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Cellular origins of serum complement receptor type 2 in normal individuals and in hypogammaglobulinaemia

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SUMMARY

A soluble form of complement receptor 2 (sCR2) is found in normal human serum. Amounts present are about 30–90 ng/ml, which is of the same order as reported for soluble CR1. Although B cells express surface CR2 and are the main peripheral blood source of sCR2 they do not appear to be the major tissue source of serum sCR2. Serum levels of sCR2 of patients with hypogammaglobulinaemia were not significantly different from those of normal individuals even in the case of two brothers with Bruton's X-linked agammaglobulinaemia (XLA) lacking ($CD19^+$) B cells. On gel filtration through Sephacryl S-300 the sCR2 from XLA serum behaved exactly like sCR2 from normal serum or sCR2 affinity purified from cell supernates of a B lymphoblastoid line or from the T-ALL line MOLT-4. In all cases a single peak appeared at the same point in the chromatogram. Possible alternative sources of serum sCR2 are follicular dendritic cells (FDC) which are known to express CR2 strongly and $T6^+$ lymphocytes within the thymus. Peripheral T cells from adults have not been reported to express CR2. However, investigation showed that cells from the Bruton's XLA cases produced small amounts of sCR2 in culture and although no CD21 was detected on the surface of the mononuclear cells by flow cytometry, the more sensitive direct antibody rosette test readily detected CD21. Further studies showed that non-B cells from control samples of cord blood or blood of young children also weakly expressed CD21.

Keywords serum CD21 serum CR2

INTRODUCTION

Complement receptor type 2 (CR2), the cell surface receptor for the C3dg fragment of the third component of the complement system and for the Epstein–Barr virus (EBV), has been extensively studied and recently reviewed (Cooper, Moore & Nemerow, 1988; Ahearn & Fearon, 1989). It is a phosphoprotein of molecular weight 145 kD belonging to a superfamily of structurally related proteins which include factor VIIIb, factor H, the interleukin-2 (IL-2) receptor, thyroid peroxidase, CR1 and several components of the complement system (Lowell *et al.*, 1989; Reid & Day, 1989). CR2 contains an extracellular domain made up of 15 or 16 tandem short concensus repeats, each of about 60 amino acids, a single transmembrane region and a cytoplasmic tail of 34 amino acids. The two *N*-terminal short consensus repeats contain the C3dg and EBV binding sites (Lowell *et al.*, 1989). CR2 has a very restricted tissue distribution. It is found on mature B cells (but not pre-B cells or plasma cells), follicular dendritic cells (FDC) (Reynes *et al.*, 1985; Ling, MacLennan & Mason, 1987) and T6-positive thymocytes (Tsoukas & Lambris, 1988). It is also frequently found on blast cells of childhood T cell acute lymphoblastoid leukaemia (ALL) (Behm *et al.*, 1989). Reports that CR2 is present on pharyngeal epithelial cells have not been confirmed (Niedobitek, Herbst & Stein, 1989). CR2 is defined antigenically by monoclonal antibodies (MoAbs) of the CD21 cluster and at least five different epitopes are detectable. The MoAbs have been used to show that EBV has a selected site on CR2 which is proximal or identical to that used by the natural ligand C3dg (Lowell *et al.*, 1989). One normal function of CR2 on B lymphocytes is the binding and transport of immune complexes. In the germinal centre, complexes bound to CR1 and CR2 on the surface of FDC effectively 'present' antigen to follicular lymphocytes (Klaus *et al.*, 1980).

A soluble form of CR2 (sCR2) has been demonstrated to be present in easily detectable amounts in culture supernates of B-lymphoblastoid cell lines (B-LCL) and in normal human serum (Lowe *et al.*, 1989). The tissue source and function of serum sCR2 are unknown.

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MATERIALS AND METHODS

Patients

Two Caucasian brothers, aged 9 and 11 years, with X-linked agammaglobulinaemia (Bruton's XLA) were studied. Both boys had marked hypogammaglobulinaemia involving IgG, IgA and IgM; absence of functional antibodies; normal cell mediated immunity; and absence of mature B cells in peripheral blood as defined by lymphocyte surface marker analysis.

