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Soluble cell adhesion molecules and von Willebrand factor in children with Kawasaki disease

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SUMMARY

Fifty-nine children with acute Kawasaki disease (KD), a childhood vasculitis, were compared with 35 children with fever due to infection and 48 healthy children. Levels of soluble E-selectin (sE-selectin), soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1) in the healthy children were double those found in adults. All three soluble cell adhesion molecules and von Willebrand factor (vWF) were higher in the children with KD than in the healthy children, but only sE-selectin, a marker for activated endothelial cells, and sICAM-1 were higher than in the febrile children. The high levels of vWF in KD appear to reflect the prominent acute-phase reaction. This information can help us to understand further the complex interactions between cytokines, circulating inflammatory cells and the vascular endothelium, and may lead to new therapeutic avenues in KD and other inflammatory diseases and vasculitides.

Keywords Kawasaki disease vasculitis von Willebrand factor cell adhesion molecules

INTRODUCTION

Leucocyte traffic across the vascular endothelium is dependent on interaction between leucocytes and endothelial cells mediated by a variety of cell adhesion molecules, including E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) [1,2]. Elevated levels of soluble forms of these molecules have been found in conditions associated with endothelial cell activation and inflammation, including systemic vasculitis [3–5]. High circulating levels of von Willebrand factor (vWF) have been proposed as a marker for endothelial cell damage [6].

Kawasaki disease (KD) is an acute self-limited childhood vasculitis of unknown etiology with clinical features of fever, rash, lymphadenopathy, oropharyngeal inflammation, conjunctivitis and peripheral desquamation [7]. Patients have elevated inflammatory cytokines, polyclonal B cell activation and T cell activation [8]. There is panvasculitis of the small and medium sized muscular arteries with endothelial oedema, necrosis, replication and desquamation, and leucocyte infiltration of the arterial wall [9,10]. A major complication is the development of coronary artery aneurysms which occurs in 15–25% of untreated patients [11].

It has been proposed that cytokine-mediated vascular endothelial cell activation and injury is a central part of KD pathogenesis [8,12]. We further hypothesized that this would lead to elevated circulating sE-selectin, sICAM-1, sVCAM-1, and vWF. We therefore measured these variables in serum from patients with KD and from three control groups. C-reactive protein (CRP) was measured as a marker of the acute-phase reaction.

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PATIENTS AND METHODS

Patients and controls

Group 1. Fifty-nine children (27 female, median age 2·5 years, range 0·4–14·2 years) with KD, diagnosed according to standard criteria [13], were studied within 28 days of fever onset. Forty were studied within 14 days. None had received intravenous gamma globulin treatment (IVIG) at the time the sample was taken. None had clinical or laboratory features of diseases which mimic KD. Sixteen (27%) developed coronary artery aneurysms, a relatively high percentage which may reflect the tertiary referral nature of our hospital. In nine patients serial samples were studied.

Group 2. Thirty-five children (15 female, 3·3 years, range 0·4–15 years) with a fever of 38·5°C or more due to infection, without underlying disease, were studied as febrile controls. None had any features of rheumatological illness or were on medication other than antibiotics. Thirteen had an organism isolated. These children had rotavirus gastroenteritis, measles, respiratory syncytial virus infection, coliform urinary tract infection, group A streptococcal tonsillitis, staphylococcal

empyema, meningococcal meningitis, *Salmonella typhi* bacteraemia, salmonella gastroenteritis, tuberculous meningitis, malaria, and mycoplasma pneumonia ($n = 2$). Twenty-two had no organism isolated, with clinical diagnoses of aseptic meningitis, osteomyelitis, gastroenteritis, tonsillitis ($n = 2$), chest infection/pneumonia ($n = 8$), upper respiratory tract infection, and non-specific fever ($n = 6$).

Group 3. Forty-eight healthy children (11 female, 4·9 years, range 0·7–14 years) were studied as afebrile controls. These children were admitted for elective surgery ($n = 43$), were bone marrow donors ($n = 3$), or were undergoing investigations for suspected ketotic hypoglycaemia or hypopituitarism. All children were afebrile, on no medication, and without intercurrent illness.

