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Free immunoglobulin light chain synthesis by lymphocytes from patients with hypogammaglobulinaemia

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SUMMARY

Lymphocytes from six individuals with reduced or deficient serum immunoglobulins of one or more class were assessed for immunoglobulin production *in vitro*. There was no clinical evidence of a secondary cause for hypogammaglobulinaemia and three of the cases were receiving immunoglobulin therapy. The predominant immunoglobulin product in the culture supernatants from four cases was free light chain. In contrast, peripheral lymphocytes from two cases with partial IgA deficiency and from normal individuals exhibited a balanced synthesis of heavy and light chains. These findings are discussed with particular reference to free light chain synthesis by immature neoplastic B lymphocytes and to normal B lymphocyte differentiation.

INTRODUCTION

Patients with variable immune deficiency and hypogammaglobulinaemia of non-Bruton's type (Fudenberg *et al.*, 1971) are characterized by the presence of variable numbers of B cells in the lymphoid tissues (Cooper, Lawton & Bockman, 1971; Grey, Rabellino & Pirofsky, 1974), and are heterogeneous both in clinical presentation and cell marker characteristics (Dickler *et al.*, 1974). Many studies on the nature of the lesion in acquired hypogammaglobulinaemia indicate a marked heterogeneity between cases, characterized by defects at various stages of the maturation of B cells into immunoglobulin-secreting cells. Cells from a subgroup of hypogammaglobulinaemic patients can be stimulated *in vitro* to produce intracellular immunoglobulin by pokeweed mitogen or by tetanus-induced lymphocyte mitogenic factor (Wu, Lawton & Cooper, 1973; Geha *et al.*, 1974). Despite stimulation, however, there appears to be a terminal block in secretion in some of these cases which has been attributed to a failure of IgG glycoslization (Ciccimara *et al.*, 1976). Waldmann *et al.*. (1974) suggest that the failure of cells to mature may not be an inherent defect of B cells alone but may also result from increased suppressor T cell activity in hypogammaglobulinaemia. There is no doubt that the nature of the lesion in hypogammaglobulinaemia is multiple (Schwartz *et al.*, 1977; de la Concha *et al.*, 1977), characterized by a failure of B cells to differentiate to plasma cells.

We have demonstrated recently that free immunoglobulin light chain is the predominant Ig product from neoplastic B cells in chronic lymphatic leukaemia, leukaemic reticuloendotheliosis and well-differentiated non-Hodgkin's lymphoma (Gordon, Howlett & Smith, 1978; Gordon & Smith, 1978). The consistency of our findings suggests that we are observing patterns of synthesis related to normal B lymphocyte differentiation. In this study we report the capacity of lymphocytes from patients with variable immunodeficiency to synthesize and secrete free light chain and discuss the relationship of these findings to our study of neoplastic disease and normal B cell maturation.

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MATERIALS AND METHODS

Patients and cell preparations. The patients WIL, KCA and SYK in this study were unrelated and had clinical histories compatible with common variable immunodeficiency. One family was investigated. Asymptomatic LCA, sister of KCA, was found to have hypogammaglobulinaemia on investigation of the family; two other siblings in this family (MCA, BRE) are also included, although neither had clinical symptoms.

SYK, KCA and LCA had vitiligo. None of the patients studied had evidence for leukaemia/lymphoma and their ages ranged from 14 to 28 years. WIL, SYK and KCA were receiving regular supplementary immunoglobulin therapy.

Peripheral blood from the patients and normal controls was collected into preservative-free heparin and layered over Ficoll–Triosil (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 (Hallberg, Gurner & Coombs, 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.

Immunoglobulin synthesis. Biosynthetic labelling techniques and the subsequent characterization and quantification of labelled Ig have been described in detail elsewhere (Gordon *et al.*, 1977). Briefly, cells at 5×10^6/ml were incubated in leucine-free medium containing ^{3}H-leucine at 50 μCi/ml for 18 hr at 37°C. Cells were separated from the supernatants by centrifugation (1500 *g*, 15 min) and lysed with phosphate buffered saline (PBS) containing detergent NP40 and proteolytic inhibitors. Both the cell lysates and supernatants were spun at 35,000 *g* to remove cell debris and dialysed exhaustively against PBS. The radioactivity incorporated in all macromolecules was determined by precipitation with 10% trichloroacetic acid (TCA). Labelled Ig was precipitated using a sandwich technique with sheep antiserum specific for human Ig as the first antibody and rabbit antiserum with activity to sheep Ig as the second antibody. Normal sheep serum was used as the first antibody in control precipitations. These Ig precipitates were washed three times with cold PBS prior to counting or preparation for gel analysis. Reduced and alkylated Ig precipitates were analysed on 9·5 cm long 7·5% SDS-polyacrylamide gel electrophoresis with radioactive myeloma Ig markers.

