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Depressed classical complement pathway activities in chronic lymphocytic leukaemia

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SUMMARY

Haemolytic activities of the classical and alternative complement pathways, and levels of C1, C4, C3, factor B and C1 inhibitor (C1-INH) were measured in 85 serum samples from 46 patients with chronic lymphocytic leukaemia (CLL). Significantly decreased mean C1 and C4 levels were found, and the haemolytic activities of these components were low or low normal in more than 50% of the sera tested. In 15 sera from 5 patients a complement profile characteristic of acquired C1-IHN deficiency was observed. These results indicate that the depression of the activity of the classical complement pathway is a frequently occurring feature in CLL.

Keywords CLL complement C1 esterase inhibitor

INTRODUCTION

Recently we performed serial complement measurements in patients with different types of leukaemia (Minh *et al.*, 1983). In acute myeloid leukaemia, acute lymphocytic leukaemia and in the chronic phase of chronic myeloid leukaemia elevated complement levels were mostly observed, whereas normal or even slightly depressed (CH_{50} and C4 levels) complement values were found in chronic lymphocytic leukaemia (CLL). Among the eight CLL cases examined, we observed a patient with recurrent herpetic eruptions who showed a profound depression of the classical pathway activities. On the basis of repeated complement measurements, a diagnosis of acquired C1-esterase inhibitor deficiency was made (Minh *et al.*, 1983b). Later a second case was found and this prompted us to carry out a systematic study on the incidence of depressed classical complement pathway activities in patients with CLL. As shown in our present work, this depression is a feature frequently seen in CLL.

MATERIALS AND METHODS

Patients and controls. Eighty-five serum samples were taken from 46 patients with CLL. Normal values for the different assays were determined from the sera of 38–67 healthy blood donors.

Collection and storage of sera. Blood samples were allowed to clot at room temperature for 90 min, then centrifuged at 2,500 min for 10 min without cooling. Sera were divided into aliquots and

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stored at −30°C for not more than 3 weeks. Samples were thawed only once, immediately before testing.

Complement measurements. Total haemolytic activity of the classical pathway (CH_{50}) was measured according to Mayer (1962). Total haemolytic activity of the alternative pathway (AH_{50}) was determined on the basis of the method of Platts-Mills & Ishizaka (1974) as described in detail in our previous paper (Minh *et al.*, 1983a). Haemolytic C1 and C4 activities were measured by the effective molecule titration method of Nelson *et al.* (1966). Concentrations of C3, factor B and C1 inhibitor (C1-IHN) were determined immunochemically by single radial immunodiffusion as outlined by Kohler & Müller-Eberhard (1967), using monospecific antisera.

C1-INH activity was determined as outlined by Lachmann & Hobart (1978) using *N*-acetyl-L-tyrosine ethyl ester as substrate for activated C1.

Measurement of the immune complex level. Levels of circulating immune complexes in the patients' and control sera were measured by a complement consumption test (Johnson, Mowbray & Porter, 1975).

RESULTS

Complement levels in patients with CLL

Classical and alternative complement pathway activities as well as concentrations of C1-IHN were measured in 85 serum samples from 46 patients with CLL (Table 1). Mean values for total haemolytic activity of the alternative pathway (AH_{50}) as well as concentrations of C3 and factor B were found to be normal. C1-IHN concentrations did not significantly differ from the normal values either. A decrease in the CH_{50} and C4 values was observed in CLL sera, of which, however, only the latter was significant. The most striking difference was found in C1 activity. The mean level of this complement component in CLL sera was less than 50% of the level in normal controls. If mean −1·5 s.d. of the control values was taken as the lower limit of the normal, then CH_{50}, C4 and C1 levels below this limit were found in 35% and 57% and 52% of the sera tested, respectively.

Complement levels in sera of CLL patients with high, normal and low C1-INH concentrations

Eighty-five sera were divided into three groups according to C1-INH content and the means and s.d.s of the other complement parameters were calculated and compared. Mean −2 s.d. and mean +1 s.d. of the control values were regarded as the lower and higher limit, respectively, for the normal C1-INH concentration. The latter arbitrary limit was chosen because sera with a C1-INH concentration exceeding mean +2 s.d. of the control values rarely occurred among the sera tested. The results are shown in Table 2.