Group 4. Fifteen healthy adults were studied (seven female, 32 years, range 21–47 years). A single sample from each was divided and serum was separated after either 1 h or 18 h in glass at room temperature. Aliquots of the serum separated at 1 h were repeatedly thawed for 45–60 min, vortex-mixed, and refrozen 11, 8, 11, and 10 times for sE-selectin, sICAM-1, sVCAM-1, and vWF, respectively.

Assay methods

Soluble cell adhesion molecules were measured by sandwich ELISA using commercial kits from R & D Systems (Abingdon, UK; previously British Biotechnology). In brief, microtitre wells coated with anti-human sE-selectin, sICAM-1 or sVCAM-1 were incubated with diluted samples, standards and controls, before incubation with conjugated anti-human cell adhesion molecules and substrate. VWF antigen was assayed by sandwich ELISA based on the method of Short *et al.* [14]. Plates were coated overnight with rabbit anti-human vWF antiserum (Dako, High Wycombe, UK). After washing, diluted samples were added and incubated for 1 h, followed by peroxidase-conjugated rabbit anti-human vWF (Dako), then substrate. The reaction was stopped by adding acid and the plate read at 492 nm in an ELISA reader (Titertek Multiskan; Flow Labs, Rickmansworth, UK). A standard curve was generated from a national standard (NIBSC, Potters Bar, UK). Interassay and intra-assay co-efficients of variation were < 10% for both high and low values. Results are reported as per cent of pooled normal plasma. One hundred percent is equivalent to 0·88 U.

CRP was measured with a commercial kit (Abbott, North Chicago, IL) using a fluorescence polarization immunoassay on a TDxFLx analyser. The lower limit of detection was 0·3 mg/dl.

Serum was stored at −70°C until analysed. Assays were run with each group tested in parallel. The study was approved by the ICH Ethical Committee.

Statistical analysis

Data were analysed on SPSS 6.0 software. The KD group was compared with the febrile and afebrile groups using the Mann–Whitney *U*-test. Correlation between variables was assessed using Spearman's rank test. The effects of delayed separation and repeated freeze–thaw cycles were analysed using paired *t*-tests.

RESULTS

Soluble cell adhesion molecules

Values for cell adhesion molecules in the healthy children were higher than those in the adults (Mann–Whitney, $P < 0·001$). Levels in the adult control group were consistent with those provided by the kit manufacturers. There was no difference in the results whether KD children studied before and after 14 days of illness were analysed separately or together; hence the KD patients were analysed as a single group. Soluble E-selectin and sICAM-1 were higher in KD patients than in the control groups, and levels in the febrile children were higher than those in the afebrile group (Table 1, Fig. 1). Within the healthy control group there was a slight but significant negative correlation of sE-selectin and sICAM-1 with age ($r = -0·31$, $P = 0·03$ and $r = -0·45$, $P = 0·002$, respectively). However, the differences between the groups persisted when levels were adjusted for the effect of age. There was no difference between the levels of sVCAM-1 in the KD group and the febrile control group, and both were higher than in the afebrile children (Table 1, Fig. 1).

Von Willebrand factor

Von Willebrand factor was significantly higher in KD patients and febrile controls than in afebrile controls (Table 1, Fig. 1). In the KD group, vWF tended to be higher for patients studied earlier in the disease ($r = -0·57$, $P < 0·001$) (Fig. 2). There was no difference between KD patients and febrile controls, whether the whole group or just those presenting in the first 14 days were compared. Results were unchanged when the highest value, from a child with malaria, was excluded from analysis.