Eight patients with common variable immunodeficiency (CVID) were studied. These patients comprised a heterogeneous group of subjects with hypogammaglobulinaemia. Patients whose hypogammaglobulinaemia was due to drugs, loss of protein, premature birth, malignancy or infections, were excluded. Patients were usually characterized by combined B and T lymphocyte immaturity or lymphopenia.

Cell lines

The following established lines were tested: five EBV-transformed B-LCL (LICR-LON-HMy, RPMI-8866, 04673, KR4 and WM1), two Burkitt lines retaining the original phenotype (EB4 and Daudi) and three of transformed phenotype (Raji, EB2 and Namalwa) (Ling *et al.*, 1989), one T-ALL line (MOLT-4), one Sezary T line (HUT-78), one erythroid line (K562), two monocytoid (U937 and M4) one pro-myeloid line (HL-60), two pre-B lines (SMS-SB and NALM-6), three plasmacytoid lines (RPMI-8226, FJNI and EJM) and one human fibroblast line. All lines were maintained in RPMI 1640 medium containing 10% fetal calf serum (FCS) as previously described (Ling *et al.*, 1989).

Blood and tonsil cells

Blood mononuclear cells (MN) were separated from heparinized blood by centrifugation through Ficoll–Isopaque and washed in HEPES-buffered RPMI medium. B-depleted or T-depleted MN cells were prepared by rosetting the MN cells with sheep erythrocytes coated with either CD19 or a CD3 MoAb and removal of the rosetted cells by centrifugation throught Ficoll–Isopaque. In a typical experiment 30×10^6 MN cells in 2 ml of H-RPMI were mixed with 6×10^8 CD19 antibody-coated sheep erythrocytes in 1·3 ml of H-RPMI to give a ratio of 20 sheep erythrocytes to one MN cell. The mixture was centrifuged at 250 ***g*** for 5 min and the cells resuspended using a broad-mouth plastic pipette. The volume of the suspension was adjusted to 5 ml with H-RPMI and layered onto 9% Ficoll–Isopaque. After centrifugation at 400 ***g*** for 15 min in a 16 × 100-mm conical tube, the cells from the interface band were harvested, washed and resuspended in medium. The success of the procedure was judged by a rosette test with CD19-antibody-coated sheep erythrocytes. Cells in the pellet from the centrifugation were discarded.

Tonsil cells were obtained from fresh tissue removed at tonsillectomy. T cells were removed by rosetting with AET–sheep erythrocytes and centrifugation through Ficoll–Isopaque. Platelets were obtained from platelet-rich plasma (kindly provided by the Regional Blood Transfusion Laboratory). Two cord blood samples were obtained from the Maternity Hospital, Birmingham.

Cell culture. Blood or tonsil cells were cultured in microtitre trays in 0·1-ml volumes of RPMI 1640 medium containing 10% FCS. Trays were placed in a CO_2 gassed incubator at 37°C.

Serum and plasma samples

Sera obtained from normal donors were kindly provided by the Regional Blood Transfusion Laboratory. Sera from patients with rheumatoid disease were kindly provided by Dr D. S. Kumararatne and from four cases of myeloma by Dr M. Hamilton. Sera were stored frozen and thawed once only since it was shown that repeated freezing and thawing reduced the CD21 titre. Titres of sera were shown to be identical to those from plasma collected at the same time.

Monoclonal antibodies

MoAb CD21. Five murine MoAbs produced in this department (BU-32, BU-33, BU-34, BU-35 and BU-36) were shown by tests on cell lines, histological tissue sections and blood cells, to have specificity for the CD21 antigen. All the MoAbs precipitated a protein of mol. wt 145 kD. The epitopes of CD21 recognized are distinct from the epitopes A, B and C recognized by the established CD21 MoAbs B2, HB5 and OKB7 (Schwarz-Albeiz & Moldenhauer, 1989). The MoAbs are all of isotype IgG1. Antibody fractions were prepared from ascitic fluids by chromatography on DEAE cellulose.

Other MoAbs. The CD19 MoAb used (BU-12, IgG1) was produced in this laboratory and its specificity confirmed in the Third International Workshop on Human Leucocyte Antigens. The CD3 MoAb was produced by the OKT3 hybridoma acquired from the American Type Culture Collection (Rockville, MD). MHM6 (a CD23 MoAb) was obtained from Dr M. Rowe.

Detection and estimation of CD21

CR2 is detected antigenically as CD21.

