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URINARY LIGHT CHAIN EXCRETION IN LEUKAEMIA AND LYMPHOMA

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SUMMARY

Immunoglobulins are produced by plasma cells. However, tissue culture and fluorescent antibody techniques have also demonstrated that other leucocytes, mainly lymphocytes, may synthesize immunoglobulins. In the aberrant protein synthesis of myeloma there is often excessive production of electrophoretically homogeneous light polypeptide chains, appearing in the urine as Bence Jones protein. Among a group of seventy-six patients with leukaemia and lymphoma, nineteen showed quantitatively increased excretion of urinary light chains (> 100 mg/24 hr). In all instances the light chain excretion of one dominating light-chain group (κ) was present. Studies of the κ/λ ratio in serum and isolated γG-globulin were made and appeared normal in the expected 2: 1 range. By cellulose acetate electrophoresis these light polypeptide chains from the leukaemia–lymphoma group appeared considerably more heterogeneous than Bence Jones proteins from multiple myeloma.

INTRODUCTION

Using radioactive amino acid incorporation techniques, van Furth (1965) has shown that normal human lymphocytes derived from peripheral blood can synthesize the three main classes of immunoglobulins—γG, γA and γM. Moreover, human cell lines from patients with leukaemia and lymphoma have recently been studied in many laboratories and shown to be capable of immunoglobulin synthesis (Fahey *et al.*, 1966; Tanigaki *et al.*, 1966; Trujillo *et al.*, 1966; Finegold, Fahey & Granger, 1967). Of considerable interest in this regard were the demonstrations by several groups that immunoglobulins produced *in vitro* by some cultured cell lines appeared to have monoclonal properties, either being electrophoretically homogeneous (Finegold *et al.*, 1967) or showing only one dominating light-chain type (Fahey *et al.*, 1966; Tanigaki *et al.*, 1966). Pertinent to the studies to be recorded here, synthesis of isolated light chains has been noted in such cell cultures in two instances (Tanigaki *et al.*, 1966).

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The light polypeptide chains of immunoglobulin molecules are normally excreted in urine (Vaughan, Jacox & Gray, 1967) although the amount excreted is only part of the light chain material being removed from the circulation by the kidneys—a large proportion being catabolized within the healthy kidney (Solomon *et al.*, 1964; Wochner, Strober & Waldman, 1967). Urinary light chains cannot be accounted for by catabolism of the whole immunoglobulin molecule (Vaughan *et al.*, 1967); they seem to constitute 'spillover' from the normal process of immunoglobulin synthesis. During a previous study dealing primarily with multiple myeloma (Lindström *et al.*, 1968), increased light chain excretion was noted in several patients with leukaemia and lymphoma; for this reason we elected to study light chain excretion in a much larger group of patients with these same disorders. Of great interest was the striking predominance of κ type urinary light chain excretion despite apparently normal ratios of κ to λ immunoglobulin molecules in serum. In addition, urinary light chains from these patients showed a more heterogeneous electrophoretic mobility than had been recorded previously in patients with multiple myeloma.

MATERIALS AND METHODS

Seventy-six patients with leukaemia and lymphoma admitted to the University of Minnesota Hospitals during a 4-month period were studied. There were forty men and thirty-six women in the group. The mean age was 46·3 and the median age 47. Renal function was assessed by urinalysis, blood urea nitrogen and creatinine determination. Twelve patients had trace to 1+ and one 2+ proteinuria. Only two patients had a slight elevation of blood urea nitrogen (BUN) and creatinine. Control subjects were directly comparable being drawn from hospitalized patients comprising the same age range and sex distribution (Lindström *et al.*, 1968). The general diagnostic groups included are indicated in Table 1. A fresh 24-hr urine sample was collected from each patient and dialysed against cold running tapwater for 24 hr using 23/32 Visking membranes (Union Carbide Co., Chicago, Illinois) capable of containing molecules of 11–15,000 molecular weight. The urines were then concentrated by lyophilization. During the time of urine collection, a blood sample was drawn and serum separated and stored at −20°C for later study. For quantitative estimation of urinary light chain excretion, an aliquot of the lyophilized urine concentrate was reconstituted in a small volume of saline, and then used in both quantitative immune diffusion and electrophoretic procedures. Quantitations of free urinary light chains as well as γG- and γA-globulins were performed using Oudin tubes (Oudin, 1962) and specific antisera against κ or λ light chains, and γG- and γA-globulins as previously described (Lindström *et al.*, 1968; Levi, Williams & Lindström, 1968). Pools of antisera prepared against κ or λ light chains were absorbed with the opposite type of light chain to render them monospecific and showed no detectable subgroup specificity.

