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Clonal Characteristics of Posttransplant Lymphoproliferative Disorders

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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 June 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 μ 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*HI, 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

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Transplant Disorders

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LYMPHOMAS

Tumors in this subgroup of 42... occurred at time intervals ranging... less than 1 month up to 26 months... The mean time of tumor onset... latter group was 6.2 months.

... previously reported, the majority of... had evidence of either primary or... Epstein-Barr virus infection.¹¹

Presentation

The presentation of PTLD was localized to... and neck region in 14 patients. This... often manifested as a mononucleo-... syndrome with marked tonsillar hyper-... and/or a cervical mass. Eleven patients... predominant in or localized to the... intestinal tract. A third category of... had solid organ involvement. In many... of solid organ presentation, the allograft... was involved by the process. A subcate-... of patients in this group presented with... multsystem disease. Eight other... presented with a variety of findings... from vague constitutional disorders to... pneumonia.

Histological Appearance

PTLDs may exist predominantly as tumor-... or infiltrative processes. In typical cases... predominant cell has features of a B... lymphocyte. All histological stages of lympho-... maturation may be seen, a condition... referred to as polymorphous or pleomorphic. Conversely, a monomorphous appearance in... which all cells appear identical to each other... may be seen. The lesions often show extensive... of necrosis and may contain large atypic-... appearing lymphoid cells.

Clonal studies

By using a combination of immunocyto-... chemical and recombinant DNA methodolo-... we have concluded that tumors were... monoclonal in 12 patients, polyclonal in 15... patients, and both monoclonal and polyclonal... (noncontiguous) 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

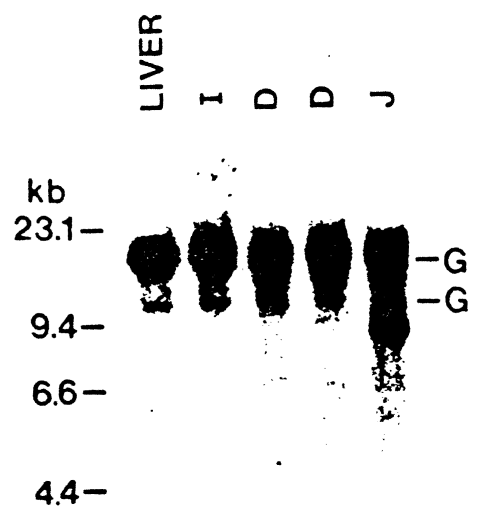


Fig 1. Autoradiogram demonstrating heavy-chain immunoglobulin gene rearrangement. Bands in each lane represent DNA hybridized to the labeled J_H probe. Liver DNA serves as a control to define unrearranged (polyclonal) pattern. Lanes I, D, D, and J show patterns obtained when using DNA obtained from tumor 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 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 Purtilo first 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

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may reflect the fact that therapy was started
too late in the course of the disease. Indeed,
patients were as likely to die from overwhelm-
ing 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 under-
way to identify additional factors that contrib-
ute to the fully developed malignant pheno-
type 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 chemother-
apy 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 con-
tinued studies of PTLDs will reveal important
underlying mechanisms of immunosurveil-
lance 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.

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