Immunology 1978 **34** 397

Free light chain synthesis by neoplastic cells in chronic lymphocytic leukaemia and non-Hodgkin's lymphoma

J. GORDON, A. R. HOWLETT & J. L. SMITH *Department of Immunology, Tenovus Research Laboratory, General Hospital, Southampton SO9 4XY*

Received 18 *April* 1977; *accepted for publication* 8 *August* 1977

Summary. Cell populations from twenty-two cases of B-cell neoplasia reflecting various stages of B-lymphocyte maturation were investigated for their ability to synthesise immunoglobulin (Ig) *in vitro*. All surface Ig positive neoplasms studied synthesised labelled Ig of the same light chain class as that expressed at the cell surface. Immature B-cell neoplasms, chronic lymphocytic leukaemia (CLL) and CLL-type lymphoma, synthesised only a minor proportion of their total protein as Ig; labelled free light chain was the only detectable secreted Ig product in thirteen cases and was in excess in the remaining four. Labelled heavy chain was detected in cell lysates in all but one case. Follicular centre cell lymphomas, neoplasms of more mature B-lymphocyte types, synthesised more of their total protein as Ig and showed a more balanced synthesis of heavy and light chains compared to the other neoplasms studied. The Ig synthesis patterns and, in particular, free light chain production, are discussed in relation to normal B-lymphocyte maturation.

INTRODUCTION

The ability of normal cells to synthesize and express or export immunoglobulin (Ig) is a reliable marker of their B-lymphocytic origin and degree of maturation (Salmon & Seligmann, 1974). Studies in mice suggest that B-cell neoplasms can also be assessed for their capacity to synthesize Ig and can be grouped accordingly (Andersson *et al.*, 1974). This concept can be extended to many human B-lymphocytic neoplasms which represent proliferations of neoplastic cells frozen at various stages of differentiation (Lukes & Collins, 1975).

Free Ig light chains in normal human serum and urine (Stevenson, 1962; Solling, 1975) have been shown to result from '*de novo*' synthesis rather than degradation of whole Ig molecules (Vaughan, Jacox & Gray, 1967). In normal rabbits and mice the production of free light chains has been demonstrated by biosynthetic studies to be associated with lymphoid populations from spleens and lymph nodes (Shapiro *et al.*, 1966; Skvortsov & Gurvich, 1968). The production of free light chains is of diagnostic significance in myeloma and in this disease are a product of mature neoplastic plasma cells (Osserman, 1965). Increased levels of urinary free light chain have been reported in leukaemia and lymphoma (Lindström *et al.*, 1969), but there is no reliable study of its frequency or monoclonality in these diseases.

It is of interest to establish the capacity of lymphoid populations to synthesize free light chain and to investigate whether this phenomenon is related to B-lymphoid maturity. For this purpose we have investigated neoplastic lymphoid populations from twenty-three patients with chronic lymphocytic leukaemia and non-Hodgkin's lymphoma.

Correspondence: Dr J. Gordon, Department of Immunology, Tenovus Research Laboratory, General Hospital, Southampton SO9 4XY.

MATERIALS AND METHODS

Cells

Lymphoma tissue was obtained at time of lymph node biopsy or splenectomy. Normal tissue was obtained from transplant donors or incidental to abdominal surgery. Biopsy tissue was finely minced and teased in cold HEPES buffered Eagle's minimal essential medium (HEPES-MEM, Biocult Laboratories, Paisley, Scotland). Cell preparations for study were obtained after Ficoll-Triosil separation (Thorsby & Bratlie, 1970). Cells collected at the interface were washed three times and the final pellet was resuspended at 5×10^6/ml in culture medium for analysis. Viability was >90% in all preparations.