An indication of the electrophoretic mobilities of various urinary immunoglobulin materials excreted was sought using cellulose acetate membranes (Grunbaum, Zec & Durrum, 1963) as well as gel electrophoresis on starch or polyacrylamide gels (Raymond, 1962). Starch gels were run in 6 M-urea medium at acid pH 3·0 (Edelman & Poulik, 1961) and at alkaline pH 8·2. Polyacrylamide gel electrophoresis used an acetic acid buffer, pH 4·5, 0·15 M.

All urines studied were tested for Bence Jones proteins using the simplified heat test at 56°C in acetate buffer, pH 4·9, as described by Putnam *et al.* (1959). In many instances concentrated urinary immunoglobulin components or light chains were examined by gel

filtration with Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala) equilibrated with phosphate buffered saline at pH 8·0. This was done in order to establish the degree of size heterogeneity of light chain materials among leukaemic or lymphoma patients' urine. Such experiments established that the pools of Bence Jones monomers and polymers used as standards for quantitations (Lindström *et al.*, 1968; Levi *et al.*, 1968) were representative of the size and ratio of light chains among the groups studied here. No evidence for increased proportions of low molecular weight light chain components (less than 15,000 molecular weight) was obtained in any of the leukaemic patients' urine studied in this fashion by gel filtration.

TABLE 1. Clinical summary of seventy-six patients* with lymphoma and leukaemia studied

Diagnostic groups	No. of patients
Malignant lymphoma Malignant lymphoblastoma Lymphosarcoma	30
Hodgkin's disease	23
Acute and subacute myelogenous leukaemia	11
Chronic myelogenous leukaemia	7
Monocytic and monomyelocytic leukaemia	2
Subacute lymphatic leukaemia	1
Chronic lymphatic leukaemia	2

* Age range 16–81, mean age 46·3 and median, 47 years. Patient group constituted forty men and thirty-six women.

Because an inordinate or marked preponderance of κ type light chain excretion was noted among nineteen patients with high 24-hr urinary light chain values, an estimate of κ to λ ratios of serum immunoglobulin molecules was made using pools of anti-κ chain and anti-λ antisera adjusted to equal precipitin titre by quantitative precipitin curves (Heidelberger & Kendall, 1932; Levi *et al.*, 1968) and appropriate dilution. Such antisera were then tested by immune diffusion against individual patients' whole serum and isolated γG-globulin obtained by DEAE-cellulose chromatography (Sober *et al.*, 1956). This gave a relative estimate of serum κ/λ ratios in such patients. In addition, Oudin tube quantitations (Oudin, 1952) of serum γG-, γA- and γM-globulins were made in all patients studied and correlations with quantitative urinary light chain excretion made by computer analysis with the assistance of Dr Robert L. Evans of the Department of Biometry, University of Minnesota Medical Center.

