Clin Exp Immunol 1995; 102:328–334

Expression of vascular adhesion molecules on human endothelia in autoimmune thyroid disorders

M. MARAZUELA, F. SÁNCHEZ-MADRID*, A. ACEVEDO†, E. LARRAÑAGA‡ & M. O. DE LANDÁZURI* *Endocrinology, *Immunology, †Pathology and ‡Surgery Services, Hospital de la Princesa, Universidad Autónoma, Madrid, Spain*

(Accepted for publication 27 June 1995)

SUMMARY

Cellular activation and expression of certain adhesion molecules within vascular endothelium is a critical event in leucocyte recruitment and emigration. A wide array of different adhesion receptors has been identified to mediate the interaction between endothelial cells (EC) and leucocyte subpopulations. In this study, the tissue expression of E-selectin, P-selectin, CD31, and endoglin endothelial cell adhesion molecules was studied on thyroid tissue from patients with Graves' disease (GD) and Hashimoto's thyroiditis (HT). We found an up-regulated expression of E-selectin in EC in GD and HT thyroids, specifically in those areas more severely inflamed, with no reactivity in control thyroids. P-selectin was basally expressed in postcapillary venules in control glands, with an increased expression in HT and GD glands. On the other hand, increased CD31 expression was found on perifollicular, small and large venule EC from GD and HT glands, that correlated with the severity of mononuclear infiltration. In addition, CD31 expression was observed in some intrathyroidal macrophages and T cells in close proximity to $CD31^+$ EC. Furthermore, a markedly enhanced expression of endoglin, a transforming growth factor-beta binding protein, was mainly located on perifollicular EC and EC from small venules as well as in adjacent macrophages from GD and HT thyroid glands. This enhanced expresion of E- and P-selectins, CD31 and endoglin by thyroid EC in GD and HT may reflect their ability to regulate leucocyte trafficking and activation.

Keywords cell adhesion molecules autoimmune thyroid disorders Graves' disease immunology vascular immunology

INTRODUCTION

Leucocyte infiltration of the thyroid in human autoimmune thyroid disorders (AITD) Graves' disease (GD) and Hashimoto's thyroiditis (HT) is probably mediated by a set of adhesion receptors on both leucocytes and endothelial cells (EC) [1]. EC are not only able to produce cytokines, but more importantly can influence leucocyte adhesiveness and homing through the expression of specific adhesion molecules [1]. These interactions are mediated by at least four distinct families of transmembrane glycoproteins: integrins, cadherins, selectins and members of the immunoglobulin superfamily [2,3]. Several groups of adhesive ligands have been described on EC: members of the immunoglobulin superfamily (intercellular adhesion molecule-1 (ICAM-1), ICAM-2, ICAM-3, vascular cell adhesion molecule-1 (VCAM-1), platelet/endothelial cell adhesion molecule-1 (PECAM-1)/CD31), selectins (E- and P-selectins), sialomucins, vascular addressins (MadCAM-1, CD34, GlyCAM), and cadherins. On the other hand, leucocytes express different integrins and carbohydrate determinants interacting with endothelial immunoglobulin members and selectins, respectively [4,5]. In general, the selectin family of adhesion proteins is primarily involved in the initial adhesive event of rolling; the binding of leucocyte integrins to endothelial immunoglobulin-like proteins mediates firm adhesion, and transendothelial migration involves leucocyte and endothelial CD31 as well as leucocyte integrins and endothelial immunoglobulin-like proteins [3].

The selectin family (CD62E, P, L), comprising the two vascular E- and P-selectins and the leucocyte L-selectin, has been involved in leucocyte migration to sites of inflammation and entry to lymphoid organs [6]. All three selectins bind to a similar, but not identical, set of glycosylated determinants [6,7]. E- and P-selectins recognize fucosylated lactosaminoglycans such as sialyl-lewis^x (sLe^x) and sialyl-lewis^a (sLe^a) [8]. A mucin-like protein designated P-selectin glycoprotein ligand (PSGL-1), has recently been identified in myeloid cells [9]. In addition, E-selectin binds to the glycosylated cutaneous lymphoid antigen

Correspondence: Dra Monica Marazuela, Servicio de Inmunología, Hospital de la Princesa, Diego de León 62, Madrid 28006, Spain.

