Clin Exp Immunol 1994; 98:240–244

Soluble endothelium-associated adhesion molecules in patients with Graves' disease

C. WENISCH, D. MYSKIW*, B. PARSCHALK, T. HARTMANN*, K. DAM* & W. GRANINGER
*Department of Infectious Diseases, Internal Medicine I, University of Vienna, Vienna, and *Department of Nuclear Medicine, Hospital of St Pölten, St Pölten, Austria*

(Accepted for publication 10 August 1994)

SUMMARY

The targeting and recruitment of inflammatory cells to vascular endothelium in Graves' disease (GD) is mediated by intercellular adhesion molecule-1 (ICAM-1), endothelial leucocyte adhesion molecule-1 (ELAM-1), and vascular cell adhesion molecule-1 (VCAM-1). We have studied serum levels of soluble ICAM-1 (sICAM-1), soluble ELAM-1 (sELAM-1), and soluble VCAM-1 (sVCAM-1) in patients with GD ($n = 21$) and in patients with iodine-deficient goitre (IDG) ($n = 23$). The serum levels of sICAM-1 were markedly elevated in patients with GD before treatment with thiamazole (median 560 ng/ml *versus* 185 ng/ml in patients with IDG). In addition, elevated serum concentrations of sELAM-1 (median 85 ng/ml *versus* 33 ng/ml, respectively) and sVCAM-1 (median 42 ng/ml *versus* 15 ng/ml, respectively) were observed in patients with GD ($P < 0.01$ for all). The serum levels of sELAM-1 and sVCAM-1 dropped significantly after initiation of therapy and were within the normal range after 4, and 8 weeks of therapy, respectively. Serum levels of sICAM-1 were elevated even after 8 weeks of therapy. Serum levels of sVACM-1 and sICAM-1 correlated with the serum concentrations of anti-thyroid-stimulating hormone (TSH)-receptor antibodies (TSHR-R) ($n = 21$; $r = 0.929$ and $r = 0.810$, respectively) and anti-thyroid peroxidase antibodies (TPO-Ab) ($n = 21$; $r = 0.673$ and $r = 0.750$, respectively). However, no correlation between sELAM-1 and TPO-Ab, TSHR-R, and anti-thyroglobulin antibodies (Tg-Ab), respectively, could be found. In addition to thyroid hormones and autoantibodies, serum concentrations of sELAM-1 and sVCAM-1, but not sICAM-1, could be useful as clinical markers for disease activity.

Keywords intercellular adhesion molecule-1 endothelial leucocyte adhesion molecule-1 vascular cell adhesion molecule-1 Graves' disease

INTRODUCTION

The endothelial vessel wall has been demonstrated to be of relevance in the immunological events of acute and chronic inflammation processes including autoimmune phenomena [1,2]. The up-regulation of adhesion molecules on vascular cells appears to play a major role in the recruitment and targeting of the inflammatory response to certain tissues [1–5]. In patients with Graves' disease (GD) an increased expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leucocyte adhesion molecule-1 (ELAM-1) was observed histologically at various sites of involved tissues [6,7]. Recently, soluble forms of these adhesion molecules were demonstrated in various diseases, but their clinical significance is still undefined [9–20].

In the present study, we determined serum levels of ELAM-1 (sELAM-1), ICAM-1 (sICAM-1) and VCAM-1 (sVCAM-1) in patients with GD before and during thiamazole therapy, in levothyroxin substituted patients with iodine-deficient goitre (IDG), and in a group of healthy controls to elucidate the relation to circulating anti-thyroid antibodies and a possible role of soluble adhesion molecules as markers of inflammatory activity.

PATIENTS AND METHODS

Sera were collected from 21 consecutive patients with GD (19 female, two male), 23 patients with IDG (four male, 19 female) on levothyroxin (T_4) therapy, and from a group of healthy controls ($n = 19$) of similar age and sex distribution.