Membrane CD21. Flow cytometric analysis was used to measure the binding of a MoAb to viable lymphocytes or cell line cells (first stage) as revealed by attachment of a fluorescein-labelled sheep anti-mouse immunoglobulin reagent (second stage). An EPICS Profile II (Coulter Electronics, Hialeah, FL) was used. In addition, a direct antibody rosette test (DAR test) was performed using sheep erythrocytes coated with an appropriate MoAb as previously described (Ling, Bishop & Jefferis, 1977).

Soluble CD21 (sCD21). The CD21 antigen is a monomeric polypeptide with only a single representation of each epitope on the molecule. Hence the detection of soluble CD21 by standard techniques requires the availability of a pair of CD21 MoAbs binding to spatially distinct epitopes. A pair of MoAbs was selected (BU-32 and BU-35) which acted synergistically in binding to the antigen. An extremely sensitive and reproducible assay was developed by coating sheep erythrocytes with each of the MoAbs and mixing the two preparations 1:1 before addition to serially diluted antigen in the test. The two unmixed suspensions were used in control titrations to detect the presence of anti-sheep erythrocytes antibodies, rheumatoid factors or other artefactual agents in the antigen preparation. The material containing antigen (e.g. culture supernates or serum) was serially diluted in 0·05-ml volumes in a microtitre tray in HEPES-buffered RPMI 1640 containing 2% FCS. After addition of 0·05 ml of coated cells the 'settled pattern' end-point was read after 2 h of settling. For titrations of sCD21 in serum or plasma, a preliminary dilution of 0·1 ml of serum in 0·9 ml of 0·1 M 2-mercapto ethanol in saline was performed to dissociate IgM antibody which is responsible for most of the background

agglutination against coated unmixed sheep erythrocytes. With this modification the background was usually negligible. Aliquots of frozen sCD21 purified by affinity and ion-exchange chromatography were used as standards to check inter-batch variations of sensitivity. The results are not claimed to be more than approximate but reproducibility was high and sensitivity was approximately 0·02 ng/ml.

Affinity purification of sCD21

A mixture of BU-34, BU-33 and BU-36 MoAbs purified by chromatography on DEAE–cellulose and dialysed against 0·1 M $NaHCO_3$ was coupled to activated Sepharose (10 mg MoAbs to 1 g of Sepharose) as recommended by the manufacturers (Pharmacia Fine Chemicals, Uppsala, Sweden). Columns were washed in phosphate-buffered saline containing 0·1% azide; 3 M KCNS was used as the eluting agent. Fractions were titrated for CD21 and positive fractions pooled and dialysed against an appropriate buffer.

Ion exchange chromatography of sCD21

Affinity-purified preparations of sCD21 were dialysed against 0·005 M phosphate buffer, pH 8·0, and passed through a column (8 × 1·4 cm) of DEAE–cellulose equilibrated with the same buffer. The column then received 200 ml of 0·01 M phosphate buffer, pH 8·0, and material absorbing at E_{280} was washed through and discarded. Most of the active material was then eluted in small volume by changing the buffers to 0·06 M and 0·1 M phosphate buffer, pH 8·0. Fractions containing high sCD21 activity were analysed, pooled, dialysed against 0·01% sucrose, and freeze-dried.

Gel filtration

Sephacryl superfine S-100 and S-300 gels (Pharmacia), bed volume 220–300 ml, were equilibrated with 0·05 M phosphate buffer, pH 7·5, containing 0·2 M NaCl and 0·02% sodium azide. Preparations for chromatography were dialysed overnight against this buffer. Samples (1 ml) were degassed before introduction into the column. Fractions of about 3 ml volume were collected at 8-min intervals. Every second fraction was screened for sCD21 by titration. Intermediate fractions were then titrated to ascertain the peak of activity. A collection of low and high molecular weight standards was run through after each antigen run and the positions of the peaks on the E_{280} plot noted.

RESULTS

Soluble CR2 in the serum of normal individuals and patients with hypogammaglobulinaemia

Soluble CR2 (30–90 ng/ml) was found in the sera of all 35 samples obtained from normal healthy individuals. These sera were separated conventionally from clotted blood and stored at 4°C. Titres remained constant over 2–3 weeks of storage. In view of a report that sera of some patient with rheumatoid arthritis contain autoantibodies against CR2 (Barel *et al.*, 1987) sera from 58 patients with rheumatoid disease were tested. Levels of sCR2 were not different from those in normal sera. Sera from four patients with multiple myeloma were tested because of the reported severe deficiency of B lymphocytes in peripheral blood in this disease (Pilarski *et al.*, 1984). Two of the cases showed reduced levels of sCR2 (values recorded: 15, 15, 60 and 60 ng/ml).