(CLA, HECA-452 antigen) expressed by T and B cells [10], and P-selectin also recognizes the non-sialylated Lewis^x (Le^x, CD15) [11]. E- and P-selectins can interact not only with neutrophils and natural killer (NK) cells, but also with certain minor T cell subsets, chronically activated T cells, at those infiltrating chronic inflammatory sites [12,13]. In addition, we have recently reported in HT the presence of sLe^x, Le^x and CLA in B cells and follicular dendritic cells (FDC), as well as the presence of E-selectin in FDC [14].

Recent reports have suggested that PECAM-1/CD31 may have an important role in leucocyte adhesion, and particularly, in transendothelial migration. CD31 has been found on endothelial cells, platelets and some leucocytes including 50% of peripheral blood mononuclear cells (PBMC), preferentially in neutrophils, monocytes, B cells and a small subpopulation of T cells [15–18]. The CD31 antigen has been shown to play a key role in homophilic adhesion of endothelial cells serving as both ligand and receptor, but can also mediate heterophilic adhesion such as monocyte, PMN and T cell–endothelial cell interactions [17]. In addition to a role for CD31 in adhesion, it has been suggested that a homophilic adhesive interaction, i.e. leucocyte CD31 binding to endothelial CD31, is involved in leucocyte transmigration [19]. Finally, binding to CD31 may also transduce activating signals to leucocytes.

Endoglin is a transmembrane disulphide-linked homodimer protein expressed mainly on vascular endothelial cells and at low levels on macrophages and other cell types [20]. Interestingly, recent experimental studies have demonstrated that endoglin is a transforming growth factor-beta (TGF-β) binding protein in human EC [21]. TGF-β is an important modulatory cytokine which regulates cellular proliferation and differentiation, as well as cell adhesion. TGF-β exerts its signalling functions by binding to membrane-bound specific receptors [22]. Endoglin has been shown to be a component of the TGF-β receptor system [21].

This study was designed to determine the expression and tissue distribution *in situ* of a repertoire of vascular adhesion receptors in HT and GD thyroid glands. We report striking differences in the expression *in situ* of several adhesion molecules between HT and GD and normal thyroid tissue. Our results demonstrate an up-regulated expression of E- and P-selectins, CD31, and endoglin in thyroid EC concomitant with an increased expression of their ligands sLe^x, Le^x and CD31 in the infiltrating leucocytes in GD and HT glands, compared with control glands.

PATIENTS AND METHODS

Patients

Surgical thyroid tissue was obtained from 10 patients with GD (eight women, two men), three female patients with HT and three female normal controls. The diagnosis was established on commonly accepted clinical, laboratory and histological criteria [23]. All GD patients had relapsed after antithyroid drug treatment and were euthyroid under carbimazole therapy at the time of surgery. All had received iodine preoperatively. All HT patients were under thyroxine therapy at the time of surgery. Normal thyroid tissues were obtained from unaffected glands of patients undergoing parathyroidectomy. All GD thyroid specimens studied showed high lymphocytic infiltration.

Monoclonal antibodies

The MoAbs used in this study were TEA 3/9 anti-E-selectin (activated endothelial cells) [14], G1 anti-P-selectin (Centocor Inc., Malvern, PA), G7C5 anti-Le^x (Dr J. S. Thompson, University of Kentucky, Lexington, KY), CSLEX-1 anti-sLe^x (Dr P. Terasaki, University of California, LA, CA), HECA-452 anti-CLA (Dr E. Butcher, Stanford University, Palo Alto, CA) [14], TP1/15 anti-CD31 [24], TEA1/31.1 anti-cadherin 5 [24], TEA 1/5.1 anti-endoglin [24], anti-CD3 (Dako, Glostrup, Denmark), anti-CD20 (Dako) and HC1/1 anti-CD11c [14]. P3 × 63 MoAb (IgG1) used as negative control is the immunoglobulin secreted by the mouse myeloma cell line P3-X63. All MoAbs were used as hybridoma culture supernatants.