Cell receptors

Rosette tests for the identification of cells with receptors for Fcγ, the C3 component of complement and for sheep erythrocytes (E) have been described fully elsewhere (Halberg, Haegert, Clein, Coombs, Feinstein & Gurner, 1973; Smith & Haegert, 1974; Payne *et al.*, 1976). Cell suspensions were stained with fluorescein-conjugated antisera to immunoglobulin heavy and light chains (Gordon *et al.*, 1977). Cell smears fixed in methanol and washed in saline were also stained with these antisera by the direct method. Controls were included in all experiments. The fluorescein labelled preparations were examined using a Leitz Orthoplan microscope fitted with a HB 200 mercury vapour Ploem illuminator.

Biosynthetic studies

Biosynthetic labelling of cells. Cells were incubated in MEM without L-leucine and supplemented with 10% heat-inactivated foetal calf serum and 1% non-essential amino acids at a concentration of 5×10^6/ml. 10–50 μCi of L-(4,5^{3}H)-leucine (58 Ci/mM) were added per 10^7 cells and the cultures were incubated in a moist 5% CO_2 atmosphere at 37° for 18 h.

The cells were separated by centrifugation and lysed in phosphate buffered saline (PBS) containing 1% Nonidet-P40 and protease inhibitors (iodoacetamide (0·25 M) and soybean trypsin inhibitor (50 μg/ml)). Lysates and extracellular fluids were dialysed for 18 h at 4° against PBS after centrifugation for 1 h at 35,000 *g* to remove cell debris.

Detection and quantification of labelled Ig. Labelled immunoglobulin was quantitated by a sandwich technique of immunoprecipitation. Five μl of sheep antiserum with specificity for human Ig was added to aliquots of both lysates and extracellular media for 30′ at 0°. Rabbit antiserum to sheep IgG was then added in sufficient amount to precipitate all the first antibody and the tubes were left for 16 h at 0° to assess total Ig production. Sheep antiserum specific for κ or λ light chains was also used as first antibody. In control experiments, antiserum specific for one light chain class precipitated radiolabelled myeloma proteins of that class only. Normal sheep serum, which had been passed down a Sepharose-4B column coupled to human Ig to remove any anti-human Ig activity, was used as first antibody in control preparations to assess co-precipitation of non-specific material.

The precipitates were washed four times by sonication in PBS containing 1 mg/ml unlabelled leucine and 0·1% NP40 before counting. The total amount of radioactivity incorporated was determined by precipitation of the culture fluids with cold 10% trichloroacetic acid.

Characterization of Ig. Immune complexes from both specific and non-specific precipitations were dissolved in 8 M urea with 0·1% SDS. After reduction and alkylation with dithiothreitol and iodoacetamide respectively, they were run concurrently with reduced and alkylated ^{125}I labelled IgM, IgG, IgA and IgD myeloma protein markers on 7·5% cross-linked polyacrylamide, sodium dodecyl sulphate gels, 95 mm in length which were subsequently cut into 80 slices and the amount of radioactivity in each slice determined (Maizel, 1966; Choules & Zimm, 1965).

RESULTS

Neoplastic cells from twelve cases of chronic lymphocytic leukaemia (CLL) synthesized detectable amounts of Ig. One case, STE, characterized as a T-cell neoplasm, did not synthesize detectable Ig (Table 1). Surface immunoglobulin (SIg) negative cases of CLL synthesized similar amounts of Ig to SIg positive cases and to those cases with detectable intracellular Ig (Table 1). Labelled light chain was the only detectable Ig product in the supernatants from ten cultures. Radioactivity was associated with both heavy and light chains in the lysates from

