

869

Clonal Characteristics of Posttransplant Lymphoproliferative Disorders

M.A. Nalesnik, J. Locker, R. Jaffe, A.J. Demetris, K. Hartle, J.A. Burnham, L. Makowka, and T.E. Starzl

CURRENT MODELS of neoplasia indicate a stepwise series of events as the usual mechanism by which normal cells become neoplastic cells.¹ However, cancer clinically presents as a fully malignant lesion capable of causing the death of the host. For this reason, it is presently not possible to trace the entire process that led to this condition.

The occurrence of lymphoproliferative disorders in immunosuppressed transplant recipients holds significant promise that a model of the early stages of neoplasia might exist in humans. These lesions, originally interpreted as non-Hodgkin's lymphomas, occur in association with Epstein-Barr virus infection in most cases.² Both polyclonal and monoclonal forms have been described.³⁻⁵

We previously observed that a significant number of posttransplant lymphoproliferative disorders (PTLDs) seen in association with cyclosporine A(CsA)-steroid immunosuppression were capable of regression after a reduction of immunosuppression.^{4,6}

Our continuing studies are designed to explore the limits of this phenomenon and define the host:tumor factors that are operative within this system. The present report describes a retrospective clonal analysis of tumor tissues by using recombinant DNA

probes specific for the immunoglobulin heavy chain region.

MATERIALS AND METHODS

Forty-four patients in the Pittsburgh-Denver transplant series received diagnoses of PTLDs between January 1980 and March 1987. Clonal designation was based originally on the results of immunoperoxidase staining of cytoplasmic immunoglobulins in paraffin-embedded tissues. The avidin-biotin method of Hsu et al⁷ was combined with pronase digestion. Antibodies of κ and λ immunoglobulin light chains were obtained from Dako Corp (Santa Barbara, CA).

Twenty-three patients whose lesions were studied by this technique also had sufficient tissue available for clonal analysis of B cells by using recombinant DNA methodology.

DNA purification and restriction endonuclease and Southern blot analyses were performed in accordance with protocols previously described from our laboratory.⁸ Frozen tissue specimens were pulverized in dry ice, freeze-dried, and solubilized in standard lysing solution before routine purification.⁹ High-molecular-weight DNA was digested with *Bam*H I, which optimally displayed heavy-chain gene rearrangements. The recombinant DNA probe for the joining region of the immunoglobulin heavy-chain gene (J_h) was kindly supplied by Dr Philip Leder, Harvard University.¹⁰

RESULTS

Patient Population

The group of 44 patients was composed of 30 males and 14 females (2.1:1). Ages at transplant ranged from 1 to 62 years, with a mean age of 23 years. There were 24 liver, 12 kidney, 5 heart, and 3 heart/lung recipients in the population who accounted for 2.2%, 1.0%, 1.8%, and 4.6% of the respective organ allograft subpopulations. Two patients initially received non-CsA-containing immunosuppressive regimens. Tumors in these patients occurred at 68 and 162 months posttransplant. In the remainder of the population CsA was part of the original immunosuppressive

From the Departments of Pathology and Surgery, Presbyterian University Hospital, Pittsburgh, and the Department of Pathology, Children's Hospital of Pittsburgh.

Supported in part by American Cancer Society Institutional Grant IN-58X (M.A.N.).

Address reprint requests to M.A. Nalesnik, MD, Department of Pathology, Veterans Administration Medical Center, University Drive C, Pittsburgh, PA 15240.

© 1988 by Grune & Stratton, Inc
0041-1345/88/2001-1103\$03.00/0

ttransplant orders

Hartle, J.A. Burnham

for the immunoglobulin

TRIALS AND METHODS

ents in the Pittsburgh-Dec-
ed diagnoses of PTLDs bet-
1987. Clonal designation
results of immunoperoxidase
noglobulins in paraffin-em-
bedding method of Hsu et al¹
digestion. Antibodies of g-
light chains were obtained from
(Irra, CA).

Patients whose lesions were stable had sufficient tissue available for B cells by using recombinant

and restriction endonucleases were performed in accordance described from our laboratory. Specimens were pulverized in dry ice, stabilized in standard lysing solution, and centrifuged. High-molecular weight DNA was digested with *Bam*H1, which optimally creates gene rearrangements. The recombinant joining region of the immunoglobulin (J_μ) was kindly supplied by Dr. J. D. University.¹⁰

RESULTS

五

4 patients was composed females (2:1:1). Ages from 1 to 62 years, with 3 heart/lung recipients accounted for 2.2%, 1.0% the respective organ allografts. Two patients initially containing immunosuppressive drugs. Tumors in these patients had 162 months posttransplantation. One member of the population Csa, one patient immunosuppressive