Correspondence: C. Wenisch MD, Department of Infectious Diseases, Internal Medicine I, University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria.

The patients with GD met the following criteria: (i) biochemical (free triiodthyronin (FT_3) > 4 pg/ml, free thyroxin (FT_4) > 20 pg/ml, and thyroid-stimulating hormone (TSH) < 0·01 μU/ml) and clinical hyperthyroidism (agitation, heat intolerance, sweating, diarrhoea, palpitations, and restlessness); (ii) elevated levels of anti-thyroid antibodies; (iii) first onset of the disease; and (iv) no clinically apparent ophthalmopathy. The patients with GD were treated with 40 mg thiamazole p.o. initially, and subsequently dose adjustment according to hormone levels and clinical response was made. Hormone levels, symptoms, and signs of hyperthyroidism resolved on average after 4–6 weeks of therapy.

The patients with IDG were classified as follows: (i) diffuse enlargement of the thyroid gland; (ii) no elevation of anti-thyroid antibodies; (iii) T_4 therapy; and (iv) biochemical euthyroidism. The blood samples were processed immediately for centrifugation, and sera were stored at −20°C until analysis. All patients and controls gave informed consent before enrolment.

Laboratory procedures

Serum concentrations for sELAM-1, sICAM-1 and sVCAM-1 were determined by enzyme-linked immunoassays (Bender Med Systems, Vienna, Austria (ELAM-1 and ICAM-1), and British Bio-technology Products Ltd, Oxford, UK). For all assays, the intra- and interassay coefficients of variation were less than 5% and 15%, respectively.

The serum levels of sICAM-1, sELAM-1 and sVCAM-1 in normal controls (median (range)) were 166 ng/ml (107–251), 25 ng/ml (6–43), and 14 ng/ml (7–20), respectively. Serum levels of anti-thyroid receptor antibodies (TSHR-R) were routinely determined by a radio receptor assay. The TSH receptors used were prepared by detergent solubilization of thyroid membranes. The receptors were preincubated with test serum. ^{125}I-labelled TSH was then added and incubation continued. The more TSHR-R present in the serum, the lower the number of TSH receptors available for binding the tracer. At the end of the incubation period, receptor-bound radioactivity was precipitated by addition of polyethylene glycol. After centrifugation, the non-bound (non-precipitated) radioactivity was removed and the precipitate counted. Non-specific binding was determined by substituting a detergent solution (lubrol solution) for the TSH receptors (Henning, Berlin, Germany). Anti-thyroglobulin antibodies (Tg-Ab) were determined by a solid-phase technique radio ligand assay using human thyroglobulin-coated tubes (Henning). For the determination of anti-thyroid peroxidase antibodies (TPO-Ab) a competitive solid-phase radioimmunoassay (RIA) using immobilized anti-TPO MoAbs as solid phase competing

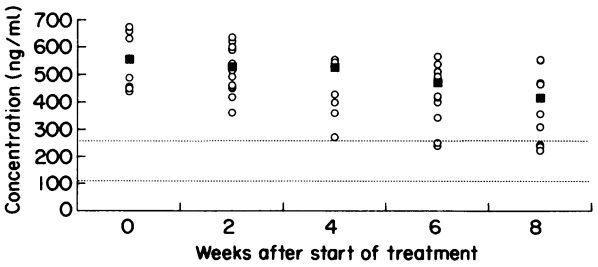

Fig. 1. Serum levels of soluble intercellular adhesion molecule-1 (sICAM-1) in patients with Graves' disease are elevated before and 8 weeks after thiamazole therapy. The black squares denote medians and the dotted lines show normal range (107–251 ng/ml).