Table 1. Ig expression and synthesis by CLL peripheral blood lymphocytes

Patient	%E rosettes	Surface Ig			Secreted Ig			Lysate Ig			
		%	Class	Strength	Ig as % of total protein synthesized	Ig synthesized (c.p.m./10^7 cells)	*Light/heavy (molar ratio)	Ig as % of total protein synthesized	Ig synthesized (c.p.m./10^7 cells)	Light/heavy (molar ratio)	†Labelled Ig class
STE	70	0	—	—	0	0	—	0	0	—	¶
MIL	4	0	—	—	0·7	7920	LC only‡	0·4	4800	LC only	k
HUT	2	0	—	—	0·4	4080	LC only	0·4	3600	13	Mκ
HOA	1	0	—	—	0·3	4370	LC only	0·2	2645	6·9	Mλ
ASM	8	0	—	—	0·6	7200	LC only	0·8	10010	3·9	Mκ
COO§	19	90	Mκ	+	0·2	2370	LC only	0·2	2120	5.8	Mκ
HAR	1	97	Mκ	+	0·4	6720	LC only	0·9	15620	3·1	Mκ
LON	2	95	Mλ	+	0·2	3200	LC only	0·1	1560	6·0	Mλ
WHI	9	90	MDλ	+	0·3	6940	LC only	0·2	4690	2·3	Mλ
PIC	2	95	D(M)λ	++	0·1	1500	LC only	0·3	4200	1·2	Dλ
PUT¶	6	0	—	—	0·2	2800	LC only	0·4	5600	0·7	Mλ
SAU**	3	0	—	—	0·1	1600	5·6	0·4	6600	0·8	Aλ
PEA††	2	0	—	—	0·4	4000	1·5	0·3	3145	n.d.	Gκ
Normal	65	12	MDκλ	++	0·9	15090	1·1	0·6	10100	1·1	GAMκλ

* Molar ratio calculated from counts associated with heavy and light chain peaks on gel electrophoresis and based on 17 leucine residues in light chain and 38 residues in heavy chain.

† Labelled heavy chain class determined from labelled heavy chain peaks on gel electrophoresis. Labelled light chain class determined by precipitation of the supernatant Ig with antiserum specific for κ or λ light chain.

‡ Labelled light chain only and no counts were associated with heavy chain on gel electrophoresis.

§ Bone marrow preparation.

¶ 50% of cells had intracellular crystalline IgMλ.

** 80% of cells had intracellular crystalline IgAλ.

†† 30% of cells had intracellular diffuse IgGκ.

n.d., not done.

eleven cultures and exclusively with light chain in one case (MIL) (Table 1).

Neoplastic cell preparations from patients with non-Hodgkin's lymphoma synthesized detectable Ig in culture but the synthesis patterns were more variable than the CLL group (Table 2). Neoplastic cells from five patients with small non-cleaved non-follicular centre cell lymphoma synthesized excess light chain; in HER, AND and COW, labelled light chain was the only detectable supernatant Ig product. In cultures of neoplastic cells from five patients with follicular centre cell lymphoma, the imbalance between light and heavy chain synthesis was less pronounced (Table 2).

Representative gel profiles from the neoplastic cell cultures are shown in Fig. 1. In all cultures the labelled heavy chain class was identified by reference to the mobility of labelled μ, γ, δ and α myeloma chains on gel electrophoresis. The labelled light chain class was determined by precipitation of the supernatant labelled Ig with antiserum specific for κ or λ chains. In the neoplastic cultures, all the labelled Ig was precipitated with antiserum specific for one light chain class only (Tables 1 and 2). In those cases where gel analysis revealed only light chain secretion, all supernatant Ig counts precipitable by polyvalent antiserum were precipitable with antiserum specific for one light chain class and no counts above background were precipitated with antisera specific for μ, γ, α or δ heavy chains.

The patterns of Ig synthesis by lymphocyte preparations obtained from normal peripheral blood, spleen and lymph node are included in Tables 1 and 2.

DISCUSSION

Labelled free light chain was the only detectable supernatant Ig product from ten cultures of CLL lymphocytes and from three cultures of non-Hodgkin's lymphoma cells. Four CLL cases were SIg negative, suggesting that free light chain secretion by these cells may not have a surface phase. Nevertheless, surface free light chains have been demonstrated in CLL (Fu, Winchester & Kunkel, 1974) and it is possible that the surface phase of these molecules may be so short as to remain undetected when using fluorescent antibodies but not when other techniques are employed (Melchers, von Boehmer & Phillips, 1975). In one SIg positive case (HER), excess light chains were demonstrable at the cell surface by lactoperoxidase iodination suggesting in this case at least, that the light chains had a surface phase (unpublished observation).