RESULTS

The patient and Ig synthesis data are summarized in Table 1. All patients had one or more serum Ig level below the normal range and all had detectable circulating surface immunoglobulin positive cells. ^{3}H-leucine was incorporated into immunoglobulin by all cell preparations studied. The incorporation into total TCA precipitable intracellular and secreted protein was similar for patients and normals.

A gel profile of the ^{3}H-leucine-labelled supernatant Ig from WIL is illustrated in Fig. 1. The gel profile from a normal peripheral blood culture is illustrated in Fig. 2 for comparison. In one case (LCA) the labelled supernatant Ig was precipitated with both k and λ light chain specific antisera; the gel profiles are shown in Fig. 3. The incorporation of radioactivity into the supernatant heavy and light chain immunoglobulin from all patients studied is given in Table 2. These ratios have been determined from the gel profiles. ^{3}H-leucine-labelled lysate Ig from the patients with severe serum Ig deficiency could not be resolved adequately on gel analysis due to low synthetic rate and relatively high background counts.

DISCUSSION

Despite a normal distribution of surface markers, lymphocytes from all patients, except BRE and MCA, synthesized less than 0·25% of their total protein as Ig *in vitro*. In comparison, normal lymphocytes synthesized more Ig as a proportion of their total protein when cultured under the same conditions and the average for six normals from this laboratory was 1·5%. These observations are consistent with earlier findings (Choi, Bigger & Good, 1972). In contrast the incorporation into total TCA precipitable protein was similar for all preparations studied thus suggesting a selective reduction of Ig synthesis in those patients with hypogammaglobulinaemia. The significant finding in this study was that the Ig synthesis by cells from WIL, SYK, LCA and KCA exhibited synthetic imbalance of combined and free

TABLE 1. Patient and Ig synthesis data

Patient	Serum Ig (g/l)			Percentage of lymphocyte surface markers				Ig synthesis (cpm/10^7 cells) Supernatant			Lysate		
	G	A	M	E	Fc	C3	SIg*	Ig†	NSS‡	Ig (% total)§	Ig	NSS	Ig (% total)
WIL	2·6	0·07	0·06	80	10	22	6	2249	1354	0·02	12,610	11,292	< 0·1
KCA**	2·8	0·03	0·05	67	39	17	16	2042	792	0·02	11,123	9083	< 0·1
LCA**	1·8	0·05	0·6	66	25	22	4	3599	1342	0·05	18,640	10,930	0·2
SYK	3·8	1·4	0·6	74	24	18	16	3749	1704	0·06	16,851	13,767	0·1
BRE**	11·6	0·08	1·3	—	—	—	7	19,432	3780	0·4	27,830	10,316	0·5
MCA**	10·4	0·5	1·1	—	—	—	9	26,642	2148	0·9	28,776	12,194	0·7
Normal 1								39,672	1834	0·9	34,328	14,784	0·5
Normal 2	7·2–13·0¶	1·0–3·6	0·5–1·5	50–70¶	15–35	10–20	7–17	24,632	2764	0·6	30,376	11,342	0·5

* Surface immunoglobulin determined after prior incubation at 37°C for 1 hr.
† Number of counts precipitated with polyvalent Ig antiserum.
‡ Number of counts precipitated with normal sheep serum.
§ Labelled Ig expressed as a percentage of labelled protein precipitated by 10% TCA.
¶ Normal adult range for our laboratory.
** Siblings.