RESULTS

Studies of urine

Quantitative data on urinary light chain excretion are shown in Fig. 1 where the separate diagnostic categories are listed in parallel. The Bence Jones heat test (Putnam *et al.*, 1959)

was negative in all seventy-six patients. In no instances were large amounts of immunoglobulin materials reacting with antisera to the Fc portion of γG-globulin or specific for γA-globulin noted. The predominant immunoglobulin components in all urines studied were related to light chains. During a previous study normal excretion of urinary light chains had generally ranged from 0 to 50 mg/24 hr. If 100 mg/24 hr is taken as the upper limit of normal for urinary light chain excretion, nineteen of our seventy-six patients (25%) showed increased excretion. Of considerable interest was the finding of marked κ-type

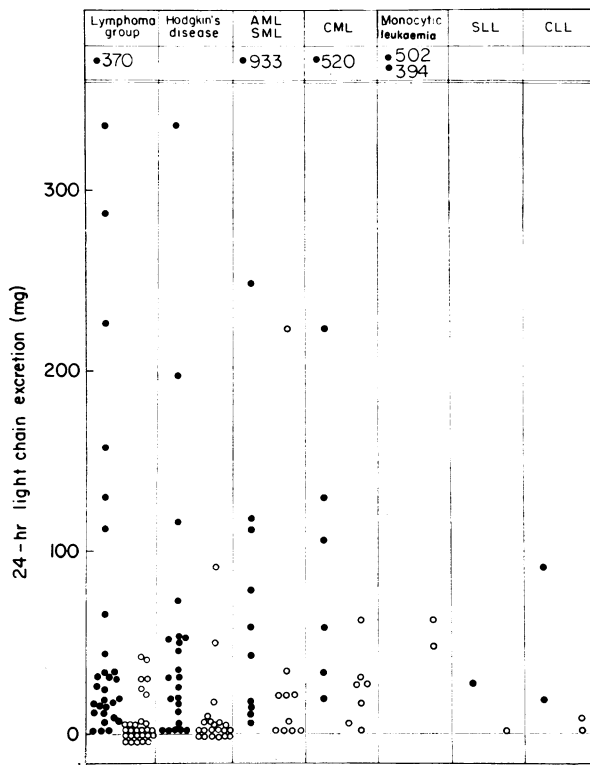

FIG. 1. Twenty-four hour urinary light chain excretion in seventy-six patients with leukaemia or lymphoma, diagnostic groups listed separately at top of figure (AML, acute myelogenous leukaemia; SML, subacute myelogenous leukaemia; CML, chronic myelogenous leukaemia; SLL, subacute lymphatic leukaemia; CLL, chronic lymphocytic leukaemia). Normal values of total 24-hr light chain excretion were 100 mg or less (9). Kappa and lambda light chains are represented by different symbols (κ, ●; λ, ○).

light chain dominance in all instances where the light chain excretion was elevated. This finding was rechecked on multiple occasions with different pools of anti-κ and anti-λ chain antisera and was found to be consistent (average κ/λ ratio among the urinary light chains was 12·5:1, ranging from 30:1 to 3·7:1). We have to date seen only one exception to this phenomenon, in a patient with chronic lymphatic leukaemia and marked hypogammaglobulinaemia studied previously (Lindström *et al.*, 1968). There was no correlation of increased urinary light chain excretion to the mild proteinuria noted in some patients. The two patients

with elevated blood urea nitrogen and creatinine levels had no quantitative increase in the amount of light chain excretion in the urine.

When the urinary concentrates from the high urinary light chain excretion cases were studied by cellulose acetate electrophoresis, it was evident that in the majority of instances excreted light chains showed broad rather heterogeneous electrophoretic mobility. In these cases the urinary light chains appeared as a wide smear-like band in the β or γ region of the strip. In this respect urinary light chain material was very similar to light polypeptide chains obtained by reduction and alkylation of Cohn Fraction II γG-globulin. In marked contrast to this was the relative electrophoretic homogeneity of many Bence Jones proteins