Labelled heavy chain with excess light chain was detected in the culture supernatants from two CLL cases belonging to atypical groups; PEA had diffuse cytoplasmic IgGk (Rudders, 1976) and SAU had IgAλ cytoplasmic crystalline inclusions (Cawley *et al.*, 1976). Similarly, labelled heavy chain with excess light chain was detected in the supernatants from five non-Hodgkin's lymphoma cultures. The light to heavy chain imbalance was more pronounced in the supernatants than in the lysates suggesting not only excess light chain production but also selective light chain secretion. There is some evidence for intracellular heavy chain degradation in neoplastic plasma cells secreting light chain (Morrison *et al.*, 1974), but in all cases studied we found no evidence for labelled heavy chain fragments on gel analysis of the culture lysates or supernatants.

At completion of this study similar findings with CLL lymphocytes were published (Maino *et al.*, 1977). These workers using similar techniques found excess light chain with heavy chain in the supernatants from seven day CLL cultures. Cell death and content release during this long incubation period may have resulted in detectable supernatant heavy chain. Nevertheless, the gel analytical technique has limited sensitivity so that trace amounts of heavy chain from SIg membrane turnover may remain undetected in short term culture.

Labelled heavy chain was identified by reference to the mobility of labelled myeloma protein markers on gel electrophoresis. However, the mobility of human surface δ chain has been reported to be similar to μ chain (Finkelman *et al.*, 1976) and for this reason labelled IgD cannot be excluded from our cultures on gel analysis alone. In one CLL case (PIC) a labelled heavy chain peak with a mobility close to the myeloma δ chain marker and appreciably faster than μ chain was demonstrable. This was confirmed as δ chain by specific precipitation with antiserum. In three SIg negative cases (HOA, HUT, ASM) the intracellular heavy chain class was exclusively μ chain by specific precipitation with antiserum. In all neoplastic cell cultures the labelled light chain was of one class and corresponded to the surface light chain of the cells cultured confirming that the Ig produced was exclusive to the neoplastic cell population.

Table 2. Ig expression and synthesis by lymph node preparations from non-Hodgkin's lymphomas

Patient	Histology*	%E rosettes	Surface Ig			Secreted Ig			Lysate Ig			Labelled Ig class
			%	Class	Strength	Ig as % of total protein synthesized	Ig synthesized (c.p.m./10^7 cells)	Light/heavy (molar ratio)	Ig as % of total protein synthesized	Ig synthesized (c.p.m./10^7 cells)	Light/heavy (molar ratio)	
HER	sm non-cl non-FCC	42	58	MDκ	+	0·2	1840	LC only	0·2	2000	1·8	Mκ
AND	sm non-cl non-FCC	6	35	MDλ	+	0·1	1030	LC only	0·2	2505	—†	—λ†
COW‡	sm non-cl non-FCC	2	90	M(G)κ	+	0·2	4380	LC only	0·2	4500	—†	—κ†
RUM	sm non-cl non-FCC	38	97	M(D)κ	+++	0·8	7950	4·5	0·7	6615	1·8	Mκ
GRE‡	sm non-cl non-FCC	23	80	M(D)κ	+++	1·0	11940	2·6	1·2	13200	1·1	Mκ
KNI	sm cl FCC	70	60	Mκ	++	0·3	5400	2·2	0·3	9000	1·1	Mκ
CPR	sm cl FCC	20	20	Gλ	++	0·2	3500	2·0	0·2	3610	1·2	Gλ
JSM	sm cl FCC	30	30	Gκ	+++	0·2	3700	1·8	1·2	24410	1·2	Gκ
CAR§	lg cl FCC	65	30	Gλ	+++	1·3	15900	1·1	1·3	15750	1·0	Gλ
COL¶	sm non-cl FCC	6	95	Mκ	++	3·3	32800	1·3	0·9	8300	1·1	Mκ
Normal Lymph Node	—	53	35	MD$\kappa\lambda$	++	0·8	7920	1·1	2·2	22900	1·0	MD6$\kappa\lambda$
Normal Spleen	—	51	45	MD$\kappa\lambda$	++	6·4	96180	1·4	2·2	33710	1·4	M$\kappa\lambda$

* Lukes & Collins (1975).
† Minimal counts associated with the heavy chain region which could not be adequately resolved by gel electrophoresis.
‡ Spleen preparations.
§ 10% of cells had intracellular diffuse IgGλ.
¶ 10% of cells had intracellular diffuse IgMκ.