Immunohistochemistry

Cryostat sections were cut from snap-frozen thyroid tissue embedded in OCT medium (Ames Co, Miles Laboratories, Elkhart, IN) stored at −80°C. Tissue sections were stained by an indirect immunoperoxidase method as described [25]. Briefly, 5 μm acetone-fixed sections were sequentially incubated with MoAb culture supernatants and peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dako). Each incubation was followed by three washes with Tris-buffered saline (Tris) isotonic buffer pH 7·6. Sections then were developed with the Graham–Karnovsky medium containing 0·5 mg/ml of 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma Chemical Co., St Louis, MO) and hydrogen peroxide. Sections were counterstained with Carazzi's haematoxylin, dehydrated, and mounted by routine methods.

For double immunostaining, the sequential method proposed by Mason *et al.* was used [25]. After the development of DAB reaction, the sections were saturated with non-specific mouse immunoglobulin from the P3 × 63 myeloma line, washed and then incubated with the second MoAb in the same conditions. Subsequently, they were incubated with a rabbit anti-mouse IgG (Dako), followed by a third incubation with the alkaline phosphatase anti-alkaline phosphatase complex (Dako). Each incubation was followed by three washes with Tris. Finally, the alkaline phosphatase reaction was developed by incubating the sections with a Tris–HCl (50 mmol/*l*, pH 8·4) buffer solution containing 0·2 mg/ml Naphtol AS-MX phosphate (Sigma) and 1 mg/ml fast blue salt (Sigma) with 10^{-5} mol/*l* levamisole as endogenous alkaline phosphatase inhibitor. The reaction product was a bright blue precipitate that contrasted with the brown colour or DAB reaction. Sections were mounted in buffered gelatin for microscopic examination.

Each section was examined under code by two independent observers without knowledge of either clinical or histological diagnoses. The expression was assessed in at least 10 different areas. Sequential sections were used to study cell distribution.

RESULTS

Immunohistochemical studies, performed in both Graves' and Hashimoto's glands, showed *de novo* expression of E-selectin in perifollicular EC and EC of small-to-medium size venules in both Graves' and Hashimoto's thyroids (Figs 1a,b). Moreover, E-selectin expression was related to the degree of inflammation, and was mainly expressed by EC in close proximity to lymphocytic infiltrates. In contrast, no E-selectin staining was found in EC of normal thyroid glands (data not shown).