Table 1. Serum sE-selectin, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), von Willebrand factor (vWF), and C-reactive protein (CRP), shown as median (inter-quartile range) for groups 1–4, and as mean (s.d.) for the values provided by the manufacturers of the cell adhesion molecule kits

	sE-selectin (ng/ml)	sICAM-1 (ng/ml)	sVCAM-1 (ng/ml)	vWF (% control)	CRP (mg/dl)
Kawasaki disease	228 (153–309)	635 (560–960)	1250 (950–1445)	230 (145–334)	6·5 (2·8–13)
Febrile controls	114 (93–186)	500 (415–545)	1295 (1010–1645)	294 (197–355)	3·6 (1·9–8·8)
Afebrile controls	86 (65–98)	418 (354–469)	1058 (950–1164)	92 (75–123)	< 0·3 (< 0·3–0·5)
Adult controls	35 (25–50)	195 (179–227)	650 (585–720)	85 (66–132)	ND
Normal adult values provided with kit	46 (17) $n = 130$	211 (48) $n = 131$	553 (160) $n = 105$	ND	ND

ND, Not done.

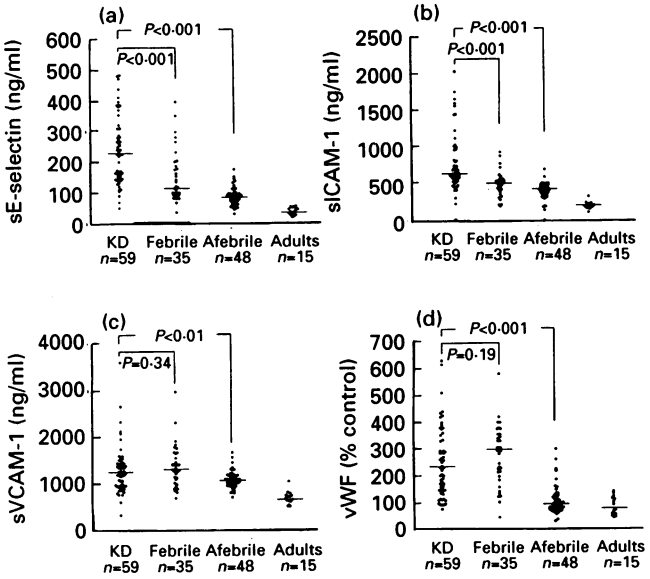

Fig. 1. Serum levels of sE-selectin, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and von Willebrand factor (vWF) in patient and control groups. Horizontal bars indicate median values. *n*, Number of subjects.

CRP

CRP was higher in KD patients than in febrile patients ($P = 0{\cdot}02$) or afebrile patients ($P < 0{\cdot}001$) (Table 1).

Correlations

Within the two disease groups vWF correlated significantly with CRP (KD, $r = 0{\cdot}62$, $P < 0{\cdot}001$; febrile, $r = 0{\cdot}41$, $P = 0{\cdot}02$) but not in the afebrile group ($r = 0{\cdot}07$, $P = 0{\cdot}64$). Within the KD group, sE-selectin correlated with CRP, but there was no correlation between sICAM-1 and sVCAM-1 and CRP. Levels of sE-selectin, sICAM-1 and sVCAM-1 were correlated with each other (Table 2). There were no differences in any variable between children with KD who did and who did not develop coronary artery aneurysms (data not shown).

Serial samples

Nine patients had serial samples studied. All received aspirin and five were treated with IVIG after the first sample. In all cases vWF, sE-selectin and sICAM-1 fell with time, regardless of IVIG treatment; sVCAM-1 fell in one patient (Fig. 3).

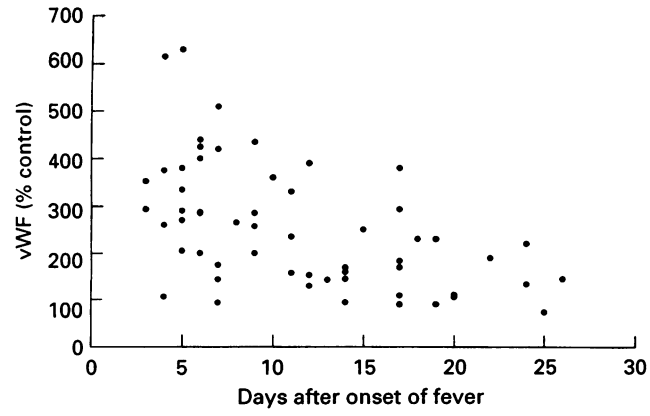

Fig. 2. Serum von Willebrand factor (vWF) levels in the Kawasaki disease group. Each point represents a different patient.