Table 1. sCD21 in the plasma of patients with common variable immunodeficiency (CVID) and Bruton's X-linked agammaglobulinaemia (XLA) compared with levels in plasmas of control donors

	Diagnosis	sCD21 (ng/ml)
Patients		
J	CVID	240
P	CVID	120
S	CVID	60
B	CVID	120
P	CVID	60
B	CVID	120
H	CVID	60
E	CVID	60
I	XLA	60
A	XLA	60
Controls		
H	Healthy	60
B	Healthy	120
Bl	Healthy	30
M	Healthy	60
T	Healthy	60
R	Healthy	60
W	IgG subclass deficiency	30

Special precautions were taken in assessing sCR2 in blood samples from patients with hypogammaglobulinaemia, in order to avoid errors due to shedding of CR2 from blood cells *in vitro* after collection and decay of existing sCR2. Blood samples freshly collected in EDTA were centrifuged and the separated plasma frozen immediately in liquid nitrogen. Plasmas were stored at −70°C and thawed just before assay. Levels of sCD21 in eight plasmas from patients with CVID were comparable to those in control plasmas from normal individuals (Table 1). A similar result was obtained with plasma samples from two

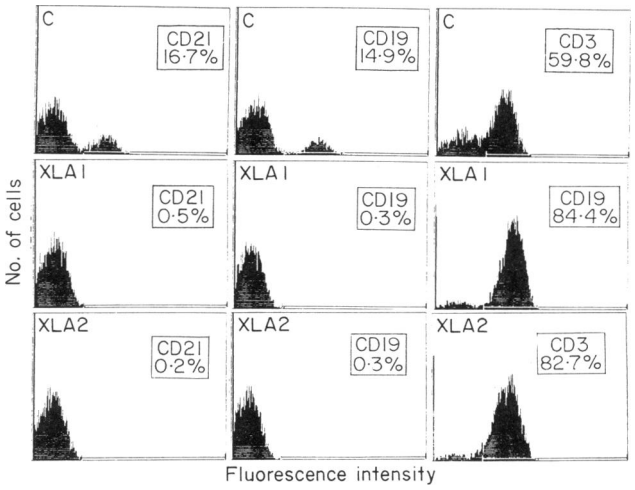

Fig. 1. Flow cytometric analysis (EPICS) of CD21, CD19 and CD3 antigens on blood lymphocytes from two brothers with Bruton's X-linked agammaglobulinaemia (XLA1 and XLA2) and a normal control child (C).

brothers with XLA. Marker analysis confirmed that cells expressing the B cell antigen were rare or absent (Fig. 1 and Table 2).

Surface expression of CR2 by blood and tonsil cells and secretion of sCR2 in short-term culture

sCR2 was present at high levels in supernates of cultured tonsil B cells. B lymphocytes from the blood of normal adults produced much less sCR2. Larger amounts were produced by B lymphocytes from cord blood but production was still well below that of T-depleted tonsil B cells (Table 3). Platelets produced no sCR2; the small amounts found at high platelet concentrations could be fully accounted for by B cell contamination.

In a comparison of sCR2 production by blood MN cells of neonates, children and adults it was found that cells of neonates and children produced greater amounts of sCR2 than cells of adults (Table 3). After removal of B cells ($CD19^+$ cells), no sCR2 was produced by MN cells of adults or children but some secretion of sCR2 by cells of cord blood remained (Table 3).