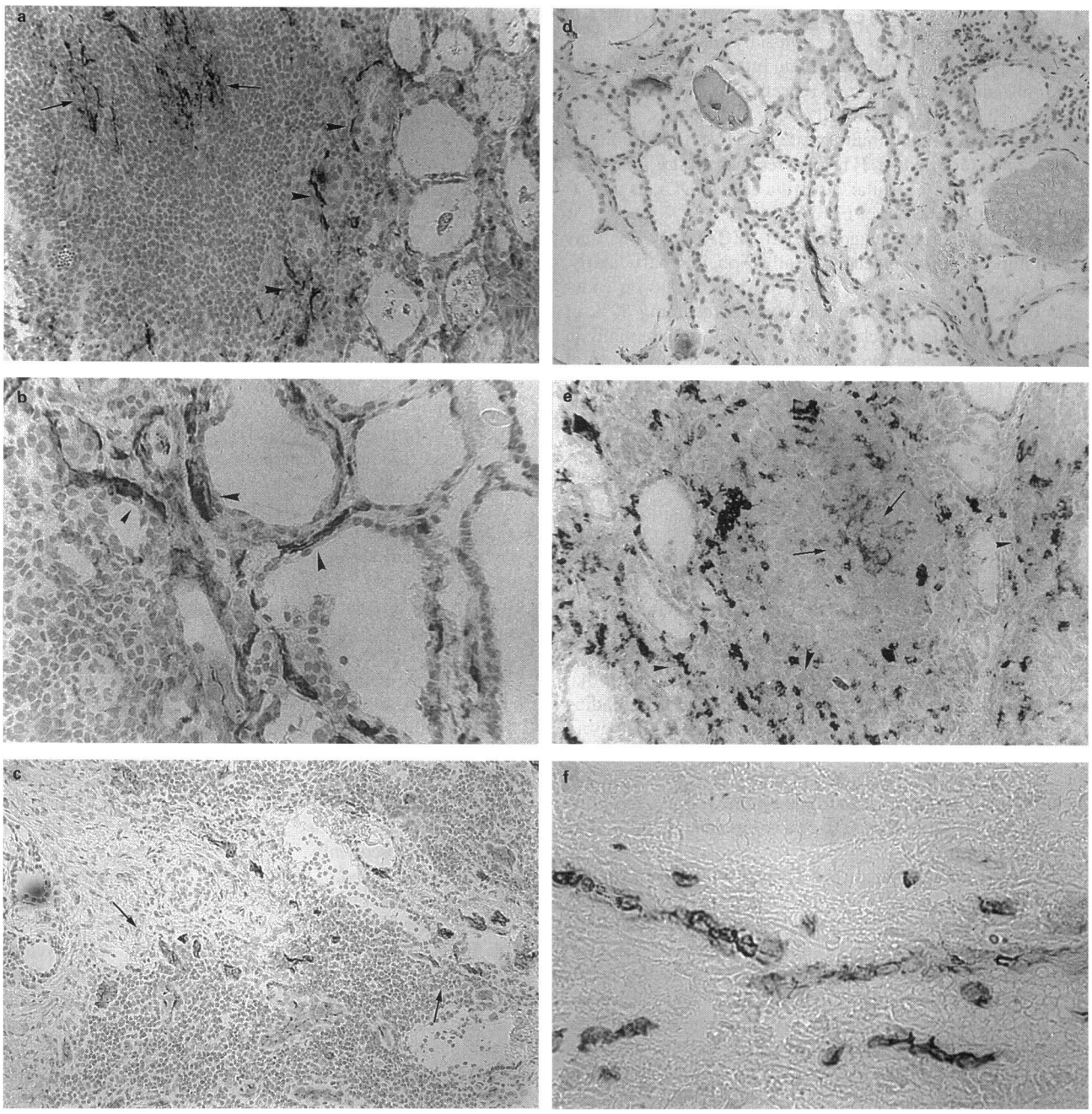


Fig. 1. Selectin expression and selectin-binding carbohydrate expression in immunoperoxidase-stained frozen thyroid sections from patients with Hashimoto's thyroiditis (HT), Graves' disease (GD) and controls. (a) E-selectin is markedly expressed on perifollicular endothelial cells (EC) (arrowheads) and follicular dendritic cells (FDC) (arrows) from a patient with HT. (b) E-selectin expression in perifollicular EC from a patient with GD (arrowheads). (c) Increased P-selectin expression in postcapilliary EC in a HT gland (arrows). (d) Faint positive P-selectin staining by normal thyroid tissue. (e) Positive staining for anti-cutaneous lymphoid antigen (CLA) HECA-452 MoAb in germinal centres on both mononuclear (arrowheads) and FDC (arrows), being more evident in those areas more severely inflamed. (f) P-selectin + EC (arrowheads) were located adjacent to Le^x (arrows) intrathyroidal leucocytes. Magnifications were ×250 (a,c,d,e), and ×500 (b,f).

Strong E-selectin staining was also found in follicular germinal centres corresponding to the expression of this selectin by FDC (Fig. 1a, arrows), as evidenced by parallel staining with the FDC-specific DRC-1 MoAb and by double-staining with both MoAbs (as previously reported [14]). Up-regulation of P-selectin was also found in EC of small-to-medium size (i.e. postcapillary venules) in GD and HT glands compared with normal control sections (Fig. 1c,d), with no expression by perifollicular EC. A positive staining for HECA-452, sLex and Lex epitopes was found in germinal centres on both mononuclear and FDC (Fig. 1e and data not shown). In order to determine the possible relationship between E- and P-selectins with their ligand counterreceptors, double immunostaining experiments were carried out. In both GD and HT a

close relationship between P-selectin and E-selectin EC with Le^x leucocyte cells was found (Fig. 1f and data not shown), being more evident in those areas more heavily infiltrated.