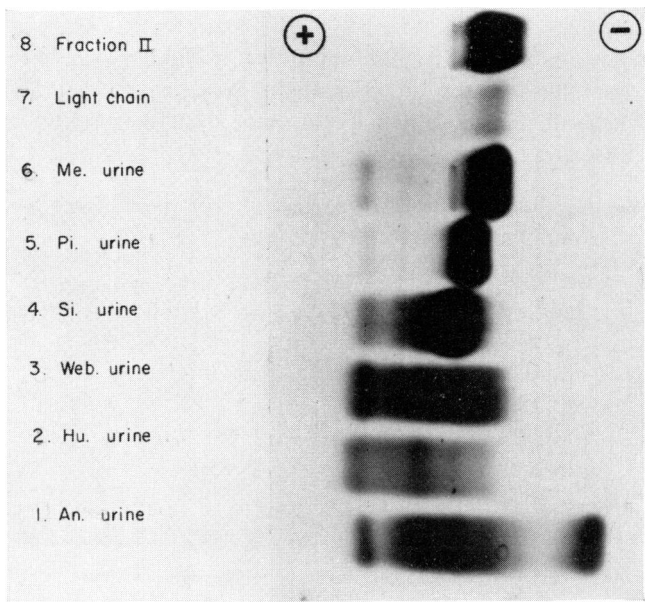

FIG. 2. Electrophoretic separation on cellulose acetate strip of urine concentrates from myeloma patients (4, 5 and 6) and patients with leukaemia (1, 2 and 3). Patient An. had monomyelocytic leukaemia and excreted high amounts of lysozyme in her urine, appearing as an extremely basic protein (cationic protein). Fraction II (8) and light chains (7) obtained by reduction and alkylation of Fraction II were also included for comparison. Note heterogeneity of light chains in leukaemia groups as compared to marked homogeneity of myeloma urinary Bence Jones proteins.

from patients with multiple myeloma and monoclonal serum or urine M-components (Waldenström, 1961). These differences are illustrated in Fig. 2. No examples of marked electrophoretic homogeneity or monoclonal banding were noted by cellulose acetate electrophoresis of urinary light chain materials among any of the patients with lymphoma or leukaemia studied.

The difference in electrophoretic patterns between relatively homogeneous urinary light chain components of myeloma and the heterogeneous electrophoretic distribution of urinary light chain materials from the patients with leukaemia or lymphoma was not reflected in detectable differences in the ratio of monomers, dimers or small fragments of

light chains between the two groups. As noted above Sephadex G-200 gel filtration of urinary γ-globulins previously separated by zone electrophoresis showed essentially similar size distributions among both leukaemia–lymphoma group and from patients with myeloma.

Studies of serum from the various patient groups

Sera from all seventy-six patients were examined by cellulose acetate electrophoresis and Oudin tube quantitations for γG-, γA- and γM-globulins. In two instances serum M-components were detected. One patient had a diagnosis of chronic myelogenous leukaemia; the other, Hodgkin's disease. Of interest was the presence in the first patient's serum of a γG M-component with λ-type light chains only, but simultaneous excretion of mainly κ-type light chains in the urine.

Estimation of κ/λ ratios of immunoglobulin molecules both in whole serum and isolated γG-globulin were completed in ten of the patients excreting more than 100 mg/24 hr of urinary light chains. In all cases dominance of urinary κ chain excretion was present and in these same instances serum κ/λ ratios averaged 2·4:1 with a range of 2:1 to 3:1. Using this

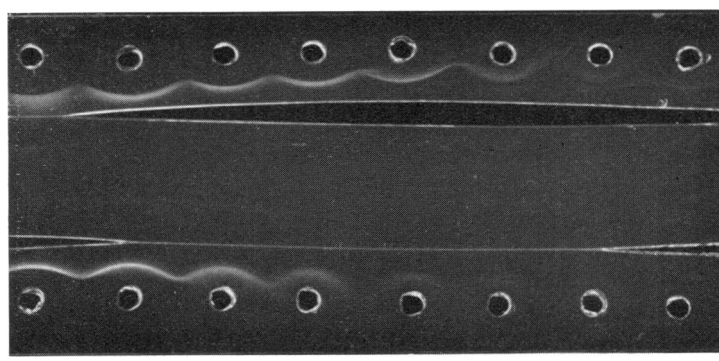

FIG. 3. Immune diffusion pattern with serial dilution of leukaemia serum in upper and lower line of wells. Anti-κ and anti-λ antisera equilibrated to equal strength by quantitative precipitin curves were used in upper and lower troughs, respectively.

