever	Liver	Allograft (?lymph nodes)	None	R31-20-0	R20-10-5	Yes	None	Expired other causes; autopsy declined	2.9

24	Retransplant for hepatic artery thrombosis	Liver	Hepatic hilar node	Resection	NC	NC	No	None	NED	22.7
25	Elevated LFT's, Fever	Liver	Allograft (?lymph nodes)	None	R31-20-0	R20-10-5	Yes	None	Expired other causes; autopsy declined	2.9

26	Tonsillitis	H/N	Tonsils, adenoids	Tonsillectomy	R26.5-0	R7.5-2.5	No	None	NED	6.9
27	Cervical mass	H/N	Nasopharynx, submandibular gland, lungs?	Biopsy	NC	NC	Yes	Cp, Vcr, Procb, XRT	Expired, sepsis	5.6
28	Renal mass	Kidney	Cervical lymph node, kidney, liver hilum	Resection	R8-2	R15-10	No	None	Expired after retransplant with microscopic tumor	4.8
29	Abdominal pain, mass adenopathy	GI/abd.	Gut and mesentery	Hemicolectomy, tumor resections	R5.5-0	R5-0	No	None	Alive with recurrence	20.4
NER										
30	Fever, increased renal function tests	Kidney	Allograft, kidney, marrow, nodes, spleen, liver	Allograft nephrectomy	R5.7-0	NC	Yes	None	Expired of sepsis; tumor at autopsy	0.5
31	Fever, tonsillitis	H/N	Cervical node, tonsils	Biopsy	NC	NC	No	None	Expired of laryngopspasm following ENT exam	15.9
32	Fever, sore throat, jaundice	Liver	Allograft, tonsils, nodes, small, large bowel, etc.	Tonsillectomy, Node biopsy	NC	NC	No	None	Died with tumor	0.1
NR										
33	Cervical mass, rectal bleeding	H/N; GI/abd	Small, large bowel, general lymph nodes, ureter	Biopsy	R8-2	R20-15	Yes	None	Expired with tumor	1.0
34	Fever, weight loss, anorexia	Other	Generalized (including brain and bone marrow)	Biopsy	R11-0	NC	Yes	Vcr, Dauno, Pred	Expired with tumor	13.3
35	Fever, diarrhea	GI/abd.	Gut, spleen, kidneys, abdominal nodes	Biopsy	R30-0	R10-0	Yes	None	Died with tumor	1.2
36	Elevated LFT's mimicking rejection	Liver	Allograft, kidney, pancreas, BM, nodes, etc	Biopsy	R8.9-3.3-0	R20-15-0	No	None	Expired with tumor	0.3
37	Fever, hepatomegaly, liver failure	Liver	Peritoneum	Biopsy	R2.9-0	R10-5	Yes	None	Expired with tumor	0.2
A										
38	Fever	Autopsy	Lung, adrenal	Biopsy	NC	NC	Yes	None	Died of CMV pneumonia (PTLD at autopsy)	0.0
39	Fever	Autopsy	Liver, spleen, lymph nodes, heart	None	NC	R25-20	No	None	Expired of pneumocystis	0.0
40	Multiorgan failure	Autopsy	Kidney, liver, gut, diffuse nodes	Biopsy	NC	NC	No	None	Died with tumor	0.0
41	Pneumocystis pneumonia	Autopsy	Diffuse lymph nodes, spleen	Biopsy	NC	NC	No	None	Died of sepsis	0.0
42	Multiorgan failure	Autopsy	Diffuse nodes, gut, pancreas	Biopsy	R13-0	R15-5	No	None	Expired with tumor at autopsy	0.0
43	Liver mycosis, sepsis	Autopsy	Diffuse nodes, spleen	Biopsy	NC	NC	No	None	Expired of sepsis, tumor at autopsy	0.0

PR, partial resolution; Rc, recurrence; NER, no evaluable response; A, autopsy diagnosis; LFT, liver function test; GI/Abd, gastrointestinal/abdominal presentation; H/N, head and/or neck presentation; BM, bone marrow; R, reduced; NC, no change; Dxrb, doxorubicin; Cp, cyclophosphamide; Vcr, vincristine; Dauno, daunomycin; NED, no evidence of disease.

Table 4—Epstein-Barr Virus and Other Infections in PTLD Patients

Patient no.	Infection type	Other infections
Regression		
1	Secondary	Gastroenteritis
2	Primary	None
3	Primary	Herpes simplex
4	Primary	None
5	Primary	None
6	Primary	None
7	Primary?	None
8	Primary	None
9	Past	Pneumocystis, CMV
10	Primary	None
Resolution		
11	Primary	Pneumocystis
12	Primary	Pneumocystis
13	Primary	CMV
14	Primary	None
15	Secondary	None
16	Past	Herpes simplex
17	Secondary	None
18	Secondary	Herpes simplex
19	Primary	None
20	Primary	None
21	Primary	None
22	Secondary	Fungal pneumonia, cholangitis
23	Primary	None
24	Primary	Hepatic enterococcus infection
25	Secondary	Herpes simplex
26	Primary	None
27	Primary	Pneumonia, (later sepsis)
28	Secondary	None
29	Primary	None
NER		
30	Primary	CMV, Aspergillus, Pseudomonas
31	Secondary	None
32	Primary	None
NR		
33	Primary	Cryptococcosis
34	Primary	Invasive aspergillosis
35	Primary	None
36	Secondary	None
37	Secondary	Herpes simplex
A		
38	Secondary	CMV pneumonia, (aspergillus brain abscess at post)
39	Secondary	Pneumocystis
40	Primary	CMV, Candidiasis
41	Primary	Pneumocystis
42	Past	Pneumocystis, Candidiasis
43	Primary	CMV, Pseudomonas sepsis

PR, partial resolution; Rc, recurrence; NER, no evaluable response; NR, no response; A, autopsy diagnosis.

Other infections

Approximately half of the patients had contemporaneous infections at the time of PTLD diagnosis. Pneumocystis carinii was found in six patients; five patients each had herpes simplex or cytomegalovirus infections. Infection was identified as the main cause of death in six patients.

Pathologic Features of Lesions

Gross Pathology

PTLDs may assume one of three general gross appearances: a solid tumor, a diffuse infiltrate of parenchymal organ, or enlargement of native lymphoid tissue.