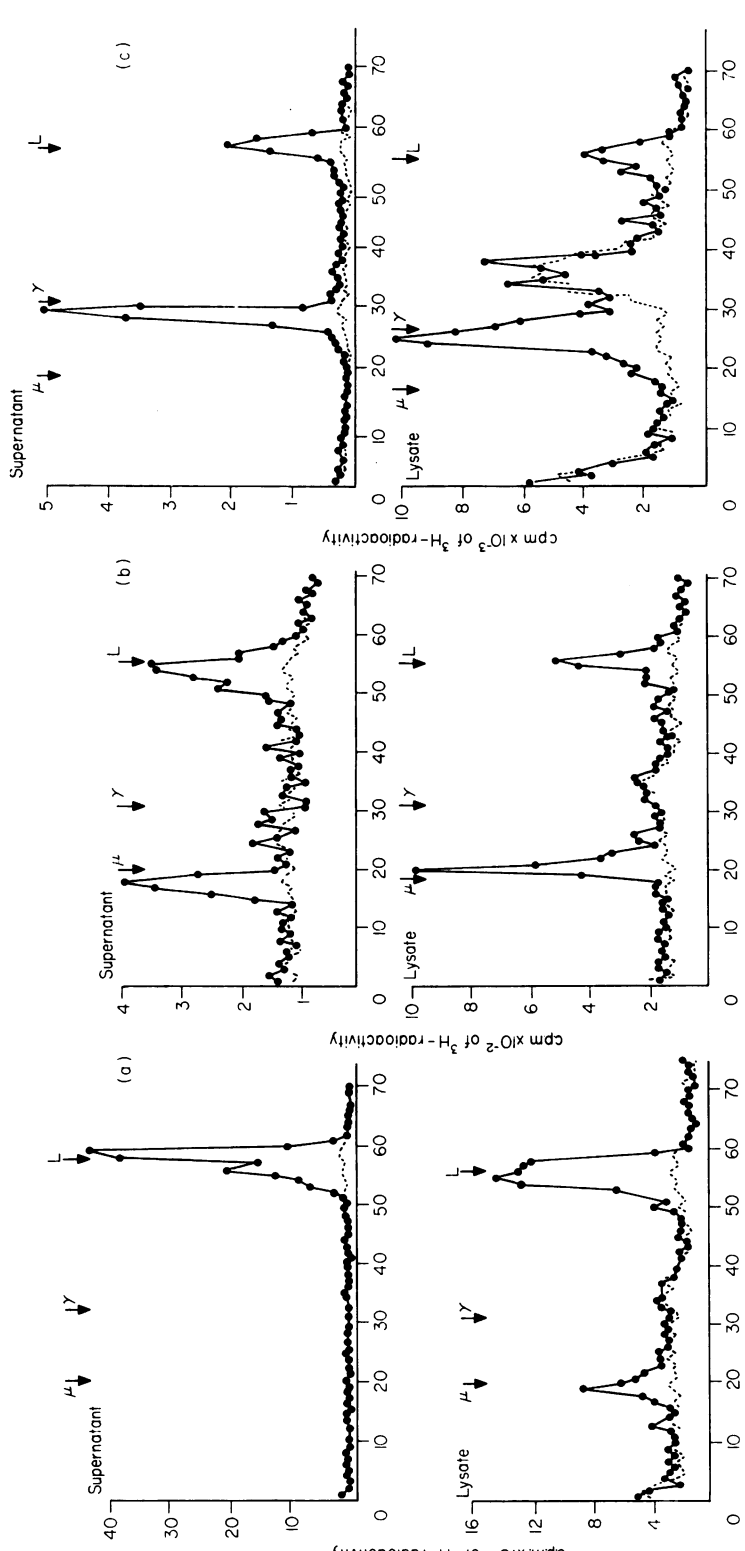

Figure 1. 7·5% polyacrylamide gel electrophoresis of fully reduced and alkylated samples showing the positions of myeloma μ, γ and light chain markers. Myeloma δ and α chain markers (not shown) had a mobility intermediate with μ and γ chains; α chain migrated slightly faster than δ chain. (a) HUT (CLL); (b) GRE (CLL-type lymphoma); (c) CAR (FCC-lymphoma).
(—) Specific precipitations; (- - - -) non-specific precipitations.

The pattern of Ig synthesis by the neoplastic cell preparations correlated with their histological type. Follicular centre cell lymphomas, neoplasms of more mature B-lymphocyte types (Lukes & Collins, 1975), synthesized more Ig as a proportion of total protein and exhibited less light chain imbalance than the other neoplasms studied. These lymphomas also stained brightly with fluorescent antibody. The most striking light chain imbalance was associated with immature B-neoplasms including CLL and CLL-type lymphomas. These immature neoplasms stained negatively or weakly for SIg consistent with the findings of Aisenberg & Wilkes (1976). All these neoplasms synthesized less Ig in culture than neoplastic plasma cells. Two cases of myeloma studied synthesized approximately 100 times more Ig than cases of CLL. In one case producing excess light chain, the secretion of free light chain was 100 times greater than seen in CLL. Reflecting this finding, we did not detect Bence-Jones protein in the urine by conventional electrophoresis of concentrated urine from the cases reported in this study.