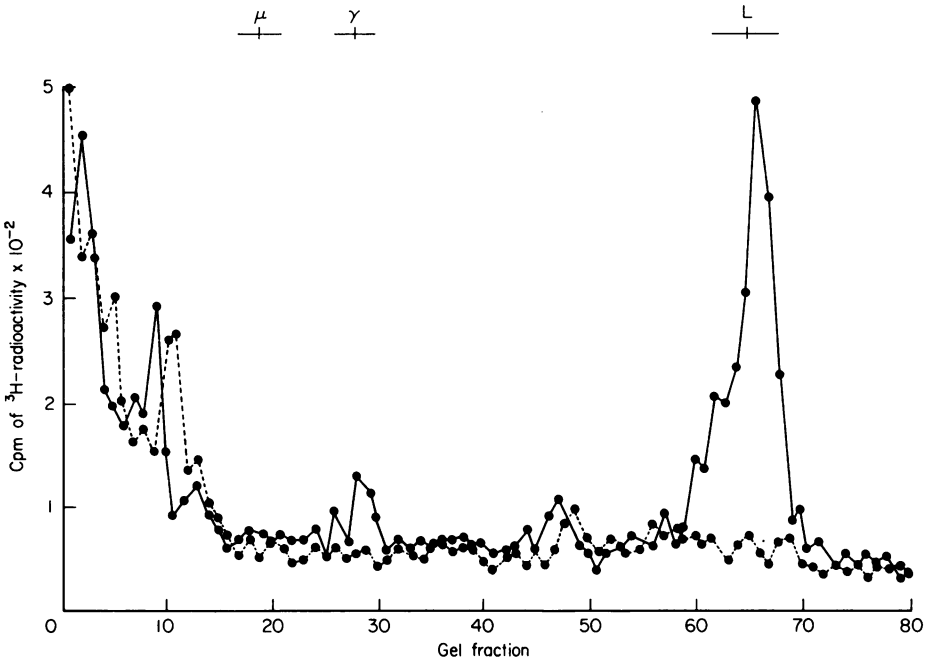

FIG. 1. 7·5% polyacrylamide gel electrophoresis of reduced and alkylated immunoglobulin from WIL supernatant. The positions of myeloma μ, γ and light chain (L) markers are also shown. (●——●) Specific precipitations. (● - - - ●) Non-specific precipitations.

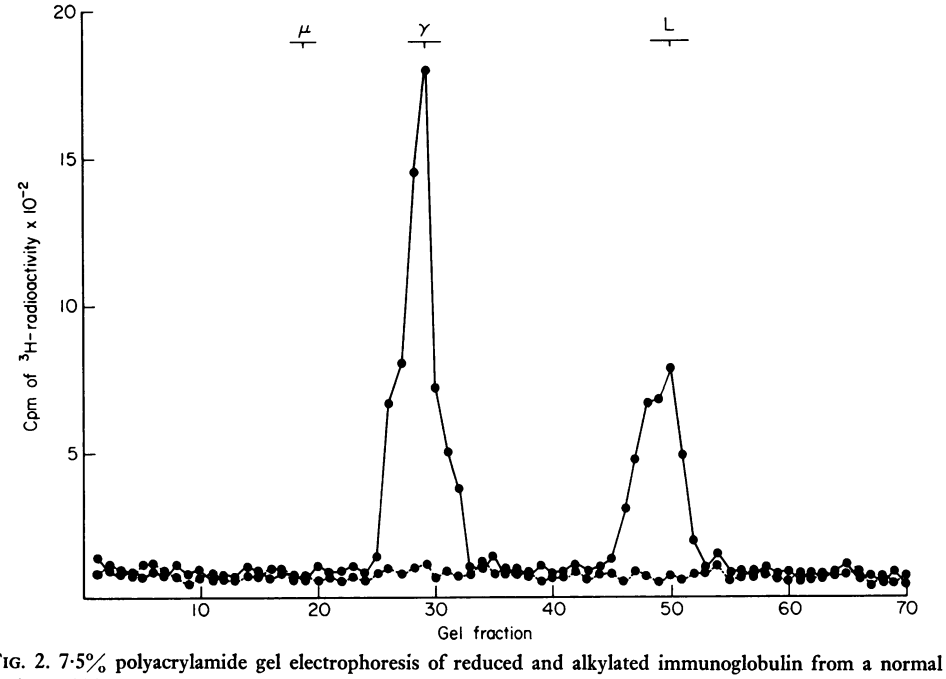

FIG. 2. 7·5% polyacrylamide gel electrophoresis of reduced and alkylated immunoglobulin from a normal peripheral blood supernatant. IgG was the predominant Ig synthesized by peripheral blood lymphocytes from five of seven normal individuals studied. κ and λ light chains were associated with labelled supernatant Ig at a ratio of 1·8:1. (●——●) Specific precipitations. (● - - - ●) Non-specific precipitations.