Tumorous PTLDs are indistinguishable from lymphoma at a macroscopic level. The lesions appear gray-white, have a vaguely lobulated appearance, and often contain extensive areas of necrosis. The total tumor bulk can be considerable due to multicentricity and the large size (up to 15 cm) of individual tumors.

The appearance of tumors may be modified when they occur in the gastrointestinal tract (Figure 1). These lesions freely ulcerate the mucosa and penetrate the muscularis propria. This can lead to frank perforation and subsequent peritoneal implants. In most cases the mucosal aspect presents an ulceronodular appearance with an overlying green-yellow pseudomembrane.

Infiltration of organs may be inapparent at a gross level, or may lead to a mottled appearance. In several cases kidneys containing PTLD were thought at the time of surgery to have an appearance consistent with severe rejection.

Microscopic Pathology (Table 5)

The microscopic appearance of PTLD is that of a diffuse proliferation of lymphoid cells that may be characterized on the basis of the degree of lymphocyte heterogeneity.

In most cases, the entire range of recognizable B lymphocyte forms is seen. Such lesions contain varying proportions of small lymphocytes, small and large cleaved and noncleaved lymphocytes, immunoblasts, plasmacytoid and plasma cells. This type of polymorphic appearance was seen in 47 of 83 histologically evaluable specimens (Table 5, Figure 2A).

Monomorphic PTLDs are composed of uniform lymphoid cells overwhelmingly at one stage of differentiation, most commonly represented by a proliferation of either small or large noncleaved lymphocytes. Plasmacellular differentiation is in general not a feature of such lesions and the appearance is indistinguishable from that of typical non-Hodgkin's lymphomas. The histologic categorization of monomorphic PTLD was applied to 12 of 83 specimens (Figure 2B).

In occasional lesions, the degree of polymorphism was considered to be slight because most of the cells appeared to be in the latter stages of the B cell differentiation pathway (ie, plasma cells and plasmacytoid

cells). Such PTLDs were categorized as displaying "minimal polymorphism" (Table 5). Twenty-four of 83 specimens showed such an appearance. However, 16 of these 24 specimens were derived from a single patient (patient 10, Table 5).

Necrosis, which may be massive, commonly occurs in wide confluent swaths (Figure 3). Reactive neutrophils and histiocytes are commonly seen in association with necrosis. Necrosis may be seen in any form of PTLD, but in the authors' experience tends to be most pronounced in the polymorphic form.

Cells compatible with "atypical immunoblasts" were seen infrequently in this series (Figure 4). They tended to occur in polymorphic PTLDs, especially near areas of necrosis. Only one specimen of monomorphic PTLD contained rare cells categorized as atypical immunoblasts.

Extranodal PTLDs manifested as either infiltrative or tumorous processes. Infiltrative PTLDs occurred in the interstitium of various organs and appeared either as discrete foci or as coalescent areas of lymphoid/plasmacytoid cells. With small infiltrates, destruction of the underlying organ was inapparent or absent. Larger infiltrates merged imperceptibly with tumorous lesions on both a gross and microscopic level.

Secondary changes were dependent on the organ involved. In the gut, the mucosal surface sustained early and marked necrosis. In larger lesions the mucosa was ulcerated and inhabited by innumerable bacteria. Solid organs could show marked parenchymal necrosis in addition to tumor necrosis. Occasionally, the extent of parenchymal necrosis overshadowed lymphoid necrosis, especially if the PTLD was of the infiltrative type. Such disproportionate organ injury was seen particularly in the liver.

In rare cases a benign-appearing, reactive diffuse plasmacytic hyperplasia was seen in lymph nodes in cases that had concurrent or subsequent PTLD elsewhere. This hyperplasia, which technically can be classified as showing minimal polymorphism according to the present classification system, is associated with retention of underlying architecture (Figure 5). Its relationship to PTLD is undefined at present.

Analysis of Tumor Clonality (Table 5)

Immunophenotypic studies were performed on 70 specimens from 34 patients, as outlined in Table 5. Cytoplasmic immunoglobulins were visualized in many but not all cases containing an obvious plasmacytoid or large cell component. Presumptive determination of clonality was based on immunoglobulin light-chain ratios as outlined in the Material and Methods section. A conclusion regarding clonality was reached in 44 specimens; in the other 26 the results were inconclusive.

Immunoglobulin gene rearrangement studies were performed in 30 tissue specimens from 20 cases. A representative series is demonstrated in Figure 6.

As expected, immunogenotypic analysis provided a more sensitive indicator of clonality than did immunophenotypic analysis. Twenty-one specimens from 18 patients were studied by both methods (Table 5). Identical conclusions were reached in 11 specimens. In seven other specimens, DNA analysis gave conclusive results whereas immunophenotypic analysis was indeterminate. Only in three specimens from cases 5, 6, and 28 was a discrepancy between the two techniques observed. In each case, DNA analysis detected a monoclonal component that had been missed by immunocytochemical means. In seven specimens that were monoclonal by immunocytochemical analysis and in which DNA analysis was performed, however, the diagnosis of monoclonality was upheld in every instance.

These results so far indicate that, of the 43 patients, 12 had monoclonal lesions, 13 had polyclonal lesions, and 5 had separate monoclonal and polyclonal lesions. No conclusions regarding clonality have yet been reached on tissues from the other 13 patients.

In those cases in which genotypic clonality was determined, it was possible to make a semiquantitative estimate of the proportion of cells that demonstrated the clonal rearrangement, which was termed "clone strength." A value of 3+ indicates a rearranged band equal to or more intense than the main germline band; 2+ indicates a strong rearranged band, weaker than the germline band, 1+ indicates a weak band, and 0 indicates that no gene rearrangement was detected. Because the region detected by the JH probe is split by heavy chain gene rearrangements, the hybridization band from the rearranged gene will be weaker than the band from the nonrearranged gene. A 1+ clone strength could thus represent a significant underestimate if only a JH probe is used. A representative determination is demonstrated in Figure 6. Results are tabulated on Table 5.

Conclusions regarding histologic appearance and clonality were available for 58 specimens. Thirty-one such specimens had a polymorphic histology. Of these, 17 were polyclonal and 14 were monoclonal. Of 22 specimens with minimal polymorphism, 4 were polyclonal and 18 were clonal. Finally, all five evaluable monomorphic PTLDs were monoclonal.

Lymph nodes that showed a diffuse reactive plasmacytic histology and were associated with PTLD elsewhere were invariably polyclonal.

