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E-rosette inhibiting substance in Hodgkin's disease spleen extracts

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SUMMARY

Patients with Hodgkin's disease often manifest impairment of cell-mediated immune responses in both *in vivo* and *in vitro* tests, as well as a markedly decreased percentage of E rosette-forming (T) lymphocytes in the peripheral blood. This report describes the inhibition of E-rosette formation by normal peripheral blood lymphocytes after incubation with extracts prepared from the spleens of patients with Hodgkin's disease. Such extracts also depressed E-rosette formation by the peripheral blood lymphocytes of patients with Hodgkin's disease after those cells had been restored to normal function by prior incubation in foetal calf serum. Similarly prepared extracts from the spleens of normal donors had no immunodepressive effect. The E-rosette inhibitory substance in the Hodgkin's disease spleen extracts was found to be a complex containing β-lipoprotein, C-reactive protein, and C1q.

INTRODUCTION

Patients with Hodgkin's disease (HD) frequently develop a depressed state of cellular immunity manifested by an impaired ability to respond to delayed hypersensitivity antigens (Eltringham & Kaplan, 1973), to chemical allergens such as dinitrochlorobenzene (Eltringham & Kaplan, 1973; Young *et al.* 1973), to reject allografts (Kelly, Lamb & Varco, 1960) and to resist certain types of infection (Goffinet, Glatstein & Kaplan, 1973; Aisenberg, 1964). Several *in vitro* correlates of this immunodeficiency have been described, including an impaired response of peripheral blood lymphocytes (PBL) to phytohaemagglutinin and concanavalin A (Con A) (Hersh & Oppenheim, 1965; Levy & Kaplan, 1974; Bobrove *et al.*, 1975), a decreased percentage of E rosette-forming PBL (Bobrove *et al.*, 1975; Khan *et al.*, 1975) which may be restored to normal by incubation in foetal calf or foetal human sera (Fuks *et al.*, 1976), and the capacity of serum fractions from patients to inhibit antigen stimulation of normal antigen-sensitive lymphocytes (Scheurlen, Schneider & Pappas, 1971; Gaines, Gilmer & Remington, 1973).

The existence of non-specific immunosuppressive factors in the circulation has been reported by several groups in the past 2 decades (Kamrin, 1959; Mowbray, 1963; Mannick & Schmid, 1967). Most of these reports describe immunosuppressive activity in an α-globulin fraction which either inhibits mitogen-induced lymphocyte activation or causes prolongation of allograft survival in lower mammals (Kamrin, 1959; Mowbray, 1963; Mannick & Schmid, 1967; Occhino *et al.*, 1973; Nimberg *et al.*, 1975).

Chisari & Edgington (1975) have reported that a serum β-lipoprotein observed in certain disease states has E rosette-inhibiting activity. Mortensen and co-workers (Mortensen, Osmand & Gewurz, 1975; Mortensen & Gewurz, 1976) have studied the *in vitro* effects of C-reactive protein (CRP), a protein which is found in the blood during a variety of disease processes involving tissue destruction or inflammation. *In vitro*, CRP binds selectively to T lymphocytes and inhibits their ability to form E rosettes with sheep erythrocytes.

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This report describes the inhibition of E-rosette formation by normal donor PBL after incubation with extracts from the spleens of patients with HD. Similarly prepared extracts from the spleens of normal donors had no inhibitory effect. When HD spleen extracts (HDSE) were incubated with PBL from patients with HD that already had a subnormal percentage of E-rosette-forming cells (ERFC), no further depression of ERFC levels was observed. However, if the percentage of ERFC in the PBL of patients with HD was first restored to normal by incubation with foetal calf serum (FCS), subsequent incubation with HDSE also depressed the ERFC levels of these cells. The E-rosette depressive substance in the HDSE has been isolated and found to be a complex of low-density β-lipoprotein, CRP, and C1q.

MATERIALS AND METHODS

Sources of spleens. Tumours were obtained from the spleens of patients with untreated HD undergoing staging laparotom (Glatstein *et al.*, 1969), all of whom were pathologically staged according to the Ann Arbor classification (Carbone *et al.*, 1971). Normal control spleens were obtained from transplantation organ donors on the cardiovascular surgery service. Al tissue was rapidly frozen in solid CO_2 immediately after removal. Frozen tissue blocks were then stored at −70°C until time of use.