PHOMAS

Tumors in this subgroup of 42 occurred at time intervals ranging than 1 month up to 26 months. The mean time of tumor onset

group was 6.2 months. Previously reported, the majority of evidence of either primary or Epstein-Barr virus infection.¹¹

Representation

resentation of PTLD was localized to the head and neck region in 14 patients. This often manifested as a mononucleosis-like syndrome with marked tonsillar hyperplasia and/or a cervical mass. Eleven patients had disease predominant in or localized to the intestinal tract. A third category of patients had solid organ involvement. In many cases solid organ presentation, the allograft was involved by the process. A subcategory of patients in this group presented with multisystem disease. Eight other patients presented with a variety of findings from vague constitutional disorders to *Coccidioides* pneumonia.

Surgical Appearance

Ds may exist predominantly as tumor-infiltrative processes. In typical cases dominant cell has features of a B-lymphocyte. All histological stages of lympho-
saturation may be seen, a condition referred to as polymorphous or pleomorphic. Usually, a monomorphous appearance in all cells appear identical to each other. Seen. The lesions often show extensive necrosis and may contain large atypical bearing lymphoid cells.

studies

using a combination of immunocytochemical and recombinant DNA methodology we have concluded that tumors were monoclonal in 12 patients, polyclonal in 15, and both monoclonal and polyclonal (contiguous) in four patients. One patient had a polyclonal tumor that later evolved into

a probable monoclonal tumor. Clonal status has not yet been assigned in 12 cases.

In 23 cases both immunoperoxidase studies and immunoglobulin heavy-chain gene rearrangement studies were performed. A representative autoradiogram is shown in Fig 1.

Five cases were categorized as monoclonal by immunocytochemical stain. All five were also shown to be monoclonal by DNA analysis. In contrast, ten cases demonstrated a polyclonal phenotype by immunoperoxidase. By using DNA analysis two of these cases were shown to contain a monoclonal element. Six other cases were inconclusive on the basis

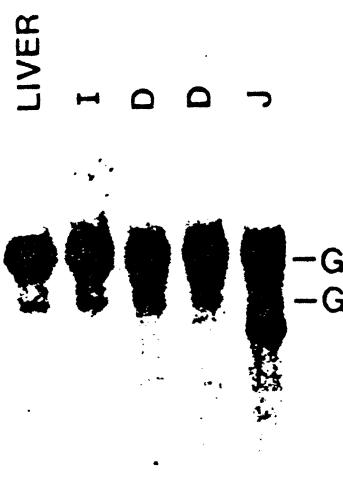


Fig 1. Autoradiogram demonstrating heavy-chain immunoglobulin gene rearrangement. Bands in each lane represent DNA hybridized to the labeled J₅ probe. Liver DNA serves as a control to define unarranged (polyclonal) pattern. Lanes I, D, D, and J show patterns obtained when using DNA obtained from tumor specimens. The extra band in lane J indicates the presence of a clone of cells with a single heavy-chain gene rearrangement. The other lanes show a germline band pattern. kb, kilobase; G, germline.

of immunoglobulin light-chain stains. DNA rearrangement patterns revealed five of these cases to be polyclonal and one to be monoclonal.

On occasion, multiple tumors were analyzed by one technique but not by another. Two cases categorized as polyclonal by phenotype had separate tumors analyzed by DNA probes. In both cases monoclonal tumors were uncovered. The converse situation was obtained in two cases in which additional tissue was available for immunoglobulin staining. Two cases categorized as either monoclonal or polyclonal on the basis of heavy-chain gene rearrangement analysis were each shown to have had separate monoclonal and polyclonal tumors when separate specimens were stained for immunoglobulin light chains.

Correlation of Histology and Clonality

A full description of the histological aspects of CsA-associated PTLDs is in preparation. In brief, a pleomorphic histology was associated with polyclonality in most but not all cases. Occasional pleomorphic cases contained a monoclonal component. In contrast, a monomorphous histology invariably implied a monoclonal tumor. In some cases, minimal pleomorphism was seen in association with prominent plasmacytoid features. These lesions were frequently associated with monoclonality.

Survival

Seven of 12 patients with monoclonal disease are alive at intervals ranging from 7 to 58 months after diagnosis. Only one of the seven received chemotherapy in the initial therapeutic regimen. There have been two cases of recurrence in these seven patients. One case of persistently recurrent disease was subsequently treated with chemotherapy. Another case resolved after reduction of immunosuppression. Five of 12 patients with monoclonal tumor died, one despite the use of chemotherapy.