CR2 expression and secretion by blood cells in hypogammaglobulinaemia

Blood MN cells from two brothers with Bruton's XLA produced significant amounts of sCR2 when cultured overnight although less than by cells of a normal child (Table 2). Flow cytometry confirmed the virtual absence of B cells ($CD19^+$) in blood obtained from the two brothers and $CD21^+$ cells were also apparently absent by this technique (Fig. 1 and Table 2). However, $CD21^+$ cells were detected by the more sensitive direct antibody rosette test (Table 2). In our experience this test is usually positive for CD21 with blood T cells from children. In agreement with this finding, the high proportion of lymphocytes rosetting in tests on cells from the brothers with Bruton's XLA indicated that substantial numbers of T cells must be expressing the CD21 antigen. Similar results were obtained with two different CD21 MoAbs directed against different epitopes on the antigen whereas the CD19 MoAb and control anti-idiotype MoAbs of the same isotype were negative confirming the specificity of the phenomenon. The sCR2 production by XLA cells in culture, although less than that of control unfractionated MN cells is well above the background level shown by B-depleted control MN cells. The most likely source of sCR2 in the XLA cultures is the T cells. No sCR2 was released in 1-h cultures which excluded cytophilically bound serum sCR2 as a source of the material. It is also noteworthy that CD23, a B cell antigen also actively produced in soluble form in culture was completely absent from XLA cultures (Table 2; data obtained by the method of Lowe *et al.*, 1989).

Surface expression and secretion of CR2 by cells of lymphoblastoid and other established cell lines

sCR2 is produced in culture by cells of most B-LCL but at very different levels (Table 4). Burkitt lines that have not undergone phenotypic drift to a B-LCL produce little or no sCR2. Pre-B cell and plasmacytoid lines are also non-producers as are peripheral T cell, myeloid and fibroblast lines. However, lines from immature T cells of T-ALL origin (e.g. MOLT-4) produce definite but low levels of sCR2. In general, secretion levels correlated roughly with surface density of CR2 as determined by flow cytometry (Table 4).

EBV and C3d-binding properties of sCR2

sCR2 coupled to the CD21 MoAb BU-32 showed strong binding to EBV particles and C3d-coated yeasts (Table 5). Lower titres were obtained with the other CD21 MoAb, presumably reflecting poor availability of binding sites after complexing.

Gel filtration characteristics of sCR2 from serum and cell sources

Profiles of sCR2 from the following sources were obtained by direct titration of antigen after fractionation on S-300 Sephacryl columns: (i) Affinity-purified from the supernate of B-LCL: (ii) affinity-purified from the T-ALL line MOLT-4; (iii) normal serum, fresh untreated; and (iv) XLA serum untreated.

The patterns obtained with the various preparations were very similar, each showing a single peak corresponding to an 'apparent' molecular weight of 320 kD (Fig. 2). This was not the true molecular weight as gel penetration is affected by the shape as well as the size of the molecule and the non-globular sCR2 penetrates the gel less readily than the globular proteins used as standards. However, the gel fractionations indicate that active material from all sources consists in its native state, of a single species with virtually identical gel filtration characteristics. The pattern was unaffected by reduction and alkylation of disulphide groups with dithiothreitol and iodoacetamide confirming that a monomeric non-aggregated form of sCR2 is being measured.

Purification and physical properties of sCR2

As shown previously (Lowe *et al.*, 1989), the CD21 activity in

Table 2. Surface membrane and secreted antigens on blood lymphocytes in XLA. Membrane antigens were measured by flow cytometry (FACS) and direct antibody rosette (DAR) assays.

Cell source	Membrane CD21		Membrane CD19		Membrane CD3		sCD21	sCD23
	FACS	DAR	FACS	DAR	FACS	DAR	18 h/24 h	18 h/24 h
Normal child	16	59	15	37	66	62	0·3/0·6	0·2/0·3
Bruton's XLA brother 1	0·8	52	0·3	0	84	86	0·15/0·10	0/0
Bruton's XLA brother 2	0·8	60	0·3	0	93	94	0·15/0·10	0/0

The results are per cent positive lymphocytes (monocytes excluded). The secretion of sCD21 and sCD23 (ng/ml) by unfractionated MN cells cultured at 10^7/ml is shown.

Table 3. sCD21 produced by cultured blood and tonsil cells of newborn, child and adult donors.

Cell type	Culture period (cell concentration)	sCD21 (ng/ml)
Blood B cells (adult, six samples)	5 days (10^6/ml)	0·6–2·4
Blood B cells (newborn, two samples)	5 days (10^6/ml)	1·2; 2·4
Tonsil non-T cells (four samples)	5 days (10^6/ml)	5·0–40·0
Erythrocytes (two samples)	24 h (10^8/ml)	<0·02
Platelets (two samples)	24 h (10^9/ml)	<0·02
Newborn (Blood MN cells)	1 h/18 h (10^7/ml)	0·07; 0·95
Newborn (B-depleted MN)	1 h/18 h (10^7/ml)	<0·03; 0·15
Child (Blood MN cells)	1 h/18 h (10^7/ml)	0·10; 0·60
Child (B-depleted MN)	1 h/18 h (10^7/ml)	<0·03; <0·03
Adult (Blood MN cells)	1 h/18 h (10^7/ml)	0·03; 0·09
Adult (B-depleted MN)	1 h/18 h (10^7/ml)	<0·03; 0·03

MN, mononuclear cells.