Preparation of tissue extracts. Cubes of frozen tissue weighing about 20 gm were homogenized for 5 min with 3 vol. of 0·01 M phosphate-buffered saline (PBS), pH 7·4, then heated to 65°C for 10 min and sedimented by centrifugation for 30 min at 48,000 *g* in a Sorvall RC2-B centrifuge. The supernatant was precipitated by 40% saturation with ammonium sulphate at pH 7·0. The precipitate was spun out by centrifugation. The supernatant was dialysed in 0·01 M potassium-phosphate buffer, pH 8·0, and fractionated on DEAE–cellulose. Fractions were eluted at 0·01 M, 0·05 M, 0·1 M and 0·5 M, at pH 8·0, to yield the test HDSE.

The homogenized spleen supernatants were also fractionated by ultra-centrifugation analytical flotation on a potassium-bromide gradient at 189,000 *g*, and lipoprotein fractions were collected according to Chisari & Edgington (1975). For further analysis the LDL fractions were pooled and banded on an isopycnic density gradient (Chisari & Edgington, 1975).

Counter-current immunoelectrophoresis (CIEP) and immunodiffusion (ID). CIEP and ID were performed on 0·6% agarose plates in barbital buffer, pH 8·2. The plates were dried and stained with amido black or oil red O. The following antisera were tested with the HDSE and spleen lipoprotein fractions: goat anti-human IgG, goat anti-human IgM, goat anti-C3, horse anti-human sera (Hyland Laboratories), rabbit anti-human IgA (Antibodies, Inc.), rabbit anti-human α_2-macroglobuy lin, rabbit anti-human transferrin, rabbit anti-human albumin, goat anti-human C-reactive protein, rabbit anti-human chorionic gonadotrophin, rabbit anti-human haemoglobin, rabbit anti-human β-lipoproteins and rabbit anti-C1q (Cappel Laboratories). Human ferritin was extracted from spleens and antibody produced in rabbits (Bieber & Bieber, 1973). All antisera were tested for specificity by immunoelectrophoresis (IEP) against human sera. Although there was no precipitation reaction with the anti-CRP and normal human sera, the anti-CRP was absorbed with normal human sera to ensure specificity.

Preparation of CRP. CRP was prepared from pleural effusions by DEAE ion exchange with 0·05 M sodium citrate and 0·1 M sodium chloride, pH 7, and eluted with 0·05 M sodium citrate and 1·5 M sodium chloride. This was followed by Sephadex G-100 chromatography and the CRP-containing fractions were collected and concentrated.

Chloroform–methanol extraction. Lipoprotein-containing solutions were layered over an equal volume of chloroform–methanol (1:1), gently agitated for 4 hr, and refrigerated overnight. The protein phase was removed and tested for lipid with oil red O dye after electrophoresis.

Protein determination. Protein was determined by the method of Lowry *et al.* (Lowry *et al.*, 1951). The protein content of the spleen extract was adjusted between 100–300 μg/ml.

Rosette-inhibition assay. PBL from patients with HD and normal donors were separated from 20 ml of heparinized blood on a Ficoll–Hypaque gradient (Böyum, 1968). After three washes in 20% FCS–RPMI 1640 medium, 0·5 ml aliquots containing 5×10^6 cells/ml were transferred into sample and control tubes. Spleen extracts, lipoprotein fractions and other proteins to be tested were added to the cell suspensions at a final concentration of 10–50 μg/ml and incubated at 37°C for 3 hr. Other controls were incubated with a 1:1 mixture of RPMI 1640 medium or 0·05 M potassium phosphate, pH 8·0. Tubes were then spun at 350 *g* for 10 min in an IEC centrifuge. The cells were washed twice in RPMI 1640 and resuspended in RPMI 1640 medium. Aliquots of 100 μl containing 5×10^6 cells/ml were then incubated for 5 min at 37°C with 100 μl of 0·5% sheep red blood cells (SRBC) (40:1 SRBC: lymphocyte ratio) and 20 μl of SRBC-absorbed, heat-inactivated pooled human AB serum. The tubes were centrifuged for 5 min at 200 *g* and the pellets incubated for 60 min at 8°C. Cells were gently resuspended and the percentage of ERFC determined by counting 200 cells in a standard haemocytometer. A rosette was scored when 3 or more SRBC were seen adhering to a lymphocyte (Jondal, Holm & Wigzell, 1972). In all instances, cell viability was determined at the beginning and end of the procedure by trypan blue exclusion. Tests in which viability was less than 95% were discarded.