On the other hand, up-regulated expression of CD31 was found in EC of all Graves' and Hashimoto's thyroid glands studied, being maximal in areas with abundant lymphocytic infiltration (Figs 2a and 3a). Positive staining was seen preferentially on perifollicular EC and on EC of small venules, but was also found on EC of large vessels. In contrast, lower numbers of $CD31^+$ vessels were found in EC of normal thyroid glands (Fig. 2b). Expression of CD31 by EC was confirmed by immunostaining of parallel tissue sections with the EC-specific antigen cadherin-5 (data not shown). Furthermore, a striking finding was the detection of $CD31^+$ mononuclear cells in lymphoid aggregates in patients with HT in close proximity to $CD31^+$ EC (Fig. 3a). To define the expression of CD31, immunostaining experiments were performed in serial biopsy sections from patients with HT using a panel of MoAbs against antigens expressed on macrophages and different lymphocyte subsets. The expression of CD31 by B cells was confirmed by immunostaining of parallel tissue sections with the lymphocyte-specific antigens CD20 (Fig. 3b). In addition, CD31 was present, although in lower percentage, in T lymphocytes, as demonstrated by parallel sections with CD3 antigen (Fig. 3c). Although CD31 was negative in most macrophages, as shown by parallel sections (Fig. 3d), it was present in some macrophages as shown by double immunostaining of macrophage-specific antigen (alkaline phosphatase) and CD31 (peroxidase) (Fig. 3d).

Endoglin, a TGF-β-binding protein on human EC, was markedly expressed on most cell types comprising inflamed areas, especially on perifollicular EC and macrophages in GD and HT thyroids, being more evident in those areas more highly infiltrated by mononuclear cells (Fig. 4a,b). By contrast, fewer vessels immunoreactive for endoglin were found on perifollicular EC and on small venules in cases of normal thyroid tissue (Fig. 4c).

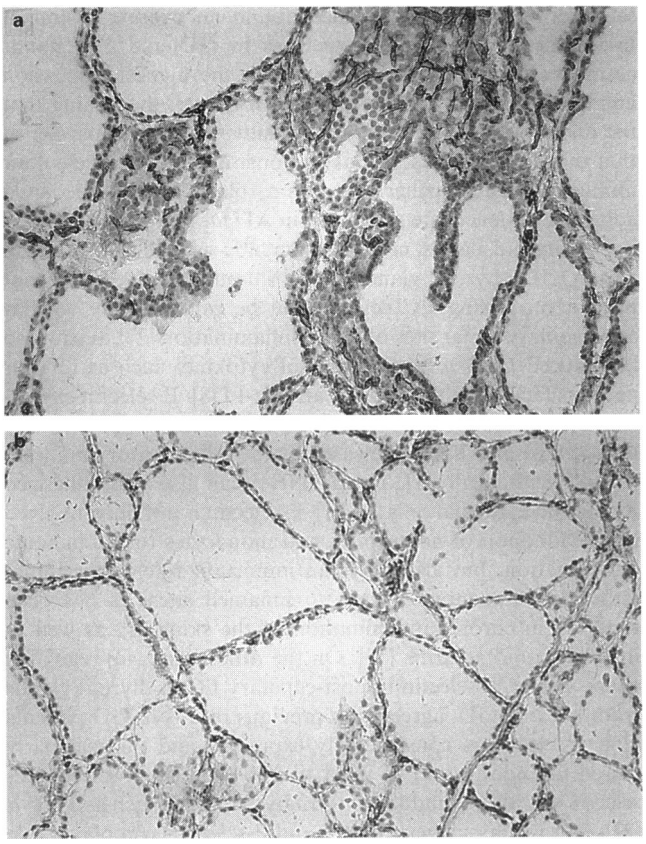
DISCUSSION

The role of antigen recognition and cell adhesion molecules in regulating thyroid immune reactions in AITD has only recently emerged [23]. The induction of the expression of adhesion molecules within vascular endothelium of target tissues is an early and selective event in the recruitment and targeting of an inflammatory response or autoimmune attack to certain tissue sites [1]. In AITD, leucocyte interaction with EC is the initial step of lymphocyte migration before their interaction with extracellular matrix (ECM) proteins and thyrocytes [1]. Thus, we have recently found that up-regulated expression of ICAM-1 and VCAM-1 on EC may contribute, through their interaction with their leucocyte counterreceptors LFA-1 and VLA-4, to the inflammatory infiltration and formation of lymphoid aggregates, which are typical findings in AITD [25].