Table 5—Histologic and Clonal Analyses of PTLDs

Patient no.	Date	Site	Genotype	Strength*	Phenotype	Poly-morphism	Plasma cells	Differentiation	Necrosis	Atypical blasts	Conclusion: histology	Conclusion: clonality
Regression												
1	4/18/83	Inguinal Node	ND		Polyclonal	2	2	Plasmacytic	1	0	Polymorphic	Polyclonal
2	1/9/87	Lung	Monoclonal	1+	Monoclonal	1	2	Plasmacytoid	1	0	Min. polymorphic	Monoclonal
3	7/26/83	Gut	ND		Indeterminate	0	0	NA	0	0	Monomorphic	Indeterminate
4	7/16/85	Tonsil	Polyclonal	0	Indeterminate	2	2	Plasmacytoid	1	0	Polymorphic	Polyclonal
5	7/8/85	Node	Monoclonal	1+	Polyclonal	2	2	Plasmacytic	2	0	Polymorphic	Monoclonal
6	3/20/85	Node	ND		Polyclonal	2	1	Plasmacytic	0	0	Polymorphic	Polyclonal
	3/20/85	Tonsils	Monoclonal	1+	Polyclonal	2	2	Plasmacytic	3	0	Polymorphic	Monoclonal
	2/6/86	Axillary node	ND		Polyclonal	2	2	Plasmacytic	3	1	Polymorphic	Polyclonal
7	3/27/86	Adenoid	ND		Polyclonal	2	0	NA	3	0	Polymorphic	Polyclonal
8	6/18/86	Adenoids	ND		ND	2	2	Plasmacytic	0	0	Polymorphic	Indeterminate
9	7/7/86	Lung	ND		ND	1	1	Plasmacytoid	1	0	Min. polymorphic	Indeterminate
10	3/26/83	Gut, peritoneum	ND		Monoclonal†	1	2	Plasmacytoid	2	1‡	Min. polymorphic†	Monoclonal†
	3/26/83	Gut	ND		Polyclonal§	1	2	Plasmacytoid	2	0	Min. polymorphic§	Polyclonal§
	5/3/83	Gut	ND		Monoclonal‖	1	2	Plasmacytic	1	0	Min. polymorphic‖	Monoclonal‖
Resolution												
11	1/29/85	Cervical node	Monoclonal	2+	Monoclonal	0	0	NA	3	0	Monomorphic	Monoclonal
12	4/22/85	Cervical node	ND		Polyclonal	2	1	Plasmacytic	3	1	Polymorphic	Polyclonal
13	7/28/83	Tonsils	Polyclonal	0	Polyclonal	2	2	Plasmacytoid	3	0	Polymorphic	Polyclonal
14	7/9/80	Gut	ND		Monoclonal	2	1	Plasmacytoid	0	0	Polymorphic	Monoclonal
15	10/5/81	Gut	ND		Polyclonal	2	0	NA	2	0	Polymorphic	Polyclonal
16	8/24/82	Submaxillary gland	ND		Indeterminate	0	0	NA	3	0	Monomorphic	Indeterminate
17	8/24/82	Prostate	ND		Indeterminate	2	1	Plasmacytoid	3	0	Polymorphic	Indeterminate
	9/10/82	Gut	ND		Indeterminate	2	1	Plasmacytoid	3	0	Polymorphic	Indeterminate
18	11/23/82	Gut (a)	Monoclonal		ND	ND	ND	ND	ND	ND	–	Monoclonal
	11/23/82	Gut (b)	ND		Monoclonal	1	2	Plasmacytoid	1	1	Min. polymorphic	Monoclonal
	11/23/82	Gut (c)	ND		Polyclonal	1	2	Plasmacytoid	1	1	Min. polymorphic	Polyclonal
19	ND	ND	ND		ND	ND	ND	ND	ND	ND	–	Indeterminate
20	10/19/86	Kidney	ND		Monoclonal	2	2	Plasmacytic	3	1	Polymorphic	Monoclonal
21	11/8/82	Gut	ND		Monoclonal	2	2	Plasmacytoid	3	1	Polymorphic	Monoclonal
22	10/7/83	Gut	Monoclonal¶	0–3†	Monoclonal**	2	1	Plasmacytoid	3	1	Polymorphic¶	Monoclonal¶
	10/7/83	Gut	Polyclonal††	0	Indeterminate	2	1	Plasmacytoid	3	1	Polymorphic	Polyclonal††
23	7/26/84	Tonsils	Polyclonal	0	Polyclonal	2	1	Plasmacytoid	3	2	Polymorphic	Polyclonal
24	11/30/85	Hepatic node	Polyclonal	0	Indeterminate	2	2	Plasmacytic	0	2	Polymorphic	Polyclonal
25	11/12/86	Liver	ND		ND	2	2	Plasmacytic	0	1	Polymorphic	Indeterminate
26	3/17/87	Tonsils	Polyclonal	0	Polyclonal	2	1	Plasmacytoid	3	2	Polymorphic	Polyclonal
27	5/12/82	Cervical Node	ND		Indeterminate	2	0	NA	3	1	Polymorphic	Indeterminate
	8/26/82	Nasopharynx	ND		Indeterminate	0	0	NA	3	0	Monomorphic	Indeterminate
28	4/11/83	Kidney	Monoclonal		Polyclonal	0	0	NA	1	0	Monomorphic	Monoclonal
	9/1/83	Liver hilum	ND		ND	2	2	Plasmacytoid	3	0	Polymorphic	Indeterminate
29	2/6/86	Gut	Monoclonal	2+	Monoclonal	0	0	NA	3	0	Monomorphic	Monoclonal
	9/11/86	Abdomen	Monoclonal	2+	Monoclonal	0	0	NA	2	0	Monomorphic	Monoclonal
	2/4/87	Abdomen	ND		ND	0	0	NA	2	0	Monomorphic	Indeterminate