method a similar κ/λ ratio was found in fifteen normal sera. A study of isolated γG-globulin confirmed these results. A representative precipitin titration using antisera carefully adjusted by quantitative precipitin curves and appropriate dilution is shown in Fig. 3. Thus, no marked aberration in serum κ/λ ratios was detected among these patients.

The effect of treatment on the urinary light chain excretion was studied in several patients. In a representative case total urinary light chain excretion dropped from 1155 to 560 mg/24 hr using 5 days of Daunomycin® treatment.

The unexpected finding of the marked κ type domination of light chains excreted by leukaemia and lymphoma patients made us try to correlate this to quantitative data regarding the three main serum immunoglobulins. This was done by computer analysis and showed little or no correlation between light chains excreted and serum γA- or γM-globulins. However, the quantities of γG-globulin correlated somewhat to the corresponding urinary light chain excretions of both κ and λ type, as demonstrated by correlation coefficients of $\rho = 0{\cdot}43$ (κ) and $\rho = 0{\cdot}42$ (λ).

DISCUSSION

The finding of many instances (nineteen out of seventy-six or 25%) of greater than 100 mg/24 hr urinary excretion of materials related to immunoglobulin light chains among patients with leukaemia and lymphoma confirms observations made on the synthesis of these chains by cell lines in tissue culture (Tanigaki *et al.*, 1966; Finegold *et al.*, 1967). However, in many of the current reports on *in vitro* leukaemic or lymphoma cell activity, most of the products seemed to be immunoglobulins in which heavy and light chains were linked together rather than isolated chains of heavy or light type. It may be that increased excretion of free light chains in the urine, as demonstrated among some leukaemic or lymphoma patients here, is a reflection of general intensified immunoglobulin synthesis and turnover. Of particular interest in this regard was the electrophoretic heterogeneity of urinary light chains from patients with lymphoma or leukaemia as studied on cellulose acetate electrophoresis. This degree of broad electrophoretic heterogeneity suggests that a wide range and variety of light chains are being produced in connection with malignant lymphoma and leukaemias and that they are by nature, therefore, essentially polyclonal as defined by Waldenström (1961). The urinary light chain heterogeneity could be related to a marked acceleration and defensive effort of the body's immune response to the leukaemic or lymphomatous process. In this connection, the interesting phenomenon of marked κ-type domination is not unique—since normally κ type immunoglobulins exceed lambda type in the ratio of 2:1 (Mannik & Kunkel, 1962); however, the striking preponderance of κ chain urinary excretion in this group of leukaemia and lymphoma patients is much more marked than would be predicted from a two to one ratio. Certainly, exceptions to the previously documented κ/λ ratio are apparent among Waldenström's γM proteins when large numbers have been studied by several groups (Wollheim & Snigurowicz, 1967; Seligmann *et al.*, 1967). The dominance of κ light chains in the urine of patients studied here cannot at present be explained. It is possible that leukaemia and lymphomatous processes affect catabolic functions for κ and λ chains in a differential manner, or that there is marked stimulation of κ chain synthesis exceeding daily fractional catabolic rate. Our studies on κ/λ ratios of serum immunoglobulins were aimed at this possibility, and showed no disproportionate ratios among the high light chain excretor patients. It is unlikely that κ chain synthesis would be accelerated beyond catabolic balance without being detectable as a shift in serum κ/λ ratios. Studies using labelled light chains and half-life determinations within individual patients are needed to clarify these points.

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