FCS Incubation. PBL from patients with untreated HD and normal donors were separated and washed as above. Lymphocytes were incubated either with HDSE or HD-spleen low-density lipoprotein (LDL) at a concentration of 20 μg/ml, or with RPMI 1640 medium alone for 3 hr. The cells were washed twice and an aliquot was tested for E-rosette formation. The

remaining lymphocytes were incubated at 37°C for 24 hr with (a), 20% FCS in RPMI 1640; (b), 20% human AB serum in RPMI 1640; (c), RPMI 1640 only. The cells were then washed twice in RPMI 1640 and tested for E-rosette formation either (a), directly, or (b), after re-incubation with HDSE or HD-spleen LDL for 3 hr at 37°C. Cell viability was determined by trypan blue exclusion.

RESULTS

ERFC levels in the peripheral blood of forty-seven normal donors ranged from 54 to 71% with a mean of 63·4% and standard deviation of 4·3%. In samples drawn from the same individual on different days, the standard deviation varied from ±1·4 to ±4·9. Duplicate variation was considerably less, averaging ±2·0 of the mean value when 200 cells were counted.

Incubation of normal donor lymphocytes with 20 μg of the 0·05 M phosphate–DEAE–cellulose fractions individually prepared from the spleens of eight different patients with HD consistently inhibited their capacity to form E rosettes. The extent of the decrease in ERFC levels produced by each of the eight extracts was highly significant ($P<0·001$), ranging from 24·6 to 43·4%, with an average of 32%, in a total of ninety determinations (Table 1). Incubation with similarly prepared extracts made from each of four normal spleens from normal donors had no significant inhibitory effect (Table 1). Control incubations of normal lymphocytes in 1:1 RPMI medium:0·05 M potassium phosphate, pH 8·0, had no effect on ERFC levels (data not shown).

TABLE 1. Depression of ERFC levels of PBL of normal donors by incubation with HDSE* and non-neoplastic conditions

Case no.	Diagnosis†	Stage‡	Spleen involved	CRP in extract	No. of donors tested	Control ERFC (mean±s.d.)	ERFC after incubation (mean±s.d.)	Mean change§	*P* valuc
1	HD, NS	IV, B	+	n.d.	17	63·6±4·9	42·2±7·6	−21·4 (−33·6)	< 0·001
2	HD, MC	IV B	+	n.d.	23	59·7±3·3	36·8±7·5	−22·9 (−38·3)	< 0·001
3	HD, NS	III A	+	n.d.	8	64·6±5·0	48·7±5·3	−15·9 (−24·6)	< 0·001
4	HD, NS	IV B	+	n.d.	12	62·7±6·7	42·6±6·5	−20·1 (−32·0)	< 0·001
5	HD, NS	III A	+¶	n.d.	7	59·2±5·2	33·5±8·9	−25·7 (−43·4)	< 0 001
6	HD, NS	II B	−	+	8	57·0±2·9	40·1±5·2	−16·9 (−29·6)	< 0·001
7	HD, NS	IV B	+	n.d.	7	60·5±4·4	43·0±6·9	−17·5 (−28·9)	< 0·001
8	HD, LP	IV A	+	+	8	63·0±4·3	36·7±9·3	−26·3 (−41·7)	< 0·001
22	CVA		—	−	8	63·3±2·3	61·8±3·1	−1·5 (−2·3)	> 0·1
23	CNST		—	n.d.	8	60·4±4·8	59·2±3·8	−1·2 (−1·9)	> 0·1
24	CNST		—	n.d.	7	61·2±4·3	59·8±4·6	−1·4 (−2·2)	> 0·1
25	CNST		—	n.d.	8	61·7±2·7	58·0±1·6	−3·7 (−5·9)	> 0·1

n.d. = Not done; + = spleen involved by HD; − = spleen not involved by HD.

* Spleen extracts prepared on 0·05 M DEAE cellulose, pH 8, and tested at 20 μg/ml.

† HD, Hodgkin's disease; NS, nodular sclerosis; MC, mixed cellularity; LP, lymphocyte predominance; CVA, cerebral vascular accident; CNST, central nervous system trauma.

‡ Stage, Ann Arbor pathological stage (Carbone *et al.*, 1971).

§ Numbers in parentheses show percentage change.

¶ Single small nodule present.

In six untreated patients with HD, the average percentage of ERFC in the peripheral blood was only 45·3±12·9%, with a range of 24–62%, in good agreement with our previously reported observations (Fuks *et al.*, 1976). Incubation of the lymphocytes from these patients for 3 hr with the splenic tumour extract from a patient with nodular sclerosing HD, stage IV-B, caused no significant change in the mean percentage of ERFC (48·3±15·5%).