	2/4/87	Abdomen	ND		ND	0	0	NA	2	0	Monomorphic	Indeterminate
NER												
30	4/14/86	Kidney	ND		Indeterminate	2	1	Plasmacytic	1	0	Polymorphic	Indeterminate
	4/28/86	Autopsy	ND		Indeterminate	2	3	Plasmacytic	0	0	Polymorphic	Indeterminate
31	8/24/82	Cervical Node	ND		Polyclonal	2	2	Plasmacytic	0	0	Polymorphic	Polyclonal
	12/13/82	Tonsil	ND		Indeterminate	2	1	Plasmacytoid	2	1	Polymorphic	Indeterminate
	12/13/82	Cervical node	ND		Indeterminate	2	2	Plasmacytoid	0	0	Polymorphic	Indeterminate
32	5/24/85	Liver	ND		ND	2	1	Plasmacytic	0	0	Polymorphic	Indeterminate
	6/11/85	Tonsils	ND		Polyclonal	1	2	Plasmacytoid	2	1	Min. polymorphic	Polyclonal
	6/13/85	Autopsy	Polyclonal	0	Indeterminate	1	2	Plasmacytoid	0	1	Min. polymorphic	Polyclonal
No response												
33	5/24/83	Cervical node	Monoclonal	2+	Monoclonal	2	1	Plasmacytoid	2	1	Polymorphic	Monoclonal
	6/18/83	Ileum	ND		Indeterminate	2	2	Plasmacytic	2	0	Polymorphic	Indeterminate
	6/24/83	Gut (multiple)	ND		Indeterminate	2	2	Plasmacytoid	1	0	Polymorphic	Indeterminate
34	12/27/84	Tonsils, adenoids	Polyclonal	0	Polyclonal	2	1	Plasmacytic	0	0	Polymorphic	Polyclonal
	1/6/86	Node	ND		Indeterminate	0	0	NA		0	Monomorphic	Indeterminate
	1/20/86	Pericardium	ND		Indeterminate	0	0	NA	0	0	Monomorphic	Indeterminate
	1/31/86	Peripancreas	ND		Indeterminate	0	0	NA	0	0	Monomorphic	Indeterminate
35	2/11/86	Node	Monoclonal	2+	Indeterminate	1	2	Plasmacytoid	1	0	Min. polymorphic	Monoclonal
	2/11/86	Multiple	ND		Indeterminate	1	2	Plasmacytoid	0	0	Min. polymorphic	Indeterminate
	2/11/86	Liver	ND		Indeterminate	2	2	Plasmacytoid	0	0	Polymorphic	Indeterminate
36	9/21/86	Liver	ND		Indeterminate	2	2	Plasmacytic	0	0	Polymorphic	Indeterminate
	9/26/86	Liver	ND		Indeterminate	2	2	Plasmacytic	0	0	Polymorphic	Indeterminate
	10/2/86	Nodes (a, b)	Monoclonal§§	3+	Indeterminate	2	2	Plasmacytoid	?	1	Polymorphic	Monoclonal‡‡
	10/2/86	Node (c)	Polyclonal	0	Indeterminate	2	2	Plasmacytoid	0	1	Polymorphic	Polyclonal
37	3/3/87	Peritoneum	Monoclonal	3+	Monoclonal	0	0	NA	1	1	Monomorphic	Monoclonal
A												
38	ND	ND	ND		ND	ND	ND	ND	ND	ND	–	Indeterminate
39	ND	Node	ND		(Monoclonal)	ND	ND	ND	ND	ND	–	Monoclonal
40	11/15/85	Soft tissue	Polyclonal	0	ND	2	2	Plasmacytoid	0	0	Polymorphic	Polyclonal
41	ND	ND	ND		ND	ND	ND	ND	ND	ND	–	Indeterminate
42	ND	ND	ND		ND	ND	ND	ND	ND	ND	–	Indeterminate
43	ND	ND	ND		ND	ND	ND	ND	ND	ND	–	Indeterminate

* See text.
† Twelve gut lesions, 1 peritoneal implant.
‡ In some tumors only.
§ Single lesion.
‖ Three gut lesions.
¶ Six gut lesions.
** One gut lesion.
†† Two gut lesions.
‡‡ Two lymph nodes.

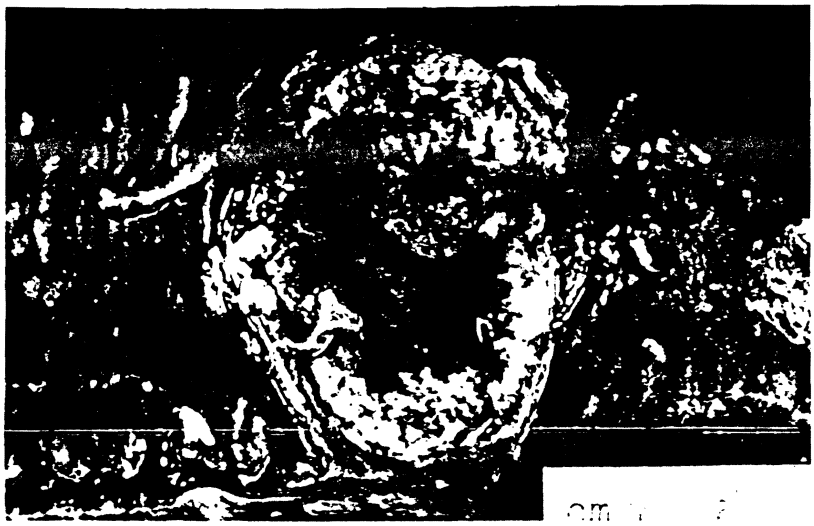

Figure 1—One of many large intestinal tumors resected from patient 10. The bowel wall has been opened longitudinally and the ulceronodular tumor is viewed from the mucosal aspect. Both monoclonal and polyclonal lesions were diagnosed. Several tumors were not resected and were observed to undergo regression during a several week period of withdrawal of immunosuppression. The patient remains free of tumor 55 months after diagnosis.

Evaluation of Multiple Specimens from Individual Patients

More than one specimen was obtained in 15 of 43 patients. Some features of interest are noted here.

Patient 10 underwent resection of multiple gastrointestinal tumors. Some of these minimally polymorphic lesions were monoclonal and others were polyclonal by immunophenotype. Clonal tumors demonstrated cytoplasmic kappa:lambda ratios ranging from 3.3:1 to 116:1. One representative lymph node was shown to have a polyclonal pattern. A tumor implant in the mesenteric fat contained lambda-containing and kappa-containing cells (intracytoplasmic) in a ratio of 92:1.

Not all tumors were removed at that time. The patient's immunosuppression was discontinued over a 5-week period, during which time he rejected his allograft. At the time of allograft nephrectomy, an attempt was made to remove residual tumors. However, it was observed that the lesions had either shrunken drastically in size or had disappeared completely. A resected length of bowel demonstrated several lesions composed predominantly of mature plasma cells in association with bowel wall repair. Cytoplasmic immunoglobulin stains of three lesions demonstrated cytoplasmic lambda:kappa ratios of 3.8:1, 25:1, and 60:1. A resected lymph node was nonclonal.