Variations in the C1-INH concentration did not affect either AH_{50} or factor B levels. C3 and C1-INH concentration showed a tendency towards a positive correlation, though this was not significant. The level of C1-INH was found to be correlated with that of CH_{50}, C1 and C4. A low inhibitor concentration was always associated with very low classical complement pathway activities, while a normal C1-INH concentration was associated with a lesser but still significant CH_{50}, C4 and C1 depression. In sera with a high normal C1-IHN level, CH_{50} values were normal and C4 activity was only slightly decreased. However, a marked depression of C1 haemolytic activity was found in this group of sera, too.

Incidence of acquired C1-INH deficiency among the patients tested.

A complement profile corresponding to an acquired deficiency of C1-INH was found in 17 serum samples from five patients out of the 85 sera from 46 patients. Criteria for this diagnosis were as follows: low (below mean −2 s.d. of control values) CH_{50}, C1, C4 and C1-INH levels, normal AH_{50} and factor B, normal or slightly depressed C3 concentration, normal or only slightly increased level of circulating immune complexes. Means and s.d. of the complement levels in these sera are shown in Table 3.

In additional experiments, 10 serum samples with low C1-INH concentration were tested for C1-INH activity and all showed depressed values. At last one EDTA–plasma sample from each

Table 1. Complement levels in sera of patients with CLL and in healthy normal controls

Subjects tested	CH_{50} (CH_{50}/ml)	AH_{50} (CH_{50}/ml)	C1 (CH_{63}/ml)	C4 (CH_{63}/ml)	C3 (mg/dl)	Bf (%*)	C1-INH (%*)
Patients with CLL ($n=85$)	71·8 ± 53·5	28·5 ± 11·3	8,232 ± 6,200	22,000 ± 18,300	122·6 ± 52·6	120 ± 44	108·5 ± 42·0
Controls	82·44 ± 18·3 $n=67$	30·4 ± 8·9 $n=38$	18,500 ± 7,300 $n=50$	37,200 ± 11,800 $n=35$	125·5 ± 27·5 $n=65$	120 ± 27 $n=64$	115·4 ± 25·3 $n=30$
P	> 0·05	> 0·1	< 0·001	< 0·001	> 0·1	> 0·1	> 0·1

* As percentage of a normal serum pool.

Table 2. Complement levels in sera of CLL patients with low, normal or high normal C1 esterase inhibitor concentrations

C1-IHN concentration	Number of sera tested	CH_{50} (CH_{50}/ml)	AH_{50} (CH_{50}/ml)	C1 (CH_{63}/ml)	C4 (CH_{63}/ml)	C3 (md/dl)	Bf (%*)	C1-IHN (%*)
Low (mean − 2 s.d. of normal values)	15	36·2 ± 15·6‡	27·1 ± 12·6	3,812 ± 5,221‡	10,000 ± 3,400‡	104·4 ± 29·1	144 ± 39	42·87 ± 17·83‡
Normal (between mean − 2 s.d. and mean + 1 s.d. of normal values)	43	67·9 ± 27·6	27·7 ± 9·6	8,400 ± 5,320‡	20,100 ± 15,100‡	116·2 ± 56·4	122 ± 45	95·44 ± 17·86
High normal or high (mean + 1 s.d. of normal values)	27	91·2 ± 31·6	28·6 ± 11·6	9,717 ± 7,183‡	27,400 ± 21,900†	137·5 ± 50·8	121 ± 44	152·70 ± 26·55

* As percentage of a normal serum pool.
Significant differences as compared to the control values (see Table 1) †$P < 0.05$; ‡$P < 0.001$.

Table 3. Complement and immune complex levels in sera of patients with CLL and acquired C1-esterase inhibitor deficiency ($n=5$)

	Patients		Controls		
Assay	No. of sera tested	mean ± s.d.	No. of sera tested	mean ± s.d.	*P*
CH_{50} (CH_{50}/ml)	15	28·1 ± 15·2	67	82·4 ± 18·3	< 0·001
AH_{50} (CH50/ml)	12	27·2 ± 9·8	38	30·4 ± 8·9	> 0·1
C1 (CH_{63}/ml)	15	2,000 ± 1,800	50	18,800 ± 7,300	< 0·001
C4 (CH_{63}/ml)	15	4,100 ± 2,200	35	37,200 ± 11,800	< 0·001
C3 (mg/dl)	15	85·8 ± 36·3	65	125·5 ± 27·5	< 0·001
Bf (%*)	12	109 ± 30	64	120 ± 27	> 0·1
C1-INH (%*)	15	47·2 ± 21·2	30	115·3 ± 25·3	< 0·001
IC (%†)	13	9·91 ± 10·4	59	2·30 ± 7·70	> 0·05

* As percentage of a normal serum pool.
† Percentage of complement consumption.

patient was also tested in parallel. No differences in the complement profiles were observed between serum and plasma.