Table 1. Serum levels of thyroid hormones, thyroid autoantibodies, and soluble adhesion molecules in patients with Graves' disease, iodine-deficient goitre, and healthy controls*

	Graves' disease (n = 21)‡	Iodine-deficient goitre (n = 23)†	Healthy controls (n = 19)†
Hormone concentrations			
FT_4 (pg/ml)	58·2 (24·3–92·2)	10·1 (6·4–23·3)	11·2 (7·5–18·9)
FT_3 (pg/ml)	16·1 (5·4–32·6)	2·7 (1·7–3·8)	2·8 (1·9–3·8)
TSH (μU/ml)	< 0·01	0·3 (0·01–4·9)	2·1 (0·5–3·7)
Thyroid autoantibodies			
TSHR-R (U/ml)	45·1 (14·4–173·1)	3·2 (0·9–9·3)	2·7 (0·7–6·6)
Tg-Ab (U/ml)	191·5 (22·0–1196·0)	59·7 (23·5–89·4)	47·4 (22·5–78·3)
TPO-Ab (U/ml)	2348 (230–9996)	54·2 (12·6–79·8)	49·8 (10·6–67·9)
Soluble adhesion molecules			
sVCAM-1 (ng/ml)	42 (23–74)	15 (6–22)	14 (7–20)
sICAM-1 (ng/ml)	560 (370–670)	185 (102–250)	166 (107–251)
sELAM-1 (ng/ml)	85 (53–238)	33 (6–48)	25 (6–43)

* Values are expressed as medians, with the ranges given in parentheses.

† None of the differences between patients with iodine-deficient goitre and healthy controls is significant.

‡ The values of patients with Graves' disease are significantly different from patients with iodine-deficient goitre, and healthy controls, respectively (P < 0·05).

FT_4, Levothyroxin; FT_3, triiodthyronin; TSH, thyroid-stimulating hormone; TSHR-R, anti-thyroid receptor antibodies; Tg-Ab, anti-thyroglobulin antibodies; TPO-Ab, anti-thyroid peroxidase antibodies; sVCAM-1, soluble vascular cell adhesion molecule-1; sICAM-1, soluble intercellular adhesion molecule-1; sELAM-1, soluble endothelial leucocyte adhesion molecule-1.

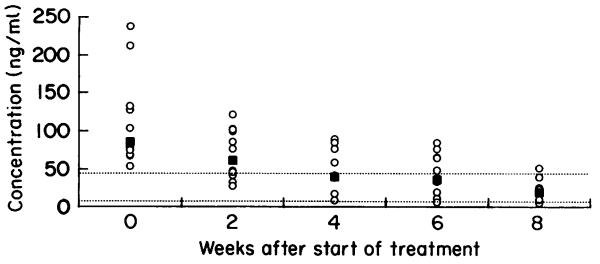

Fig. 2. Serum levels of soluble endothelial leucocyte adhesion molecule-1 (sELAM-1) in patients with Graves' disease are elevated before treatment and drop to normal values after 4 weeks of treatment. The black squares denote medians and the dotted lines show normal range (6–43 ng/ml).

with anti-TPO antibodies in serum was used (Henning). Serum FT_4, FT_3, and TSH were routinely determined by RIA (Behring Werke AG, Marburg, Germany). Normal values are shown in Table 1.

Statistical analysis

Non-parametric tests were used. For comparison between patients and controls Kruskall–Wallis and Mann–Whitney–Wilcoxon *U*-tests were used. Serum levels at different days were compared with the Wilcoxon rank sum test. For correlation analysis Spearman's test was used. All the analyses were two-sided, and differences with $P < 0.05$ were considered statistically significant.

RESULTS

Soluble ICAM-1

The serum levels of sICAM-1 were markedly elevated (Fig. 1) in patients with GD compared with patients with IDG and normal controls (Table 1). A decrease of sICAM-1 levels was observed only 4 weeks after thiamazole treatment. Levels of sICAM-1 remained elevated up to 8 weeks after thiamazole treatment. No significant differences in serum levels of sICAM-1 between IDG patients and normal controls were observed.

Soluble ELAM-1

The levels of sELAM-1 were markedly elevated (Fig. 2) in patients with GD before treatment compared with IDG patients and normal controls (Table 1). The serum levels of sELAM-1 dropped to normal values 4 weeks after therapy was started. Again, no significant differences in serum levels of sELAM-1 between patients with IDG and normal controls were observed.