Patient 34 had a polymorphic PTLD in December 1984. She continued to have recurrent viral episodes over the following year and in 1986 presented with a widespread monomorphic tumor. This lesion was unresponsive to belated modulation of immunosuppression. Infectious complications in association with the tumor led to the patient's demise.

Patient 29 had a monomorphic tumor on several occasions. Temporary remission was obtained by surgical resections, only to have an identical-appearing tumor recur. This tumor was unresponsive to modulation of immunosuppression. The patient is currently alive with tumor.

Recurrence of a polymorphic PTLD also occurred in patient 6, who remains well to date with no further evidence of disease. The recurrence resolved under the influence of surgery and reduced immunosuppression.

Clinicopathologic Correlation

Clinical Presentation and Extent of Tumor

The manner of presentation of an individual patient offered a reasonable guide to the location of the pathologic process. Of the 12 patients who had gastrointestinal/abdominal complaints, 11 were subsequently shown to have mass lesions involving the gastrointestinal tract. The 12th patient in this category, who had more constitutional symptoms, had enlarged retroperitoneal nodes in addition to involvement of the tonsillar and cervical region.

Likewise, the 13 patients who had symptoms referable to the head and neck regions had PTLD located predominantly located at these sites. The same situation obtained in the ten patients who had evidence of organ dysfunction. A separate patient who initially had ileum perforation, was also found to have prostate obstruction (17). Subsequent biopsy revealed widespread involvement of this organ by tumor.

Although the clinical presentation is useful in localizing the major sites of PTLD, it does not necessarily

Figure 2A—Representative pleomorphic (polymorphic) proliferation from patient 23. Lymphocytes at all stages of differentiation are present in a diffuse pattern. A small area of necrosis is seen in the lower right corner. H & E, original magnification ×312.

define the extent of the disease. Patients 30 and 32–36 indicate this. Because prospective staging was not uniformly performed in these patients, the true pathologic extent of PTLD in this population can only be approximated.

A gastrointestinal/abdominal presentation is associated with a good prognostic outcome at our institution. Ten of 12 patients with this presentation are alive with follow-up times ranging from 20 to 88 months. Only one patient in this group had recurrence of tumor. The two patients who died (No. 33, 35), did so 1 month after diagnosis and had tumors at multiple sites at autopsy.

Ten of 13 patients with a head and neck site also are alive at time intervals ranging from 7 to 62 months following diagnosis. Recurrence was seen in only one patient. One of the three deaths in this group was due to unrelated causes 17 months after diagnosis. No tumor was found at autopsy (No. 13). In another patient (No. 27), tumor was eradicated by chemotherapy, but death supervened due to sepsis. Finally, a third patient (No. 31) died of laryngospasm following an ENT exam.

Presentation with organ dysfunction was not associated with a similar high survival rate. Four of 10 patients in this category are alive from 9–23 months after diagnosis. In each survivor, tumor was apparently restricted to the individual symptomatic organ, sometimes involving its contiguous lymph nodes. In contrast, nonsurvivors were generally found to have tumor at sites well-removed from the organ of presentation.

Relation of Clonal Status to Onset Time

Of the patients initially immunosuppressed with CsA, there was no apparent difference in time duration between transplant and tumor diagnosis, regardless of whether the lesion was clonal or nonclonal. The median time of onset in patients with non-clonal lesions was 4.4 months (range, 1.1–26.2), and in patients with clonal lesions this value was 4.1 months (range, 0.7–25.1).

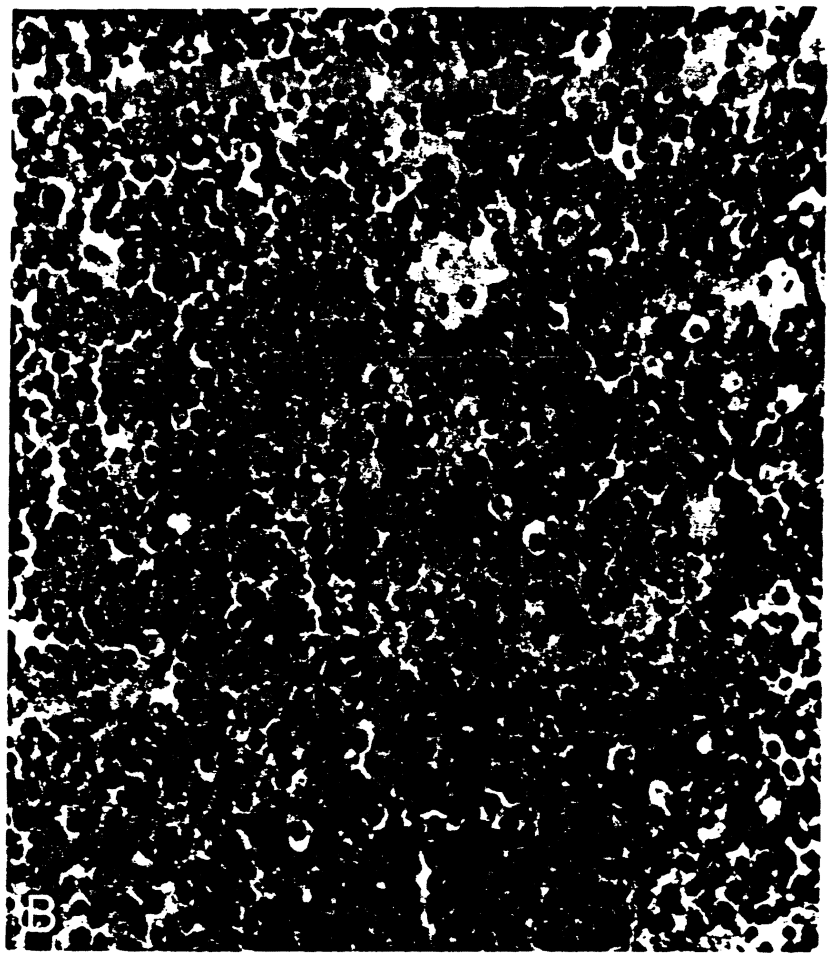

Figure 2B—Monomorphic proliferation from patient 29. Lymphoid cells are all at the same stage of development. The only heterogeneity in appearance is provided by mitotic figures, macrophages and individual necrotic cells. H & E, original magnification ×312.