However, when the lymphocytes from two patients with HD who initially had an abnormally low percentage of ERFC were first incubated for 24 hr in 20% FCS–RPMI 1640, the percentage of ERFC

rose to the normal range. Restoration to normal ERFC levels was not observed when the cells of patients with HD were incubated with RPMI 1640 medium alone or with RPMI 1640 containing 20% adult human AB serum. When FCS-incubated lymphocytes were washed and reincubated with HDSE for 3 hr, the percentage of ERFC was again depressed (Table 2).

Incubation of normal lymphocytes with FCS caused no significant change in the percentage of ERFC. However, the ERFC levels of lymphocytes from two normal donors which had been depressed by prior incubation with HDSE were restored to their control values after incubation with 20% FCS. The restoration induced by FCS could not be duplicated by incubation with RPMI 1640 with or without added 20% adult human AB serum (Table 2).

TABLE 2. Effect of HDSE on E-rosette formation by lymphocytes from normal donors and patients with HD*

Treatment of PBL†	Normal donor 1	Normal donor 2	HD patient stage II A	HD patient stage II B
None	65	60	49	45
3 hr HDSE‡	40	32	46	36
24 hr FCS	63	63	61	67
24 hr FCS, then 3 hr HDSE	41	33	44	42
24 hr human AB serum	61	63	41	47
24 hr human AB serum, then 3 hr HDSE	n.d.	n.d.	42	43
3 hr HDSE, then 24 hr FCS	65	66	62	69
3 hr HDSE, then 24 hr AB serum	50	40	53	50

Treatment of PBL	Normal donor 3	Normal donor 4	HD patient stage III B	HD patient stage I B
None	59	62	43	50
3 hr HD-LDL	44	40	40	53
24 hr FCS	59	57	64	65
24 hr FCS, then 3 hr HD-LDL	46	40	48	33
24 hr human AB serum	61	60	46	55
3 hr HD-LDL, then 24 hr FCS	64	65	65	68
3 hr HD-LDL, then 24 hr AB serum	55	60	51	55

n.d. = Not done.

* All figures percentages of E rosette-forming PBL.

† Abbreviations as in text and Table 1 except HD-LDL: Hodgkin's disease LDL–CRP–C1q complex.

‡ HDSE were used at 20 μg/ml, HD-LDL were used at 20 μg/ml, FCS and human AB serum at 20% concentration.

By CIEP and ID, the 0·05 M DEAE fraction from two HD spleens was found to contain β-lipoprotein, C1q and CRP, as well as ferritin and albumin. The normal spleen fractions did not contain CRP or C1q. We investigated the possibility that the E-rosette inhibitory substance might be an abnormal β-lipoprotein or lipoprotein complex. Seven HD spleens, eight lymphoma spleens, and two normal spleens were homogenized and separated by analytical flotation at 189,000 *g* for 18 hr. E-rosette inhibitory activity was present only in the LDL fractions. After isopycnic banding, E-rosette inhibitory activity was found in lipoproteins with densities between 1·050–1·075 g/cm^3 from all seven of the HD spleens and two of the eight lymphoma spleens (Table 3). Further characterization by CIEP and IEP of the E-rosette inhibitory HD-spleen-LDL fractions revealed that they contained β-lipoprotein, C1q and CRP. The CRP and the C1q contained in the HD-spleen LDL migrated as β proteins on IEP and the immunoprecipitate stained for lipid. This indicates that the CRP and C1q in the HD-spleen LDL were associated with lipoprotein. γ-Migrating CRP purified from pleural effusions could be detected by CIEP at a concentration of 1 μg/ml, but no γ-migrating (free) CRP was detected in the spleen-lipoprotein fraction.

TABLE 3. Depression of ERFC levels of PBL of normal donors by LDL fraction of isopycnic density-gradient-fractionated spleen extracts from patients with HD, non-Hodgkin's lymphomas and non-neoplastic conditions