One of the important pathways for leucocyte adhesion is the initial interaction through the binding to vascular E- and P-selectins [1]. In AITD we have found an overexpression of E-selectin by endothelial structures in GD and HT glands compared with normal controls, with the highest expression adjacent to lymphocyte infiltrates. Moreover, the finding that the distribution of Le^x and sLe^x-positive leucocytes paralelled that of selectin expression, as demonstrated by double-colour immunostaining, further supports a role of selectin-mediated adhesions in leucocyte migration in AITD. In a previous study in GD thyroid glands, no expression of E-selectin was found on EC in GD thyroid glands [26]. Although E-selectin is not present on resting EC cells, it can be expressed by venular and capillary EC at sites of active inflammation [27]. It can also be induced *in vitro* by a variety of cytokines such as tumour necrosis factor-alpha (TNF-α) and IL-1 [28]. E-selectin expression on thyroid EC may have been enhanced by local production of IL-1 and TNF-α released by the inflammatory infiltrate [29]. Parenthetically, IL-1 and TNF-α can also be synthesized by thyroid epithelial cells [30,31]. E-selectin is not only involved in the adhesion of neutrophils and monocytes to EC in acute inflammation, but also plays an important role in the initial tethering of memory T cells to inflamed sites, as has been reported in chronic inflammation of the skin [32], as well as in rheumatoid arthritis [13]. On the other hand, up-regulated expression of P-selectin in post-capillary EC in thyroid glands from HT and GD, agrees with previous results in GD patients [26]. P-selectin is constitutively expressed and intracellularly stored in endothelium of most tissues [9]. Like E-selectin, P-selectin can also by induced *in vivo* by cytokines such as TNF-α [33]. Our results suggest that E- and P-selectins can play a role in leucocyte recruitment to organs undergoing autoimmune attack, such as the thyroid in AITD. Therefore, the induction of E- and P-selectins in thyroid gland EC mediated by cytokines may play an important role in localizing and perpetuating the autoimmune response in AITD.

PECAM-1 (CD31) functions preferentially as an homophilic adhesion molecule and mediates not only intercellular recognition and adhesion between EC, but also contributes to leucocyte–endothelial cell interactions [15,16]. We have found increased CD31 expression not only in EC but also in activated thyroidal B and T cells located surrounding $CD31^+$ EC of GD and HT thyroids. These findings confirm previous studies performed in peripheral blood lymphoid cells showing that CD31 was differentially expressed by monocytes, B cells as well as on subsets of circulating T cells, preferentially $CD8^+$ cells [18]. AITD could represent an *in vivo* situation were CD31 has a role in lymphocyte recruitment during an autoimmune attack, allowing homotypic and heterotypic intercellular interactions. Moreover, CD31 can function as an adhesion amplifier, activating integrins in an 'adhesion cascade', being particularly effective in inducing the function of VLA-4 integrin [17]. It is increasingly apparent how important VLA-4 is in T cell interactions with endothelium in lymphocyte recruitment in AITD [25]. In addition, adhesion of leucocyte CD31 to endothelial CD31 may play a role in transmigration, and help to control the efflux of cells and soluble molecules during the inflammatory response [18].