Table 2. Correlations between variables in the Kawasaki disease (KD) group, shown as Spearman correlation matrix, $r(P)$

	CRP	sE-selectin	sICAM-1
sE-selectin	0·40 (0·004)		
sICAM-1	0·08 (0·56)	0·47 (0·001)	
sVCAM-1	0·02 (0·87)	0·37 (0·004)	0·56 (0·001)

Effect of sample handling

There were small increases in sE-selectin levels after delayed separation, and in sICAM-1 levels after delayed separation and multiple freeze–thaw cycles (Table 3). There was no change in vWF or sVCAM-1 levels. These changes are not sufficient to explain the differences between the groups described above.

DISCUSSION

We have demonstrated elevated levels of sE-selectin and sICAM-1 but not sVCAM-1 or vWF in children with KD compared with children with fever caused by a range of infectious diseases, and have shown that the differences between groups are unlikely to be due to a difference in the handling of specimens.

E-selectin (CD62E, ELAM-1), ICAM-1 (CD54) and VCAM-1 (CD106) are cell surface adhesion molecules present on vascular endothelial cells, that are up-regulated by inflammatory cytokines and are critical in regulating the adhesion and migration of leucocytes [1,2]. Circulating adhesion molecules appear to be formed by cleavage of the membrane-bound form and release into the circulation of the extracellular domain [15–17]. Elevated levels of circulating adhesion molecules have been reported in numerous inflammatory conditions, including systemic vasculitis [3–5]. ICAM-1 and VCAM-1 are widely distributed, while E-selectin is specific to endothelial cells and only minimally expressed in the resting state [2]. An elevated serum level of sE-selectin, as reported here, seems to be a specific marker of endothelial cell activation. In KD there are elevated levels of the pro-inflammatory cytokines IL-1, tumour necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ) and IL-6 [8]. E-selectin expression is increased in skin vessels in acute KD [12], and elevated soluble levels were recently reported in 24 patients compared with healthy children and children with Henoch–Schönlein purpura [18]. Furukawa *et al.* demonstrated elevated levels of sICAM-1 in patients with acute KD compared with healthy children, children with measles, and children with Henoch–Schönlein purpura, and showed that high levels correlated with the presence of coronary artery aneurysms [19]. We found similar high levels but no correlation with the presence of coronary artery aneurysms, despite studying a larger group. We found high levels of sVCAM-1 in KD, to our knowledge not previously reported, but equally high levels in infection. A recent study found elevated levels of sVCAM-1 in patients with active Wegener's granulomatosis compared with healthy controls [4]. However,