While the possibility cannot be excluded that excess light chain synthesis is a consequence of neoplasia, the incidence and consistency of our findings in immature B-lymphocytic neoplasms, suggests that we are observing patterns of Ig synthesis related to normal cells. We have been able to demonstrate that normal human spleen and lymph node preparations synthesize excess light chain (unpublished observations) consistent with animal studies (Shapiro *et al.*, 1966; Skvortsov & Gurrvich, 1968). We believe that further investigation and elucidation of the normal B-cell populations responsible are warranted.

ACKNOWLEDGMENTS

We would like to acknowledge the Leukaemia Research Fund, the Wessex Oncology Fund and the Wessex Regional Health Authority for financial support.

REFERENCES

AISENBERG A.C. & WILKES B. (1976) Lymphosarcoma cell leukemia: the contribution of cell surface study to diagnosis. *Blood*, **48**, 707.

ANDERSSON J., BUXBAUM J., CITRONBAUM R., DOUGLAS S., FORNI L., MELCHERS F., PERNIS B. & SCOTT D. (1974) IgM producing tumours in the BALB/c mouse: a model of B cell maturation. *J. exp. Med.* **140**, 742.

CAWLEY J.C., SMITH J., GOLDSTONE A.H., EMMINES J., HAMBLIN J. & HOUGH L. (1976) IgA and IgM cytoplasmic inclusions in a series of chronic lymphocytic leukaemia. *Clin. exp. Immunol.* **23**, 78.

CHOULES G.L. & ZIMM B.H. (1965) An acrylamide gel soluble in scintillation fluid: Its application to electrophoresis at neutral and low pH. *Anal. Biochem.* **13**, 279.

FINKELMAN F.D., BOXEL J.A., ASOFSKY R. & PAUL W.L. (1976) Cell membrane IgD: demonstration of IgD on human lymphocytes by enzyme—catalysed iodination and comparison with cell surface Ig of mouse, guinea-pig and rabbit. *J. Immunol.* **116**, 1173.

FU S.M., WINCHESTER R.J. & KUNKEL H.G. (1974) Occurrence of surface IgM, IgD and free light chain on human lymphocytes. *J. exp. Med.* **139**, 451.