Response to Therapy

Clinical regression of PTLD was seen in ten patients who received therapeutic intervention based on a reduction of immunosuppression (No. 1–10). Two patients received acyclovir and one patient had additional resection, which demonstrated involuting monoclonal tumor. Most of the tumors had a polymorphic appearance with mild to extensive necrosis.

Within this group, two patients had clonal tumors, two had clonal and nonclonal tumors, three had nonclonal lesions, and in three the clonality was indeterminate. In those cases in which an estimate of the relative proportion of cells with a rearranged phenotype was possible (band strength, Table 1), it was found to be low in comparison with control non-Hodgkin's lymphomas.

Nineteen patients (No. 11–29) had resolution of tumor. Of these 19, only four received chemotherapy or radiotherapy. The remainder were treated with reduced immunosuppression coupled with surgical resection of grossly visible tumor. The histologic and clonal characteristics of these tumors were in general similar to those of the first group. As a whole, gross disease was more advanced in this group of 17 patients. This accounted for the more aggressive surgical intervention (Table 3).

No response to therapy was seen in five patients (No. 33–37). All five patients had disease at more than one site, and usually at multiple sites (Table 3). Clonal analysis was performed in four patients and demonstrated clonal rearrangements in every case. The intensity of the rearranged bands in this group was much stronger than in those cases which had undergone regression.

One patient (No. 29) had several instances of recurrent disease. This patient had a large but well-circumscribed involvement of the abdomen by a monomorphic clonal tumor.

Six patients received a diagnosis of PTLD at autopsy (No. 39–44), precluding comment on clinical behavior. These patients had in common the presence of multiple, severe, opportunistic infections (Table 4).

Figure 3—Extensive necrosis appears as amorphous areas in this low-power photomicrograph. H & E, original magnification ×50.

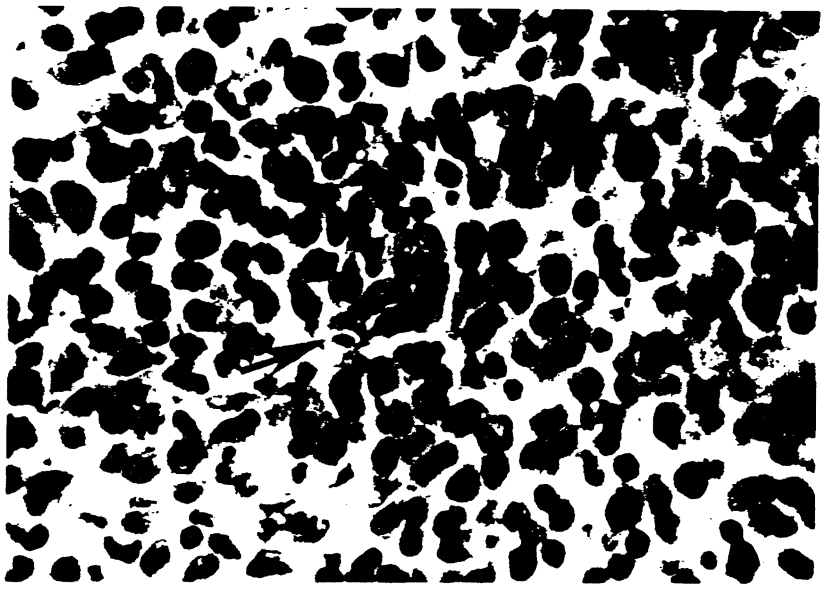

Figure 4—Large atypical blast ("atypical immunoblast") from patient 18. The multilobed nucleus is partially out of the plane of section due to its large volume. Portions of several nucleoli are also observed within this cell. The background cells demonstrate a minimal degree of polymorphism due to a preponderance of plasmacytoid forms. H & E, original magnification ×500.