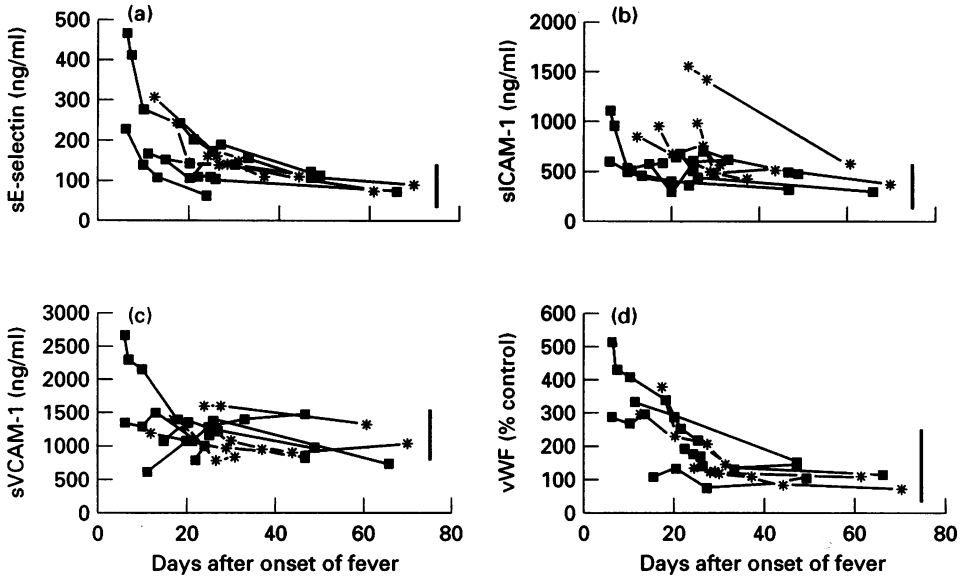

Fig. 3. Serial levels of sE-selectin, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and von Willebrand factor (vWF) in nine patients. Vertical bars indicate fifth to ninety-fifth centile of the control group. ★, Patients who did not receive intravenous gamma globulin (IVIG); ■, patients who received IVIG after the first blood sample.

sVCAM-1 levels did not distinguish between active disease and infectious episodes in these patients.

A discordance between elevation of different soluble cell adhesion molecules has been reported in systemic lupus erythematosus (SLE) [20] and systemic vasculitis [3,4]. Pall *et al.* found high sICAM-1 and sE-selectin but not sVCAM-1 in patients with active systemic vasculitis [3], although Stegeman *et al.* reported relatively greater elevations of sICAM-1 and sVCAM-1 than sE-selectin at the time of diagnosis of patients with Wegener's granulomatosis [4]. This discordance could reflect the different kinetics of expression of each molecule, and their differential up-regulation on different cells by various combinations of cytokines and other stimuli such as thrombin and oxygen radicals [2]. For example, IL-4 seems to inhibit cytokine-induced expression of E-selectin and augment the expression of VCAM-1 [2,21]. The relative levels of circulating soluble endothelial cell molecules may indicate which cells and mediators are most involved in the inflammatory reaction [20,21]. In addition, differences in expression and regulation of these molecules in the endothelium of different vascular beds may help explain the relative specificity of vessel involvement in the different types of systemic vasculitis.

We have shown that sE-selectin, sICAM-1, and sVCAM-1 in healthy children are approximately double adult values, and that levels in a wide range of infections are higher than in healthy children. Adult levels in this study are consistent with those supplied by the kit manufacturers and with those previously reported [5]. Levels of these molecules do not change with age between 18 and 65 [4,15,17,20], but Furukawa *et al.* found sICAM-1 in children less than 3 years old to be higher than in school age children [19].

Von Willebrand factor is an adhesive multimeric glycoprotein found in platelets, megakaryocytes and endothelial cells. The circulating plasma pool is derived mainly from endothelial cells [22]. It is an acute-phase reactant and is elevated in infectious diseases [23], numerous inflammatory and vasculitic diseases, after surgery, in pregnancy, and after exercise [22,24–26]. Levels are also high in atherosclerosis [24] and essential hypertension [27] in the absence of an acute-phase reaction. High levels of vWF have been proposed as a specific marker for endothelial damage [6]. However, vWF levels are an indirect measurement of the effects of a number of diverse stimuli, including inflammatory cytokines, on the endothelial cell, and high levels can reflect activation or damage.

VWF has been previously found to be elevated in small groups of children with KD compared with afebrile controls

Table 3. The effect of sample handling on serum sE-selectin, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and von Willebrand factor (vWF) in adult controls ($n = 15$)

	sE-selectin (ng/ml)	sICAM-1 (ng/ml)	sVCAM-1 (ng/ml)	vWF (% control)
Mean effect of delayed separation	+2 ng/ml*	+12 ng/ml*	−2 ng/ml	−1%
(95% CI, *P* value)	(+0·4 to +4, 0·02)	(+0·4 to +23, 0·04)	(−47 to +43, 0·94)	(−9 to +7, 0·75)
Mean effect of freeze/thaw cycles	+1 ng/ml	+13 ng/ml*	+13 ng/ml	−3%
(95% CI, *P* value)	(−2 to +3·6, 0·55)	(+2 to +24, 0·03)	(−51 to +77, 0·67)	(−8 to +3, 0·31)

*$P < 0·05$ compared with samples separated at 1 h and defrosted once.