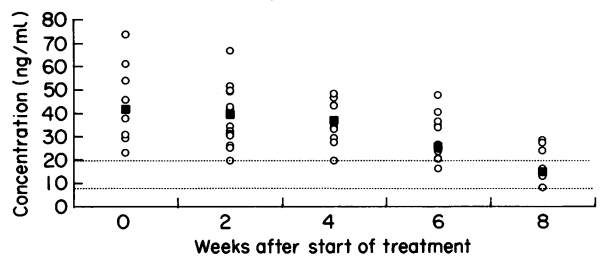

Fig. 3. Serum levels of soluble vascular cell adhesion molecule-1 (sVCAM-1) in patients with Graves' disease are elevated before treatment and drop to normal values after 8 weeks of treatment. The black squares denote medians and the dotted lines show normal range (7–20 ng/ml).

Table 2. Correlation between soluble adhesion molecules and thyroid hormones in patients with Graves' disease ($n = 21$)

Parameter	sVCAM-1	sELAM-1	sICAM-1
FT_4	0·324*	0·051	0·133
FT_3	0·431	0·283	0·115

* Correlation coefficient, $P < 0.05$ for all.

sVCAM-1, Soluble vascular cell adhesion molecule-1; sELAM-1, soluble endothelial leucocyte adhesion molecule-1; sICAM-1, soluble intercellular adhesion molecule-1; FT_4, levothyroxin; FT_3, triiodthyronin.

Soluble VCAM-1

In patients with GD, significantly elevated levels of sVCAM-1 before therapy were observed (Fig. 3 and Table 1). The levels of sVCAM-1 normalized within 8 weeks after therapy was initiated.

Serum levels of sVCAM-1 and sICAM-1 correlated with the serum concentrations of TSHR-R ($r = 0.929$ and $r = 0.810$, respectively; $n = 21$) and TPO-Ab ($r = 0.673$ and $r = 0.750$, respectively; $n = 21$), whereas no correlation between sELAM-1 and TPO-Ab, and TSHR-R, respectively, existed. Serum levels of Tg-Ab and sVCAM-1, sELAM-1 and sICAM-1 did not correlate. In the subgroup of GD patients with elevated Tg-Ab no correlation to soluble adhesion molecules could be detected. Also, no correlation between serum levels of adhesion molecules and thyroid hormones existed (Table 2).

DISCUSSION

The expression of adhesion molecules on vascular endothelium and adjacent extravascular structures is suggested to target the inflammatory response to a particular tissue site [1,2]. ICAM-1, ELAM-1, and VCAM-1 are co-expressed on activated endothelium by inflammatory mediators [8]. Various cytokines and inflammatory mediators have been implicated in enhancing the expression of vascular adhesion molecules [3,21,22]. The expression of ICAM-1 was shown to be up-regulated on thyroidal perifollicular endothelial cells [3], fibroblasts [3], and epithelial cells, including thyrocytes [6,23–28] in autoimmune thyroid diseases both *in vitro* and *in vivo*. Dendritic-like cells, present within lymphocytic infiltrates in thyroid glands of patients with GD were positive for VCAM-1 [6]. In addition, vascular endothelium of retroocular tissue of patients with Graves' ophthalmopathy was strongly positive for ELAM-1 and VCAM-1.

A shedding of soluble determinants of adhesion molecules has been observed [8,11,13,17,29] and, *in vivo*, increased levels of circulating adhesion molecules have been demonstrated in patients suffering from malignancies, inflammatory bowel disease, septic shock, neonatal sepsis, malaria, and autoimmune disorders [9–20,30,31].