Resolution of polyclonal lesions was seen in nine of 11 cases. One patient required subsequent radiotherapy for control of a lesion morphologically consistent with Hodgkin's disease. The remainder of the nine patients responded to a reduction of immunosuppression. Two of the nine later died of other causes with no evidence of tumor. One patient had recurrent polyclonal disease that again resolved. One patient developed unresponsive, widespread, presumably monoclonal disease and died of aspergillosis in association with chemotherapy.

The 5-year survival rate for the entire group of 44 patients is 63%. No difference is seen when these figures are segregated according to clonality.

DISCUSSION

The increased incidence of lymphoproliferative disorders in transplant patients was first recognized by Starzl in 1968.¹² Purtile et al suggested that Epstein-Barr virus might contribute to this disorder.¹³ This was found to be the case in studies of transplant patients at the University of Minnesota.²

These prior studies were based on patients receiving conventional immunosuppression. Differences in tumor localization, tumor onset time, and reversibility of tumors are seen when CsA-containing immunosuppressive regimens are used.¹⁴

The present studies indicate that both monoclonal and polyclonal tumors occur in association with CsA immunosuppression. Analysis of immunoglobulin genes is shown to be a more sensitive indicator of clonal status than is analysis of cytoplasmic immunoglobulin expression.

It is important to emphasize that clonal status alone does not constitute the dividing line between benign and malignant lesions in these cases. In our experience modulation of immunosuppression results in resolution of most polyclonal lesions and some monoclonal lesions. The lack of response in some cases

polyclonal lesions was seen in one patient required surgery for control of a lesion consistent with Hodgkin's. The remainder of the nine patients had reduction of immunosuppression; nine later died of other causes of tumor. One patient had additional disease that again developed unresponsiveness to presumably monoclonal disease, sarcoidosis in association with survival rate for the entire group 63%. No difference is seen as are segregated according to

DISCUSSION

The incidence of lymphoproliferative disorders in transplant patients was first reported by Starzl in 1968.¹² Purtilo first suggested that Epstein-Barr virus might contribute to the disorder.¹³ This was found to be true in a series of transplant patients at the University of Minnesota.² These studies were based on patients receiving high doses of immunosuppressive drugs, nor localization, tumor onset, or histology of tumors are seen in patients receiving immunosuppressive drugs.¹⁴

These studies indicate that both polyclonal and monoclonal tumors occur in patients receiving CsA immunosuppression. The presence of immunoglobulin genes is shown to be a reliable indicator of clonal status of cytoplasmic immunoglobulins.

It is important to emphasize that clonal status does not constitute the dividing line between benign and malignant lesions in our experience modulation of immunosuppression results in resolution of some polyclonal lesions and some monoclonal lesions, and lack of response in some cases.

This may reflect the fact that therapy was started too late in the course of the disease. Indeed, patients were as likely to die from overwhelming polyclonal disease as from overwhelming monoclonal disease (Starzl et al, unpublished data).

In a distinct minority of cases it is likely that the tumor had progressed to a stage that placed it beyond the reach of host control mechanisms. Prospective studies are underway to identify additional factors that contribute to the fully developed malignant phenotype in these tumors.

The clinician caring for a transplant patient with this syndrome should be aware that the

majority of cases will respond to a careful reduction of immunosuppression with control of local complications. Aggressive chemotherapy in these immunosuppressed patients should only be used in those rare cases in which tumor cannot be controlled by other means.

The ultimate lessons of these lesions have not yet been realized. It is likely that continued studies of PTLDs will reveal important underlying mechanisms of immunosurveillance and tumor progression in the human. Thus the importance of PTLDs may be far in excess of their sporadic occurrence in this highly select group of patients.

REFERENCES

1. Lenoir GM, Bornkamm GW: Adv Cancer Res 37:1-113, 1987
2. Saemundsen AK, Purtilo DT, Sakamoto K, et al: Cancer Res 41:4237, 1981
3. Frizzera G, Hanto DW, Gajl-Peczalska KJ, et al: Cancer Res 41:4262, 1981
4. Starzl TE, Nalesnik MA, Porter KA, et al: Lancet 1:583, 1984
5. Cleary ML, Sklar J: Lancet 1:489, 1984
6. Nalesnik MA, Starzl TE, Porter KA, et al: Transplantation 43:592, 1987
7. Hsu SM, Raine L, Fanger H: J Histochem Cytochem 29:577, 1981
8. Kunnath L, Locker J: Nucleic Acids Res 10:3877, 1982
9. Graham DE: Anal Biochem 85:609, 1978
10. Ravetch JV, Siebenlist U, Korsmeyer SJ, et al: Cell 27:583, 1981
11. Ho M, Miller G, Atchison RW, et al: J Infect Dis 152:876, 1985
12. Starzl TE: Ann Surg 168:416, 1968
13. Purtilo DT: Lab Invest 51:373, 1984
14. Penn I: Transplantation 43:32, 1987