[28,29], and we have confirmed this. However, levels were no different in KD from those in febrile controls. It is difficult to define the relative contribution of endothelial cell activation and damage to the elevation of vWF in KD. We found that vWF in the two disease groups correlated well with the acute-phase reactant CRP. In a small number of serial samples in patients with KD, levels of vWF, sE-selectin and sICAM-1 were high early in the disease and fell to the normal range after 2–4 weeks. This is the duration of maximal inflammatory changes, fever and acute-phase reaction in KD, and it seems likely that high vWF levels in the KD group were mainly due to the acute-phase reaction.

It is important not to over-interpret measurement of circulating molecules, which may not reflect biological activity or levels in the microenvironment. The role of soluble adhesion molecules *in vivo* is not defined, although *in vitro* they bind to ligands on other cells [15–17] and may be competitive inhibitors of adhesion [30]. There is great therapeutic potential in modulating inflammation by interfering with leucocyte–endothelial cell adhesion by using specific antibodies or non-specific inhibitors [2,31]. An acute self-limited disease such as KD may be particularly suited to antibody therapy, as the cost and inconvenience of such therapy and development of anti-idiotypic antibodies are less of a problem than in chronic disease. The clinical relevance of measuring sE-selectin, sICAM-1, sVCAM-1 and vWF in KD is limited, as they do not predict coronary artery aneurysms and discriminate poorly between KD and other febrile illnesses. However, together with other inflammatory mediators and markers, they can help us to understand the complex pathogenesis of inflammatory diseases and vasculitides.