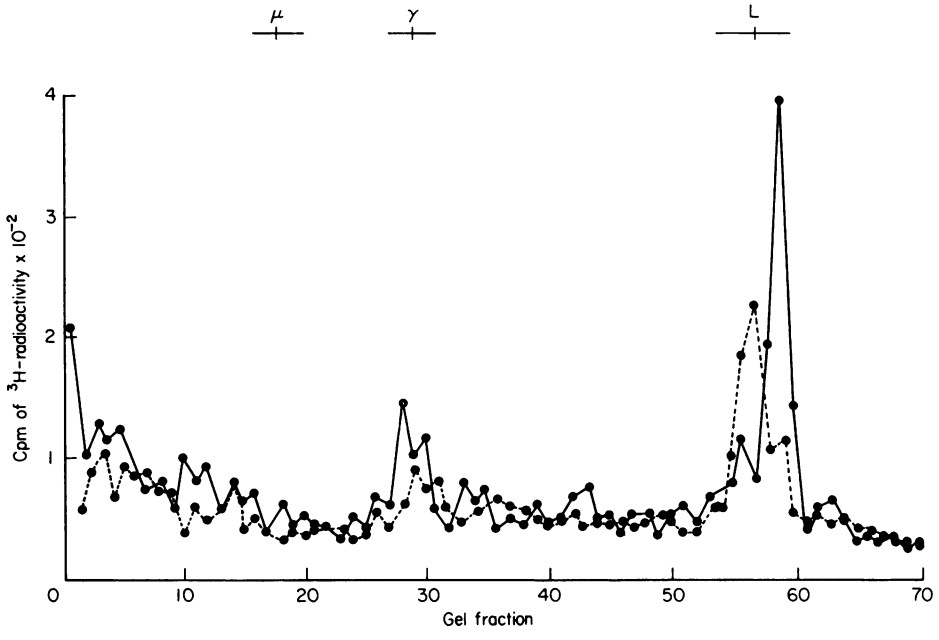

FIG. 3. 7·5% polyacrylamide gel electrophoresis of reduced and alkylated immunoglobulin precipitated with light chain class-specific antisera from LCA supernatant. (●——●) Precipitated with κ light chain specific antiserum. (● - - - ●) Precipitated with λ light chain specific antiserum.

TABLE 2. Gel profile analyses

Patient	Percentage supernatant Ig radioactivity associated with:*		Supernatant $\frac{\text{light}}{\text{heavy}}$ chain molar ratio†
	Heavy chain	Light chain	
WIL	12	88	17·8
KCA‡	19	81	9·9
LCA‡	25	75	7·2
SYK	27	73	6·3
BRE‡	60	40	1·5
MCA‡	68	32	1·1
Normal 1	68	32	1·1
Normal 2	64	36	1·3

* Calculated from peaks on gel electrophoresis.
† Based on seventeen leucine residues in light chain and thirty-eight residues in heavy chain.
‡ Siblings.

light chain, and free light chain was the predominant supernatant-labelled Ig product, Within the family studied, BRE and MCA, who both had partial serum IgA deficiency, did not exhibit synthetic imbalance in contrast to LCA and KCA. In one patient (LCA) the labelled free light chain was precipitated by both *k* and λ light chain specific antiserum and not by one class exclusively, suggesting in this case at least, that excess light chain was not the product of an isolated clone.

Marked synthetic imbalance was not observed in cultures of normal peripheral blood (Table 2). In our

laboratory the normal range for the molar light to heavy chain ratio for at least six individuals was, for peripheral blood 1·0–1·5, for spleen 1·4–2·3 and for lymph node 1·2–5·6 (Gordon & Smith, 1978; Gordon, PhD. thesis, 1978). These findings do suggest that synthetic imbalance occurs in normal lymph node and spleen which is consistent with animal studies (Shapiro et al, 1966; Skvortsov & Gurvich, 1968), but the B cell populations responsible have yet to be investigated.

It is significant to compare the findings in this paper with the Ig synthesis patterns of neoplastic lymphocytes from patients with chronic lymphocytic leukaemia and non-Hodgkin's lymphoma. Neoplastic lymphocyte cultures from patients with chronic lymphocytic leukaemia and non-follicular centre cell lymphoma, synthesize and secrete predominantly free light chain, in contrast to neoplasms of other B lymphocytic types, which exhibit a more balanced synthesis of heavy and light chains (Maino *et al.*, 1977; Gordon *et al.*, 1978). The lymphoma classification scheme proposed by Lukes & Collins (1975), together with cell surface Ig and marker expression (Aisenberg & Wilkes, 1976; Payne *et al.*, 1977) and neoplastic cell capacity to synthesize Ig (Andersson *et al.*, 1974), suggest that we are observing a pattern of Ig synthesis related to immature B lymphocytic neoplasms (Gordon & Smith, 1978).

There is no doubt that the lesion in hypogammaglobulinaemia is multiple (Schwartz *et al.*, 1977) and while the possibility cannot be excluded that B lymphocytes in some cases of hypogammaglobulinaemia may be abnormal (de la Concha *et al.*, 1977), the fact that cells from some patients can be stimulated to differentiate to Ig production *in vitro* suggests that in these cases at least, B cells are arrested at an early stage of maturation (Wu *et al.*, 1973; Geha *et al.*, 1974). Furthermore, the striking similarity between the Ig synthesis patterns of neoplastic B lymphocytes and B lymphocytes from the cases of immunodeficiency studied, suggest that we may be observing patterns of synthesis related to normal B lymphocyte differentiation. Further studies are required to establish the significance of this phenomenon in hypogammaglobulinaemia and in the ontogeny of normal B lymphocytes.

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