Elevated levels of sICAM-1 have been found in patients with Graves' ophthalmopathy [9] which normalize within 3 months after glucocorticoide treatment. In our study, patients with GD without ophthalmopathy were investigated. However, a similar decrease of elevated serum levels could be observed after 2 months of thiamazole treatment. In addition, Heufelder & Bahn demonstrated different effects of sICAM-1 on cell adhesion *in vitro* [9]. sICAM-1 from patients' sera acted as a soluble ligand capable of enhancing mononuclear cell binding to retroocular fibroblasts.

In contrast to sICAM-1, sELAM-1 and sVCAM-1 dropped to normal values within 4 and 8 weeks of therapy, respectively. This could reflect different distributions and/or biological half lives of these molecules. Thus, ICAM-1 can be found on various activated and non-activated cells, whereas the expression of sELAM-1 and sVCAM-1 is limited predominantly to endothelial cells [1,2]. The different kinetics of serum levels of sELAM-1, sVCAM-1, and sICAM-1 could reflect the variable time of appearance and shedding, which was demonstrated in experimental models of inflammation [1,2,8,32]. The observation of accelerated normalization of sELAM-1 in contrast to the slow return to lower levels for sICAM-1 corresponds with the finding that enhanced ICAM-1 expression on endothelial cells can be sustained in the continuous presence of inflammatory cytokines, whereas ELAM-1 expression rapidly returns to baseline levels [33]. This is similar to other inflammatory diseases where a sustained elevation of sICAM-1 had been observed even after clinical improvement, whereas levels of sELAM-1 paralleled clinical cure [12,15–18].

In vivo, elevated serum levels of soluble compounds of adhesion molecules in patients' sera could function as competitive inhibitors of membrane-bound forms, thereby focusing cell adhesion at the site of inflammatory tissue activation. Conversely, in patients with GD, serum levels of sELAM-1 and sICAM-1 are elevated, whereas no significant expression of ELAM-1 and VCAM-1 on thyroid perifollicular endothelial cells could be found [6]. This could implicate thyroid hormones in the activation of endothelial cells [34]. Thus, thyroid hormones could activate vascular endothelium at non-related sites and/or prolong the metabolization of adhesion molecules, and thereby cause an elevation of circulating adhesion molecules. However, in various other conditions with no elevation of thyroid hormones elevated serum levels of adhesion molecules were demonstrated. In contrast to fibronectin, angiotensin-converting enzyme, and factor VIII-related antigen, which are elevated in non-immunologically mediated hyperthyroidism [34], an elevation of soluble adhesion molecules could be specific for immunopathological effects of the vessel wall.

In conclusion, markedly elevated serum levels of sICAM-1, sELAM-1 and sVCAM-1 could be observed in patients with GD compared with normal controls. Serum concentrations of sICAM-1 and sVCAM-1 correlated closely to serum levels of TSHR-R and TPO-Ab. Serum levels of sELAM-1 and sVCAM-1 paralleled clinical improvement during thiamazole treatment, and reached normal levels within 4 and 8 weeks after therapy, respectively, whereas sICAM-1 remained elevated even 8 weeks after start of treatment. Serum levels of sELAM-1, sICAM-1 and sVCAM-1 were not elevated in patients with iodine-deficient goitre and T_4 therapy. The extent of intrathyroidal antibody production and/or expression of adhesion molecules within thyroid glands of patients with GD may not be well reflected by serum levels of antibodies [35]. Serum concentrations of sELAM-1 and sVCAM-1, but not sICAM-1, could be useful as clinical markers for disease activity in addition to thyroid hormones and autoantibodies.

ACKNOWLEDGMENTS

We are grateful to the patients and nursing staff of the Department of Nuclear Medicine, Hospital of St. Pölten, Austria, for their kind cooperation. We are also indebted to Manuela Schmutz and Eva Otter for their skilful assistance.