Table 4. Surface and secreted CD21 produced by cells of various lines

Cell Line		CD21	
Name	Type	Surface	Supernatant (ng/ml)
EB4	Burkitt1	–	<0·02
DAUDI	Burkitt 1	–	0·15
EB2	Burkitt 2	++	1·4
RAJI	Burkitt 2	++	3·0
LICR-LON-HMy	B-LCL	+++	12
04673	B-LCL	+	0·7
RPMI-8866	B-LCL	±	0·08
KR4	B-LCL	+	0·7
WM1	B-LCL	++	1·2
MOLT-4	T-ALL	++	1·2
HUT-78	T-Sézary	–	<0·02
K 562	Erythroid	–	<0·02
U 937	Monocytoid	–	<0·02
MLI	Monocytoid	–	<0·02
HL-60	Pro-myeloid	–	<0·02
SMS-SB	Pre-B	–	<0·02
RPMI-8226	Plasmacytoid	–	0·08
FJN-1	Plasmacytoid	–	<0·02
EJM	Plasmacytoid	–	<0·02

Burkitt 1 lines retain the original Burkitt phenotype whereas Burkitt 2 have undergone phenotypic drift towards a B-LCL phenotype. Surface CD21 was assessed by flow cytometry.

culture supernatant and sera was not diminished by centrifugation at 100 000 *g*. A purified preparation of sCR2 for use as a standard was obtained by affinity and ion-exchange purification of 500 ml of culture supernate from a B-LCL. The sCR2 was comparatively stable to heat, retaining activity up to 80°C.

Table 5. Epstein–Barr virus (EBV) and C3d binding properties of sCR2 from a B-LCL

MoAb on sheep erythrocytes	EBV	C3d yeasts
BU-34	0	2
BU-33	0	2
BU-36	4	4
BU-35	8	2
BU-32	32	32
MHM6	0	0

All wells contained 0·05 ml of affinity-purified sCR2 (50 ng/ml). u.v.-inactivated EBV particles (10^4 infective U/ml, kindly provided by Dr L. Young) or fixed baker's yeast coated with human C3d ($0·7 \times 10^8$/ml) were serially diluted in the sCR2 and 0·05 ml of sheep erythrocytes coated with one of the five CD21 MoAb or a control MoAb (MHM6) were added and the settled end-point read two hours later. Other controls, 'no antigen' titrations and titrations with uncoated yeasts.

Purified sCR2 from 500 ml of culture supernatant was dialysed against 0·01% sucrose and freeze-dried. The freeze-dried material was taken up in 0·05 ml of SDS, boiled and subjected to SDS–PAGE analysis under reducing conditions. In the SDS gel shown in Fig. 3, the sCR2 is probably the major band slightly below the bovine albumin standard, corresponding to a molecular weight of approximately 67 kD. Attempts to confirm this by electroblotting and immunoperoxidase development were unsuccessful because denaturation of the freeze-dried material by heating with SDS destroyed antigenicity of sCR2. Further studies are necessary to ensure that break-down of higher molecular weight material has not occurred, since CR2 is very susceptible to cleavage.

DISCUSSION

The B lymphocytes of peripheral lymphoid tissues constitute a large population of cells expressing CD21 and it is reasonable to