GORDON J., HOUGH D., KARPAS A. & SMITH J.L. (1977) Immunoglobulin expression and synthesis by human haemic cell lines. *Immunology*, **32**, 559.

HALBERG T., GURNER B.W. & COOMBS R.R.A. (1973) Opsonic adherence of sensitized ox red cells to human lymphocytes as measured by rosette formation. *Int. Arch. Allergy.* **44**, 500.

LINDSTRÖM F.D., WILLIAMS R.C. & THEOLOGIDES A. (1969) Urinary light chain excretion in leukaemia and lymphoma. *Clin. exp. Immunol.* **5**, 83.

LUKES R.J. & COLLINS R.D. (1975) New approaches to the Classification of the Lymphomata. *Brit. J. Cancer*, **31** Suppl. **ii**, 1.

MAINO V.C., KURNICK J.T., KUBO R.T. & GREY H.M. (1977) Mitogen activation of human chronic lymphatic leukemia cells. I. Synthesis and secretion of immunoglobulin. *J. Immunol.* **118**, 742.

MAIZEL J.V., JR (1966) Acrylamide gel electrophoresis by mechanical fractionation: radioactive adenovirus protein. *Science* (*Wash.*), **151**, 988.

MELCHERS F., VON BOEHMER H. & PHILLIPS R.A. (1975) B-lymphocytes subpopulations in the mouse: organ distribution and ontogeny of immunoglobulin-synthesising and of mitogen-sensitive cells. *Transplant Rev.* **25**, 26.

MORRISON S.L., BAUMAL R., BIRSHTEIN B.K., KUEHL W.M., PREUD'HOMME J.L., FRANK, L., JASEK T. & SCHARFF M.D. (1974) The identification of mouse myeloma cells which have undergone mutations in immunoglobulin production. *Cellular Selection and regulation in the immune response* (ed. by G.M. Edelman), p. 233. Raven Press, New York.

OSSERMAN E.F. (1965) Multiple myeloma. *Immunological Diseases* (Ed. by M. Samter and H. L. Alexander), p. 353. Little, Brown and Co., Boston, Massachusetts.

PAYNE S.V., JONES D.B., HAEGART D.G., SMITH J.L. & WRIGHT D.H. (1976) T and B lymphocytes and Reed-Sternberg cells in Hodgkin's disease lymph nodes and spleens. *Clin. exp. Immunol.* **24**, 280.

RUDDERS R.A. (1976) B-lymphocyte sub-populations in chronic lymphocyte leukaemia. *Blood*, **47**, 229.

SALMON S.E. & SELIGMANN M. (1974) B-cell neoplasia in man. *Lancet*, **ii**, 1230.

SHAPIRO A.L., SCHARFF M.D., MAIZEL J.V. & UHR J.W. (1966) Synthesis of excess light chains of gamma globulin by rabbit lymph node cells. *Nature*, **211**, 243.

SKVORTSOV V.T. & GURVICH A.E. (1968) Relative ratio of synthesis of immunoglobulin and light chains in rabbit spleen cells during secondary response. *Nature*, **218**, 377.

SMITH J.L. & HAEGART D. (1974) B- and T-lymphocyte markers on transformed lymphocytes from mitogen stimulated cultures of normal and CLL lymphocytes and on tonsil blasts. *Clin. exp. Immunol.* **17**, 547.

SOLLING K. (1975) Free light chains of immunoglobulins in normal serum and urine determined by radioimmunoassay. *Scand. J. clin. Lab. Invest.*, **35**, 407.

STEVENSON G.T. (1962) Further studies of the gamma-related proteins of normal urine. *J. clin. Invest.* **41**, 1190.

THORSBY E. & BRATLIE A. (1970) A rapid method for preparation of pure lymphocyte suspensions. *Histocompatibility Testing* (ed. by P. Terasaki), p. 655. Munksgaard, Copenhagen.

VAUGHAN J.H., JACOX R.F. & GRAY B.A. (1967) Light and heavy chain components of gamma globulins in urines of normal persons and patients with agammaglobulinemia. *J. Clin. Invest.* **46**, 266.