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REFERENCES

1 Springer TA. Adhesion receptors of the immune system. Nature 1990; **346**:425–34.
2 Carlos TM, Harlan JM. Leukocyte–endothelial adhesion molecules. Blood 1994; **84**:2068–101.
3 Pall AA, Adu D, Drayson CM, Richards NT, Michael J. Circulating soluble adhesion molecules in systemic vasculitis. Nephrol Dial Transplant 1994; **9**:770–4.
4 Stegeman CA, Cohen Tervaert JW, Huitema MG, de Jong PE, Kallenberg CGM. Serum levels of soluble adhesion molecules intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and E-selectin in patients with Wegener's granulomatosis. Arthritis Rheum 1994; **37**:1228–35.
5 Gearing AJH, Newman W. Circulating adhesion molecules in disease. Immunol Today 1993; **14**:506–12.
6 Anonymous. Factor-VIII-related antigen and vasculitis. (Editorial) Lancet 1988; **i**:1203–4.
7 Kawasaki T, Kosaki F, Okawa S, Shigematsu I, Yanagawa H. A new infantile acute febrile mucocutaneous lymph node syndrome (MLNS) prevailing in Japan. Pediatrics 1974; **54**:271–6.
8 Leung DYM. The potential role of cytokine-mediated vascular endothelial activation in the pathogenesis of Kawasaki disease. Acta Paediatr Jpn 1991; **33**:739–44.
9 Hirose S, Hamashima Y. Morphological observations on the vasculitis in the mucocutaneous lymph node syndrome. Eur J Pediatr 1978; **129**:17–27.
10 Fujiwara H, Hamashima Y. Pathology of the heart in Kawasaki disease. Pediatrics 1978; **61**:100–7.
11 Newburger JW, Takahashi M, Burns JC *et al.* The treatment of Kawasaki syndrome with intravenous gamma globulin. N Eng J Med 1986; **315**:341–7.
12 Leung DYM, Cotran RS, Kurt-Jones E, Burns JC, Newburger JW, Pober JS. Endothelial cell activation and high interleukin-1 secretion in the pathogenesis of acute Kawasaki disease. Lancet 1989; **ii**:1298–301.
13 Dajani AS, Bisno AL, Chung KJ *et al.* Diagnostic guidelines for Kawasaki disease. Am J Dis Child 1990; **144**:1218–9.
14 Short PE, Williams CE, Picken AM, Hill FGH. Factor VIII related antigen: an improved enzyme immunoassay. Med Lab Sci 1982; **39**:351–5.
15 Newman W, Beall LD, Carson CW *et al.* Soluble E-selectin is found in supernatants of activated endothelial cells and is elevated in the serum of patients with septic shock. J Immunol 1993; **150**:644–54.
16 Rothlein R, Mainolfi EA, Czajkowski M, Marlin SD. A form of circulating ICAM-1 in human serum. J Immunol 1991; **147**:3788–93.
17 Wellicome SM, Kapahi P, Mason JC, Lebranchu Y, Yarwood H, Haskard DO. Detection of a circulating form of vascular cell adhesion molecule-1: raised levels in rheumatoid arthritis and systemic lupus erythematosus. Clin Exp Immunol 1993; **92**:412–8.
18 Kim DS, Lee KY. Serum soluble E-selectin levels in Kawasaki disease. Scand J Rheumatol 1994; **23**:283–6.
19 Furukawa S, Imai K, Matsubara T *et al.* Increased levels of circulating intercellular adhesion molecule 1 in Kawasaki Disease. Arthritis Rheum 1992; **35**:672–7.
20 Mason JC, Kapahi P, Haskard DO. Detection of increased levels of circulating intercellular adhesion molecule 1 in some patients with rheumatoid arthritis but not in patients with systemic lupus erythematosus. Arthritis Rheum 1993; **36**:519–27.
21 Thornhill MH, Haskard DO. IL-4 regulates endothelial cell activation by IL-1, tumor necrosis factor, or IFN-γ. J Immunol 1990; **145**:865–72.
22 Blann A. Von Willebrand factor and the endothelium in vascular disease. Br J Biomed Sci 1993; **50**:125–34.
23 Pottinger BE, Read RC, Paleolog EM, Higgins PG, Pearson JD. Von Willebrand Factor is an acute phase reactant in man. Thromb Res 1989; **53**:387–94.
24 Belch JJF, Zoma AA, Richards IM, McLaughlin K, Forbes CD, Sturrock RD. Vascular damage and factor-VIII-related antigen in the rheumatic diseases. Rheumatol Int 1987; **7**:107–11.
25 Woolf AD, Wakerley G, Wallington TB, Scott DGI, Dieppe PA. Factor VIII related antigen in the assessment of vasculitis. Ann Rheum Dis 1987; **46**:441–7.
26 Ateş E, Bakkaloğlu A, Saatçi Ü, Söylemezoğlu O. Von Willebrand factor antigen compared with other factors in vasculitic syndromes. Arch Dis Child 1994; **70**:40–43.
27 Blann AD, Naqvi T, Waite M, McCollum CN. Von Willebrand factor and endothelial damage in essential hypertension. J Hum Hypertension 1993; **7**:107–11.
28 Irazuzta JE, Elbl F, Rees AR. Factor VIII related antigen (von Willebrand's factor) in Kawasaki disease. Clin Ped 1990; **29**:347–8.
29 Bowyer SL, Ragsdale CG, Sullivan DB. Factor VIII related antigen and childhood rheumatic diseases. J Rheumatol 1989; **16**:1093–7.
30 Shingu M, Hashimoto M, Esaki I, Nobunaga M. Effect of cytokine-induced soluble ICAM-1 from human synovial cells on synovial cell–lymphocyte adhesion. Clin Exp Immunol 1994; **97**:46–51.
31 Kavanaugh AF, Davis LS, Nichols LA *et al.* Treatment of refractory rheumatoid arthritis with a monoclonal antibody to intercellular adhesion molecule 1. Arthritis Rheum 1994; **7**:992–9.