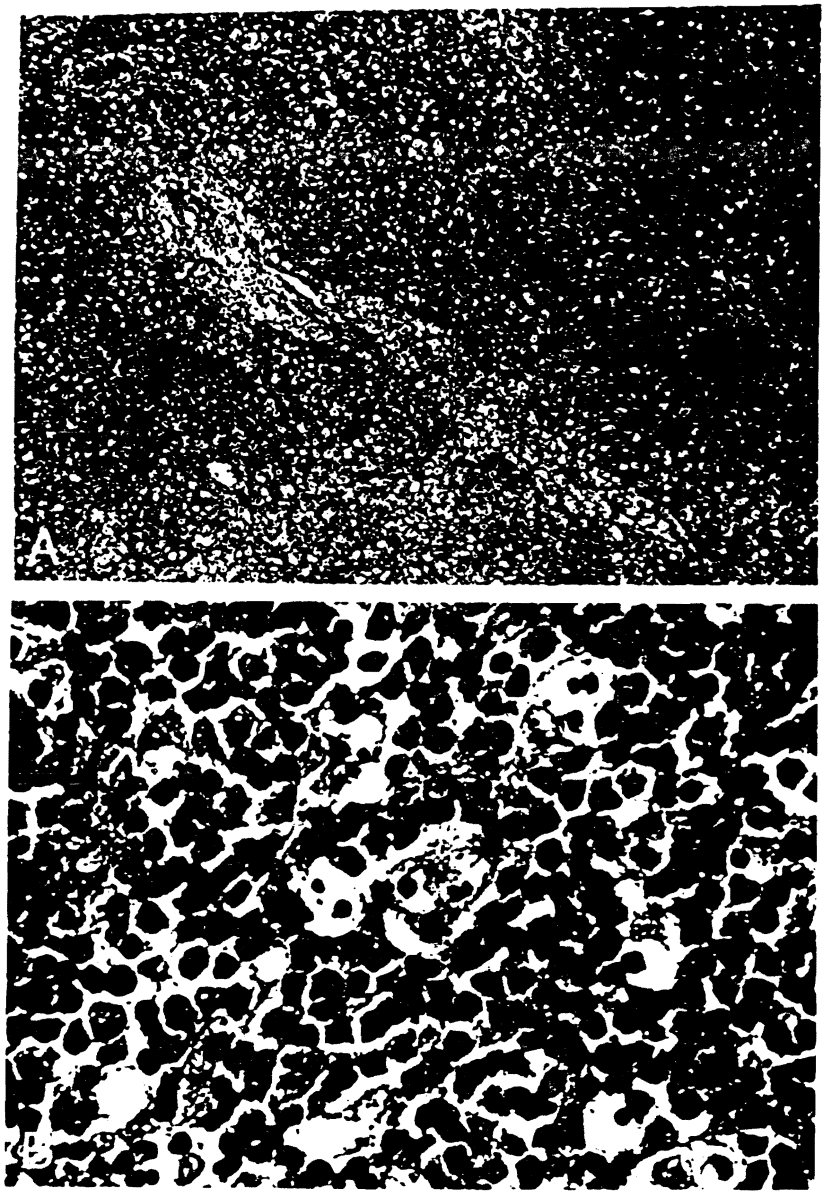

Figure 5A—Low power photomicrograph of a lymph node from patient 31. The diffuse nature of the proliferation, the "starry sky" appearance, and the preservation of sinusoids are all apparent in this low-power photomicrograph. The lesion was diagnosed as reactive hyperplasia and the patient represented several months later with more typical PTLD. H & E, original magnification ×50. B—Higher magnification of A. Several large macrophages are surrounded by smaller mature plasma cells. The lesion appears histologically benign. However, the diffuse plasmacellular infiltrate should suggest the possibility of early PTLD or the presence of PTLD elsewhere and prompt a search for EBV infection. Reduction of immunosuppression at this stage will almost invariably be associated with clinical remission.

Tumor was found in more than one site in each case (Table 3).

Discussion

The 43 allograft patients described in this report developed posttransplant lymphoproliferative disorders in conjunction with CsA-containing immunosuppressive regimens. The overall tumor incidence of 1.7% is in close agreement with figures derived from non-CsA immunosuppressed transplant populations,[18] and indicates that immunosuppression *per se* is the common denominator in these cases.

Minor differences of tumor frequency exist among the various organ allograft subpopulations in our series, however. Kidney recipients have the lowest (1.0%) and heart/lung recipients the highest (4.6%) rate of PTLD in this population. Although these differences are most likely due to differing intensities of immunosuppression in the various subpopulations, the authors wish to stress the low incidence in heart/lung recipients in contrast to previous estimates (8).

A major difference between PTLDs arising in CsA-immunosuppressed and conventionally immunosuppressed patients relates to the time of onset. The median time interval between transplant and tumor in patients initially immunosuppressed with CsA is 4.4 months in this series. Two of the 43 patients who received azathioprine but not CsA as part of the initial

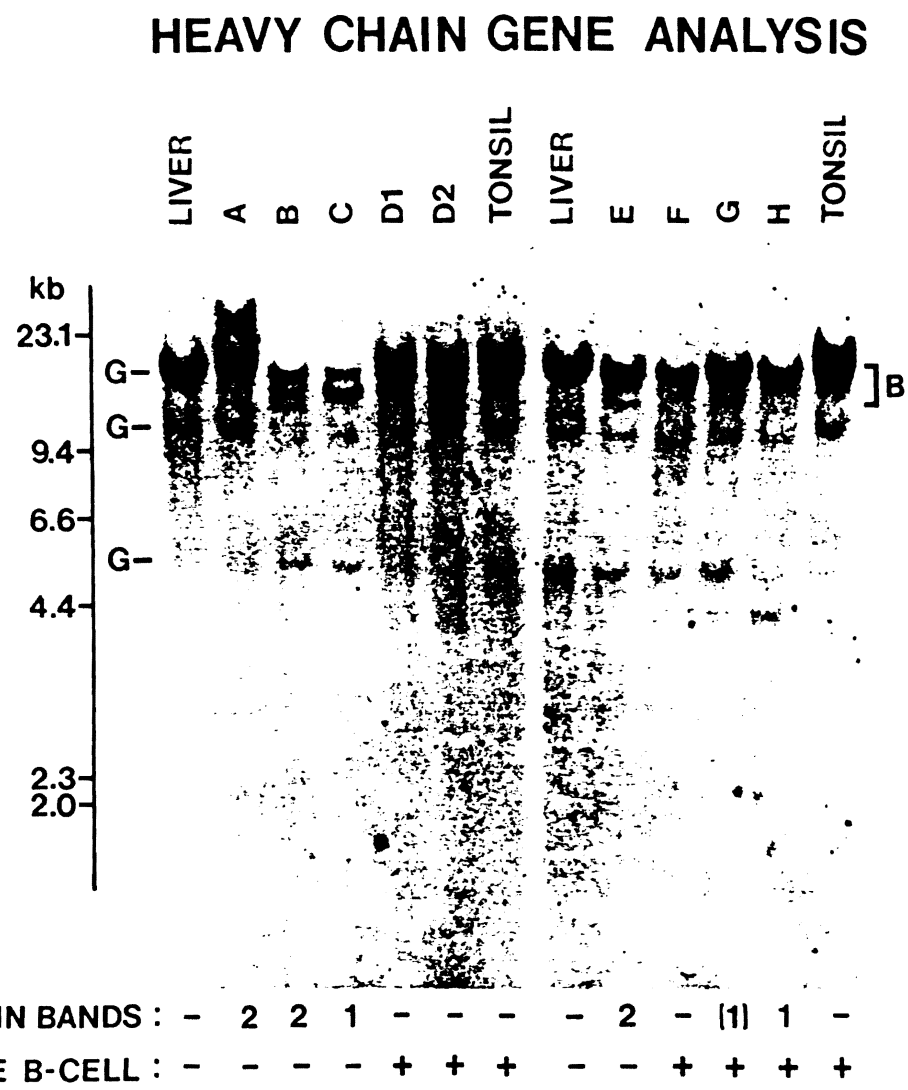

Figure 6—Immunoglobulin heavy chain gene analysis. Five micrograms (or less) of each DNA was digested with Bam HI, resolved on an 0.7% agarose gel, blotted to nitrocellulose, and hybridized to 7.5×10^5 d/min/ml of ^{32}P-labeled J_H probe. Detailed hybridization protocols have been described. Control DNAs were obtained from normal liver and hyperplastic tonsil. Both showed one prominent and two light germline bands (G). The tonsil specimen also showed a blur beneath the main germline band, caused by nonclonal gene rearrangements. This blur is characteristic of reactive B cell populations. Individual patients are designated A–H. Two separate tumors from patient D were analyzed. The DNA from patient C's tumor shows a weak germline band, suggesting deletion of the nonrearranged heavy chain gene.

immunosuppression had significantly longer intervals to tumor development. This phenomenon has been observed by others.[18] The underlying pathophysiologic mechanisms of this difference are obscure.