REFERENCES

1 Springer TA. Adhesion receptors of the immune system. Nature 1990; **346**:424–5.
2 Makgoba MW, Bernard A, Sandees ME. Cell adhesion/signalling: biology and clinical applications. Eur J Clin Invest 1992; **22**:443–53.
3 Heufelder AE, Bahn RS. Modulation of intercellular adhesion molecule-1 (ICAM-1) by cytokines and Graves' IgGs in cultured Graves' retroocular fibroblasts. Eur J Clin Invest 1992; **22**:529–37.
4 Hynes RO. Integrins: versability, modulation, and signaling in cell adhesion. Cell 1992; **69**:11–25.
5 Albelda SM. Endothelial and epithelial cell adhesion molecules. Am J Respir Cell Mol Biol 1991; **4**:195–203.
6 Miyazaki A, Mirakian R, Bottazzo GF. Adhesion molecule expansion in Graves' thyroid glands; potential relevances of granule membrane protein (GMP-140) and intercellular adhesion molecule-1 (ICAM-1) in the homing and antigen presentation processes. Clin Exp Immunol 1992; **89**:52–57.
7 Heufelder AE, Bahn RG. Elevated expression *in situ* of selectin and immunoglobulin superfamily type adhesion molecules in retroocular connective tissues from patients with Graves' ophthalmopathy. Clin Exp Immunol 1993; **91**:381–9.
8 Pigott R, Dillon LP, Hemingway LH, Gearing ASA. Soluble forms of E-selectin, ICAM-1, and VCAM-1 are present in the supernatants of cytokine-activated cultured endothelial cells. Biochem Biophys Res Commun 1992; **187**:584–9.
9 Heufelder AE, Bahn RS. Soluble intercellular adhesion molecule-1 in sera of patients with Graves' ophthalmopathy and thyroid diseases. Clin Exp Immunol 1993; **92**:296–302.
10 Shijubo N, Imai K, Hirasawa M. Circulating intercellular adhesion molecule-1 (ICAM-1) antigen in sera of patients with idiopathic pulmonary fibrosis. Clin Exp Immunol 1992; **89**:58–62.
11 Tsujisaki M, Imai K, Hirata T. Detection of circulating intercellular adhesion molecule-1 antigen in malignant diseases. Clin Exp Immunol 1991; **85**:3–8.
12 Turnkawa S, Imai K, Matsubara H. Increased levels of intercellular adhesion molecular in Kawasaki disease. Arthritis Rheum 1992; **35**:672–7.
13 Harning R, Mainolfi E, Bystryn JC, Henn M, Merluzzi VJ, Rothlein R. Serum levels of circulating intercellular adhesion molecule-1 in human malignant disease. Cancer Res 1991; **51**:5003–5.
14 Campeter ER, Kishimoto TK, Rothlein R. Elevated levels of circulating adhesion molecules in IDDM patients and in subjects at risk for IDDM. Diabetes 1992; **41**:1668–71.
15 Hviid L, Theander TG, Elhorsan IM, Jansen JB. Increased plasma levels of soluble ICAM-1 and ELAM-1 (E-selectin) during acute *Plasmodium falciparum* malaria. Immunolog Lett 1993; **36**:51–8.
16 Wenisch C, Looareesuwan S, Parschalk B, Graninger W. Soluble vascular cell adhesion molecule is elevated in patients with *Plasmodium falciparum* malaria. J Infect Dis 1994; **169**:710–11.
17 Gearing AJH, Hemingway I, Rigott R, Hughes J, Rees AJ, Cashman SJ. Soluble forms of vascular adhesion molecules, E-selectin, ICAM-1, and VCAM-1: pathological significance. N Y Acad Sci 1992; **667**:324–31.

18 Banks RE, Gearing AJ, Hemingway IK, Norfalk DR, Perren TJ, Selby PJ. Circulating intercellular adhesion molecule-1 (ICAM-1), E-selectin and vascular cell adhesion molecule-1 (VCAM-1) in human malignancies. Br J Cancer 1993; **68**:122–4.