C1-INH deficiency was repeatedly observed in four patients. It lasted for more than 1 year in two patients and for 6 months in another. Two patients died during the period of the study, both from severe infections.

DISCUSSION

An acquired C1-INH deficiency characterized by profound depression of CH_{50}, C1, C4 and C1-INH levels but normal or nearly normal AH_{50}, Bf, C3 and immune complex concentrations, was found in five of 46 CLL patients investigated in the present work. If the eight patients previously tested by us (Minh *et al.*, 1983a), were also included then this deficiency was found in six of 54 cases or in more than 10% of CLL patients. Acquired C1-INH deficiency was first described by Caldwell *et al.* (1972) and was claimed to be a rare condition. At the time of Gelfand *et al.* (1979) review 15 cases had been recorded and only a few further cases have been published since then (Laprotier *et al.*, 1981). Most were patients with lymphoproliferative disorders, including some CLL patients (Day *et al.*, 1976; Oberling *et al.*, 1975).

Our present results indicate that acquired C1-INH deficiency occurs more frequently in CLL patients than is usually assumed. This discrepancy with the data from the literature can be best explained by the lack of systematic complement measurements in CLL or other types of lymphoma. To the best of our knowledge, studies on only a limited number of patients have been published. For example, five, eight, 20 and 13 patients with CLL were investigated by Southam & Goldsmith (1951), Rottiho & Levy (1959), Baltsch *et al.* (1960) and Yoshikawa, Yamada & Yoshido (1969), respectively and only one or two types of complement assay were used by these authors.

Previous investigators of the acquired C1-INH deficiency syndrome have demonstrated that the low level of inhibitor is a secondary phenomenon induced by an intensive *in vivo* activation of the classical complement pathway. CP activation can be triggered by 7S IgM monoclonal immunoglobulin (Caldwell *et al.*, 1972), circulating immune complexes (Day *et al.*, 1976) or by the tumour cells (Schreiber *et al.*, 1976; Hauptmann *et al.*, 1976). No monoclonal immunoglobulin of 7S IgM or any other type was found in our six patients with C1-INH deficiency and the level of circulating immune complexes determined by a complement consumption assay was also normal or only slightly elevated. Thus it seems probable that CP activation leading to profound hypocomplementaemia

and secondary inhibitor consumption is induced by the CLL cells in these patients. Overall significantly decreased C1 and C4 mean levels were found in the CLL sera. Low or normal C1 and/or C4 activities were detected in more than a half of the sera tested. These findings suggest that *in vivo* CP activation is not limited to patients with acquired C1-INH deficiency but that the phenomenon also occurs in at least some of the other CLL patients. Because monoclonal immunoglobulins were found in only two of 46 CLL patients and no correlation between immune complexes and complement levels were found (data not shown) it seems probable that the *in vivo* interaction between CLL cells and the complement system was responsible for the CP activation. It can be assumed that tumour cells bind and activate C1 and activated C1 molecules cleave their substrate, C4 and consume their inhibitor, C1-INH. When C1 activation is less marked, it is revealed by a decreased C4 level, whereas with more intensive C1 activation, C1-INH is also consumed. The low C1 level found in sera with low C1-INH content and the less marked but still significantly low C1 in sera with normal or high normal C1-INH concentration (Table 2) supports this conclusion. These assumptions are in agreement with our previous results (Füst *et al.*, 1976) and those of others (Kitamura, Toshima & Day, 1978; Bach-Mortensen, Osther & Stroyer, 1975) showing that normal and CLL lymphocytes interact with C1 and this interaction may lead to C1 activation and in turn to C4 consumption. However, an effective C3 convertase enzyme does not seem to be formed during CP activation triggered by CLL tumour cells and this is probably why no decrease in C3 and alternative pathway levels is seen in the patients.

It is difficult to draw any firm conclusions as to the clinical significance of our findings from the data obtained so far. That is why we began a prospective study in 1983 during which serial complement measurements will be compared with the clinical course of the disease, the development of infectious complications and other laboratory parameters. Three of six patients with acquired C1-INH defeiciency have died from severe infections. Thus, hypocomplementaemia must be seriously considered as an additional predisposing factor for severe infections in CLL.

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