Case no.	Diagnosis*	Stage	Spleen involved	CRP in extract	No. of donors tested	Control ERFC (mean ± s.d.)	ERFC after incubation (mean ± s.d.)	Mean change†	*P* value
6	HD, NS	II B[a]	−‡	+§	6	62·6 ± 4·1	48·2 ± 6·2	−14·4 (−23)	< 0·01
8	HD, LP	IV A	+	+	8	62·2 ± 5·2	42·6 ± 7·2	−19·6 (−31·5)	< 0·001
9	HD, NS	IV B	+	+	15	64·2 ± 3·5	40·1 ± 5	−24·1 (−37·5)	< 0·001
10	HD, NS	III B	+	+	10	64 ± 4·4	44·4 ± 5·2	−19·6 (−30·6)	< 0·001
11	HD, NS	III	+	+	8	63·2 ± 5·3	47·2 ± 6·3	−16 (−25·3)	< 0·01
12	HD, U	III	+	+	6	62·6 ± 4·1	51·1 ± 7·6	−11·5 (−18·3)	< 0·05
13	HD, MC	III	+	+	6	62·6 ± 4·1	45·4 ± 5·8	−17·2 (−27·4)	< 0·001
14	Lymphoma, NLPD		+	+	8	62·1 ± 3·9	40 ± 5·3	−22·1 (−35·5)	< 0·001
15	Lymphoma, LtL		+	+	6	60·3 ± 4·9	49·1 ± 6·2	−11·2 (−18·5)	< 0·05
16	Lymphoma, NLPD		+	−	6	60·3 ± 4·9	56·6 ± 3·5	−3·7 (−6·1)	> 0·1
17	Lymphoma, LtL		+	−	6	60·3 ± 4·9	58·1 ± 7·4	−2·2 (−3·6)	> 0·1
18	Lymphoma, DHL		−	−	6	60·3 ± 4·9	57·4 ± 4·6	−2·9 (−4·8)	> 0·1
19	Lymphoma, DHL		+	−	6	60·3 ± 4·9	55·5 ± 5	−4·8 (−7·9)	> 0·1
20	Lymphoma, DHL		−	−	6	60·3 ± 4·9	58 ± 3·1	−2·3 (−3·8)	> 0·1
21	Lymphoma, NLPD		−	−	6	60·3 ± 4·9	57·2 ± 3·2	−3·1 (−5·1)	> 0·1
22	CVA		—¶	−	6	61·4 ± 4·8	60·2 ± 2·8	−1·2 (−1·9)	> 0·1
26	CNST		—¶	−	6	62·6 ± 4·1	60·7 ± 3·8	−1·9 (−3)	> 0·1

* Abbreviations as in text and previous tables, except: U, unclassified; NLPD, nodular lymphoma; LtL, Lennert-type lymphoma; DHL, diffuse histiocytic lymphoma.

† Numbers in parentheses show percentage change.

‡ +, Spleen involved by tumours; −, spleen not involved by tumours.

§ +, CRP present; −, CRP absent.

¶ No tumour.

With the exception of CRP, C1q, albumin and β-lipoproteins, no other serum proteins could be identified by CIEP or IEP in any of the HD-spleen-LDL fractions with the antisera employed (see Materials and Methods). Normal spleen-LDL fractions contained β-lipoprotein and albumin. Using anti-CRP antiserum, CRP was detected in the HD-spleen-LDL fractions with densities of 1·055–1·075 g/cm^3.

The HD-spleen-LDL fraction at concentrations of 20–30 μg/ml inhibited E-rosette formation by lymphocytes from normal donors and from patients with HD after their rosetting ability had been restored to normal with 20% FCS incubation (Table 2). The capacity of lymphocytes from both normal and HD donors to form E rosettes after depression by HD-spleen LDL could be restored to normal by incubation for 18 hr in 20% FCS–RPMI 1640 (Table 2).

In order to determine what component of the LDL–CRP complex was responsible for the E-rosette inhibition, C polysaccharide (CPS) at a concentration of 20 μg/ml and HD-spleen LDL at a concentration of 50 μg/ml were added at the same time to PBL. Lipid-free CRP and CRP–CPS were also assayed at similar concentrations (Mortensen *et al.*, 1975). Although free CRP inhibited E-rosette formation, this response was prevented by CPS, whereas CPS failed to block E-rosette depression by the HD-spleen LDL complex (Table 4). After the HD-spleen LDL was extracted with chloroform–methanol, the remaining protein, at a concentration of 20 μg/ml, still inhibited E-rosette formation. However, the E-rosette inhibitory activity of the extracted protein, unlike that of the complex, was neutralized by CPS, indicating that the activity of the lipid-extracted protein was due to CRP (Table 4).