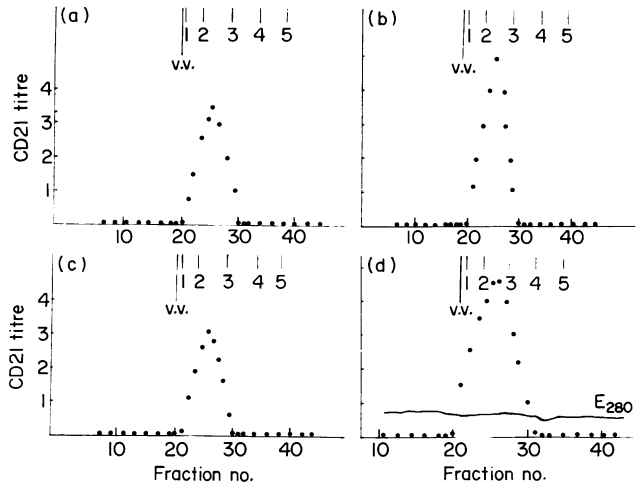

Fig. 2. (Legend) Gel filtration of (a) untreated human serum; (b) untreated serum from a case of Bruton's hypogammaglobulinaemia; (c) affinity-purified sCD21 from the supernate of the MOLT-4 T-ALL cell line; (d) affinity-purified sCD21 from the supernate of the B-LCL HMy-LICR-LON. The same Sephacryl S-300 column was used in all cases under the conditions described in the Methods section. V.V., void volume corresponding to the position of blue dextran in the E_{280} read-out. The molecular weight standards 1–5 were: 1, thyroglobulin (669 kD); 2, ferritin (440 kD); 3 catalase (232 kD); 4, aldolase (158 kD); and 5, BSA (67 kD). On the left hand scale the titre of CD21 plotted is the $\log_2$ titre using 0·05 ml of the 3-ml fraction. Affinity-purified preparations of sCD21 showed no measurable rise in E_{280} absorption in active fractions as shown in the B-LCL plot.

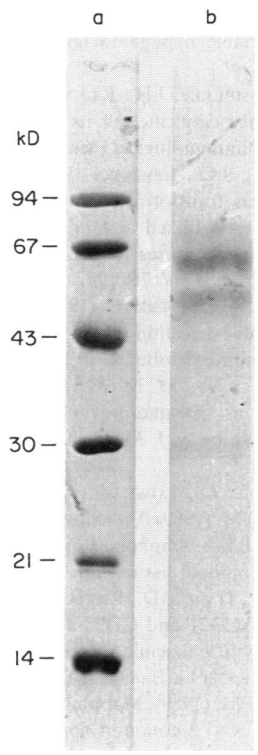

Fig. 3. Separation of freeze-dried affinity-purified sCR2 from B-LCL by SDS–PAGE on a 12·5% gel under reducing conditions. The material was of very high activity and low protein content. Stain, Coomassie blue. a, Standards of known molecular weight; b, LICR-LON-HMy. A control of culture medium put through the same procedure showed no bands.

presume that they are a major source of serum sCR2. The fact that serum sCR2 levels were not reduced in patients with CVID or XLA indicates that other cell types must also be making a strong contribution. Another population of cells showing strong expression of CR2 is also widespread in lymphoid tissue, namely follicular dendritic cells (FDC). The CR2 is found in the cytoplasm as well as on the surface of FDC. Cytophilic uptake from B cells is excluded because of the strength of expression of CR2 and the fact that isolated FDC have been shown to contain CR2 (Sellheyer, Schwarting & Stein, 1989). Because of the difficulties in obtaining viable FDC free of B cells it has not been possible to test whether a soluble form of CR2 is exported from these cells but it is clearly a possibility.

In those patients possessing a significant number of B lymphocytes, these cells, even though they are abnormal with respect to immunoglobulin production, may still be producing sCR2. In fact B cell lines have been established by EBV transformation of cells of patients with Bruton's XLA (Lau *et al.*, 1989). One possibility is that the lines were derived from 'immature' B-lineage cells. 'Immature' B cells, negative for surface CD21, were found in the circulation in three cases of XLA reported by Golay & Webster (1986). In the two cases studied here the cells lacked not only cell surface immunoglobulin but also CD19 which is present on pre-B cells. Another possibility, that the sCR2 arises from non-T non-B cells such as monocytes is also excluded because these cells do not express surface CD21.

The sCR2 produced in cultures of peripheral blood mononuclear XLA cells could be produced by T cells of these patients. Our direct antibody rosette tests suggest that CD21 is present at low density on a substantial number of the T lymphocytes of normal children. A possible explanation of the result is that T cells of the XLA children have retained or acquired the capacity to secrete sCD21 as well as express it on their surface. It is interesting that T lymphocytes of XLA patients have been reported to show metabolic abnormalities consistent with immaturity (Webster, 1982).

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