19 Küstner H, Regitz K. Circulating ICAM-1 in neonatal sepsis. Lancet 1993; **341**:506.

20 Neumann W, Beall LD, Carson CW. 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.

21 Heufelder AE, Bahn RJ. Graves' immunoglobulins and cytokines stimulate the expression of intercellular adhesion molecule-1 (ICAM-1) in cultured Graves' orbital fibroblasts. Eur J Clin Invest 1992; **22**:529–37.

22 Zheng RQH, Abney ER, Genbeck-Loebenstein B, Dayan C, Maini RN, Feldmann M. Expression of intercellular adhesion molecule-1 and lymphocyte-function-associated antigen-3 on human thyroid epithelial cells in Graves' and Hashimoto's disease. J Autoimmun 1990; **3**:727–36.

23 Tolasa E, Roura C, Cabalfomo M. Expression of intercellular adhesion molecule-1 in thyroid follicular cells in autoimmune, non-autoimmune, and neoplastic of the thyroid gland: discordance with HLA. J Autoimmun 1992; **5**:107–18.

24 Bagnasco M, Caveblo A, Olive D, Pedini B, Candica GW, Bebterle C. Expression of intercellular adhesion molecule-1 (ICAM-1) on thyroid epithelial cells in Hashimoto's thyroiditis but not in Graves' disease or papillary thyroid cancer. Clin Exp Immunol 1991; **213**:809–313.

25 Weetman AP, Cohen S, Makgoba MW, Borysiewicz LK. Expression of an intercellular adhesion molecule, ICAM-1, by human thyroid cells. J Endocrinol 1989; **122**:185–91.

26 Martin A, Huber GK, Davies TF. Induction of human thyroid cell ICAM-1 (CD54) antigen expression and ICAM-1 mediated lymphocyte binding. Endocrinology 1990; **127**:651–7.

27 Focater PD, Tacker M, Whitley GS, Meager A, Nussey SS, Johnstone AP. Expression of intercellular adhesion molecule-1 (ICAM-1) on human thyroid cell lines correlated with their binding of lymphoblasts. Mol Cell Endocrinol 1990; **71**:55–61.

28 Fukazawa H, Yoshida K, Ichinohasama R *et al.* Expression of the Hermes-1 (CD44) and KAM-1 (CD54) molecule on the surface of thyroid cells from patients with Graves's disease. Thyroid 1993; **3**:285–9

29 Becker JC, Dummer R, Hartmann AA, Burg L, Schmidt RE. Shedding of ICAM-1 from human melanoma cell lines induced by IFN-gamma and tumour necrosis factor-alpha. J Immunol 1991; **147**:4398–401.

30 Staunton DE, Ockenhouse CF, Springer TA. Soluble intercellular adhesion molecule-1 immuno-adhesion mediates phagocytosis of malaria infected erythrocyte. J Exp Med 1992; **176**:1471–6.

31 Berendt AR, Simmons DI, Tansey J, Newbold CI, Marsh K. Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for *Plasmodium falciparum*. Nature 1989; **341**:57.

32 Norris P, Poston RN, Thomas DM, Thornhill M, Hawk J, Hoskaral D. The expression of endothelial leukocyte adhesion molecule-1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in experimental cutaneous inflammation: a comparison of ultraviolet B erythema and delayed hypersensitivity. J Invest Dermatol 1991; **96**:763–70.

33 Pober JS, Limbrone Jr MA, Lapierre LAl. Overlapping patterns of activation of human endothelial cells by interleukin-1, tumor necrosis factor, and immune interferon. J Immunol 1986; **137**:1893–9.

34 Graninger W, Pirich KR, Speiser W, Deutsch E, Waldhäusl WK. Effect of thyroid hormones on plasma protein concentrations in man. J Clin Endocrinol Metabol 1986; **63**:407–11.

35 Poschke R, Vogg M, Sevillens S, Usodel KH. Correlation of microsomal antibodies with the intensity of the intrathyroidal autoimmune process in Graves' disease. J Clin Endocrinol Metabol 1993; **77**:939–43.