DISCUSSION

Although the ability of a lymphocyte to bind SRBC in the 'late' or cold incubation E-rosette test is accepted as a specific marker for human T cells (Jondal *et al.*, 1972; Bach, 1973), the immunological

TABLE 4. ERFC from six normal donors after incubation for 3 hr at 37°C with HD-spleen LDL and other preparations

Preparation*	ERFC	Mean change†
RPMI control	66·4%±4·6%	0
HD-LDL, 50 μg/ml	39%±3·6%	−27·4 (−41%)
HD-LDL, 50 μg/ml+CPS, 20 μg/ml	40·2%±3·2%	−26·2 (−39%)
Lipid-extracted HD-LDL, 30 μg/ml	39·6%±6·5%	−26·8 (−40%)
Lipid-extracted HD-LDL, 30 μg/ml+ CPS, 20 μg/ml	61·4%±4%	−5 (−7%)
CRP, 50 μg/ml	30·2%±3·2%	−36·2 (−54%)
CRP, 50 μg/ml+CPS, 20 μg/ml	62·4%±3·9%	−4 (−6)

* Abbreviations as in text and other tables.

† Numbers in parentheses show percentage change.

significance of this response is still unknown. It has been reported that E-rosette formation by peripheral blood T cells of patients with HD is depressed (Bobrove *et al.*, 1975; Fuks *et al.*, 1976). Further studies of the mechanisms of this E-rosette depression have now revealed that extracts prepared from the spleens of thirteen patients with HD consistently and significantly depressed the E-rosette levels of peripheral blood lymphocytes from normal donors to the low values typically found in patients with HD. However, these HDSE did not further depress the abnormally low ERFC levels observed in the PBL of patients with HD.

Normal lymphocytes pre-incubated with HDSE and the hyporesponsive lymphocytes from patients with HD could both be restored to normal ERFC levels by incubation with 20% FCS. After restoration by FCS, PBL from patients with HD became susceptible to ERFC inhibition by re-incubation with HDSE or HD-spleen LDL. There is thus an apparent correlation between the *in vitro* effect of HDSE on normal lymphocytes and the naturally impaired ERFC responses found *in vivo* in patients with HD with respect to the extent and the dynamics of E-rosette depression and restoration. The E-rosette inhibitory substance was not found in normal spleens but was present in two out of eight non-Hodgkin's lymphoma spleens tested.

Further partial purification of the E-rosette inhibitory substance in the spleen extracts from seven patients with HD revealed that it contains β-lipoprotein, C1q and CRP. On IEP and CIEP, the CRP and C1q migrate in the β region and with isopycnic banding ultracentrifugation the CRP and C1q were found in the lipoprotein fraction with a density of 1·055–1·075 g/cm^3.

The HD-spleen E-rosette inhibitory lipoproteins, containing CRP–C1q, were isolated in a density range, 1·055–1·075 g/cm^3, which overlaps that of LDL-2, 1·019–1·063 g/cm^3, and that of the lighter high density lipoproteins (HDL), 1·063–1·075 g/cm^3. This density range coincides with that of a lipoprotein class known as LP(a) or HDL-1. LP(a) lipoprotein has the density of HDL but a lipid:protein ratio characteristic of LDL. The apoproteins in LP(a) are composed of 65% apoprotein B (the predominant apoprotein in LDL) and 20% LP apoprotein, which is an uncharacterized apolipoprotein not found in any other class of lipoproteins. The physiological significance of this lipoprotein class is unknown (Levy, Blum & Schaeffer, 1976).

The occurrence of CRP bound to lipoprotein (Wood, 1963) and of C1q bound to CRP–lecithin has previously been reported (Kaplan & Volanakis, 1974). Recently, CRP has been shown by Mortensen *et al.* (1975, 1976) to bind to T cells, to depress E rosettes, and to inhibit the mixed lymphocyte reaction and the generation of cytotoxic lymphocytes. All of these effects of CRP could be blocked by CPS.

The inability of CPS to block the HD–LDL–CRP inhibition of E-rosette formation indicates either that the complex does not bind to the cell by means of the CRP molecule, or that the lipoprotein blocks CRP binding to CPS but not to the lymphocyte membrane. It is possible that, once bound to the cell by lipoproteins, the CRP is effective at lower concentrations than the free molecule in depressing E-rosette formation. Preliminary experiments have shown that the LDL fractions from three HD spleens inhibit

the response of normal PBL in a mixed lymphocyte culture, and the *in vitro* response to streptokinase–streptodornase (SK–SD) antigens, indicating that the lipoprotein–CRP–C1q complex may have immunosuppressive, as well as E-rosette inhibitory, activity. Further studies of these and other *in vitro* immune responses will be required to determine whether the HD-spleen LDL–CRP–C1q complex plays a significant role in the impairment of cell-mediated immunity which is so characteristically observed in patients with untreated HD.

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