Presentation of PTLD falls into several general categories. Patients presenting with localized symptomatology demonstrate predominant involvement of the head and neck, gastrointestinal tract, or solid organ. Head and neck disease may range from a syndrome indistinguishable from infectious mononucleosis to a localized tumor mass. It is likely that some cases included in this category do indeed represent infectious mononucleosis. Because only a portion of transplant patients with active Epstein–Barr virus infection ever receive the generic diagnosis of "posttransplant lymphoproliferative syndrome," many probably have subclinical disease that resolves despite continued immunosuppression. The detection of a clonal rearrangement in at least one patients with a presentation otherwise consistent with infectious mononucleosis suggests caution in this regard, however.

There appears to be an affinity of PTLD for the allograft organ in cases of isolated organ involvement. It has been suggested that continued antigenic stimulation may play a role in facilitating development of these lesions. While the present study sheds no further

light on this topic, it does point out to the pathologist that this lesion must always be considered in the histologic differential diagnosis of organ allograft rejection.

The association of PTLD with Epstein–Barr virus infection, alluded to above, has been repeatedly stressed. The present series again emphasizes this relationship. More detailed analysis of the role of EBV in this transplant population is provided by Ho et al.[30]

Posttransplant lymphoproliferative tumors may be single or multiple lesions. It is important to realize that, in the case of multiple tumors, some masses may represent clonal proliferations, whereas other, concurrent lesions may represent nonclonal growths. Further, multiple clonal proliferations may or may not show identical immunoglobulin gene rearrangement patterns. The presence of an identical gene rearrangement pattern in more than one tumor is strong evidence in support of a metastatic process. In those cases in which noncontiguous clonal tumors demonstrate unique gene rearrangement patterns, the conclusion is not as clear. This result may be due to the presence of multiple independent primary tumors. Alternatively, it may represent continuing gene rearrangements within tumors that have evolved from a progenitor clone.[19] Preliminary studies from this laboratory, using a DNA probe unrelated to the immunoglobulin genes, have suggested that at least some patients have multiple clonal tumors as the result of the latter mechanism (Locker J., et al, in preparation).

Presentation with multiple tumors has been observed in other series.[12,19] The most notable cases of multiple EBV-associated posttransplant lymphoproliferative tumor presentation was that of the "bubble boy",[27] who developed this disease after receiving a T cell depleted histioincompatible bone marrow transplant. There are striking similarities between that case and some cases within the present series, including the presence of concurrent clonal and nonclonal tumors, numerous (>20) colonic lesions, and a polymorphic lymphoid tumor histology. In that case the authors demonstrated that some of the phenotypically polyclonal proliferations were genotypically oligoclonal, and one had a monoclonal component. They logically suggested a progression sequence in which a monoclonal tumor evolved from a polyclonal background.

In typical infectious mononucleosis in the nonimmunosuppressed individual, the B lymphocyte proliferation is polyclonal.[28] However, Brichacek et al, analyzing DNA from tissues of patients with fatal infectious mononucleosis, found several examples of clonal B cell proliferative infiltrates in their cases.[29] Of further interest, all of their specimens were obtained from patients who died within 1–20 weeks of symptom onset. Thus, the results indicate that the emergence of clonal proliferations may occur early in cases of fatal infectious mononucleosis. The early emergence of monoclonal lesions is also a feature of the present series, as noted above.

Perhaps the most controversial aspect of these lesions relates to claims of tumor regression under reestablished host immune controls. This report expands the initial observation of this behavior and also serves to set an upper limit on this phenomenon.

A previous histopathologic classification of PTLD was proposed by Frizzera[7] on the basis of the Minnesota transplant population. That group suggested a progression from "polymorphic diffuse B cell hyperplasia" to "polymorphic diffuse B cell lymphoma" (PBL) based on the presence of necrosis and atypical immunoblasts in the latter category. The designation of PBL was used to indicate a malignancy arising from a reactive background. With the diminution and eventual disappearance of follicular center cells the lesion would be diagnosed as an immunoblastic sarcoma.

In the authors' experience, necrosis and atypical immunoblasts do not serve as a correlate of malignant transformation in these lesions. Rather, the direction of malignant progression, in a general sense, appears to be from a polymorphic to a monomorphic histologic picture. This pathway corresponds to some degree with the distribution of clonal (monoclonal) and nonclonal (polyclonal) lesions. That is, nonclonal proliferations appear polymorphic and clonal proliferations appear either polymorphic or monomorphic. The authors have not observed this theoretical pathway of polymorphic to monomorphic evolution to occur in any individual lesion in this series, a point worth stressing.

These results indicate that nonclonal PTLDs and a subpopulation of clonal PTLDs are both capable of regression following reduced immunosuppression. Widespread clonal PTLDs do not respond in the same manner and can rapidly cause the death of the host. Most of the cases of widespread tumor in this series also were notable for delays in diagnosis, often with elevated immunosuppression during these delays. It is impossible to draw conclusions regarding the relative roles of intrinsic tumor nonresponsiveness to host control mechanisms versus massive tumor burden leading to host unresponsiveness in such cases. It is likely that some cases fall into either category. A high level of clinical suspicion may serve to reduce the occurrence of such widespread cases in the future. In the interim, it is observed that polymorphic/polyclonal

and polymorphic monoclonal tumors in general appear to be more responsive to a conservative therapeutic regimen than do monomorphic/monoclonal tumors. Monoclonal tumors that are "minimally polymorphic", ie, have a predominant plasmacellular component, also appear to be more responsive to treatment than do monomorphic/monoclonal tumors that have essentially no evidence of a plasmacytic component.

It is difficult at this point to clearly separate "malignant" from "nonmalignant" PTLDs. Clearly, in the immunosuppressed host, many of these lesions will act in a malignant fashion. In particular, monoclonal tumors that are capable of invasion, tissue destruction and metastasis should be considered malignant regardless of subsequent demonstration of regression. It is important to mentally dissociate the concepts of "malignancy" and "regression" in these tumors.

That only a subpopulation of monoclonal lesions retain the capability of regression may point to rapidly evolving tumor progression in this category of lesions.[31,32] Karyotypic analysis was not performed in many of these cases and is not reported in this study. However, Hanto et al[11] have reported clonal karyotypic abnormalities in several of their patients with PTLD. They noted regression of tumor in one patient who received CsA-containing immunosuppression, despite the presence of karyotypic abnormalities. To what degree the host and tumor each contribute to this neoplastic progression remains to be investigated. The results of investigations of this system may have benefits well beyond those applicable to this highly restricted group of patients.

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