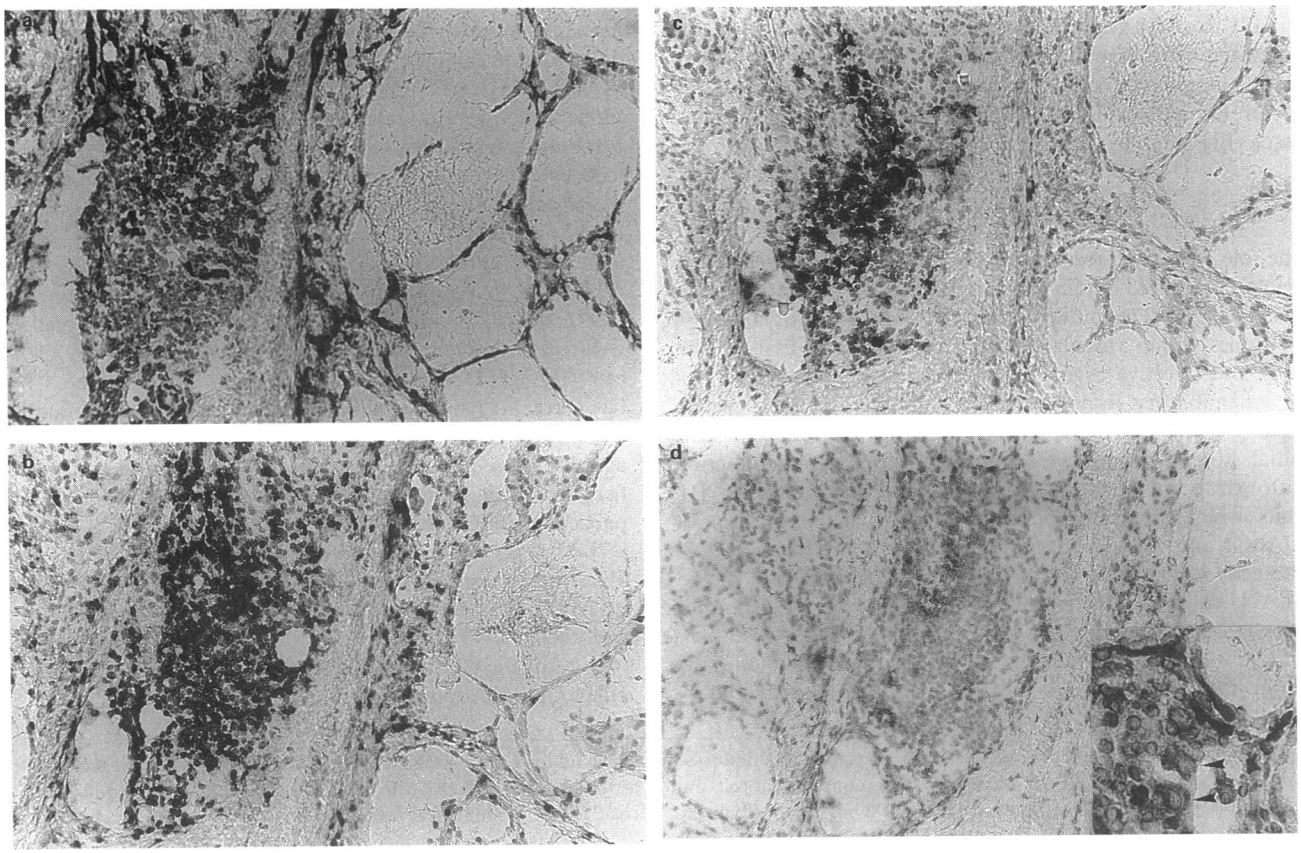
Fig. 3. (bottom of next page) Parallel tissue sections on a Hashimoto's thyroid gland for CD31, CD20, CD3 and CD11c by immunohistochemistry. (a) Positive staining for CD31 in an aggregate of mononuclear cells and in perifollicular endothelial cells (EC). (b) Most $CD31^+$ mononuclear cells are $CD20^+$ B cells. (c) Focal infiltrate with $CD3^+$ T lymphocytes. (d) Most $CD31^+$ mononuclear cells are not $CD11c^+$ macrophages. Double immunostaining for anti-CD31 and anti-CD11c demonstrates expression of CD31 by some of macrophages (arrowheads). Magnifications were ×250.



Finally, we have also observed a marked expression of endoglin in EC and macrophages in inflamed areas in HT and GD thyroid glands. Recent reports have demonstrated that endoglin is a TGF-β binding protein in human EC [21,22], and it is well defined that TGF-β stimulates proliferation of endothelial cells *in vivo* inducing angiogenesis [22]. In the thyroid system, TGF-β suppresses *in vitro* proliferation of thyroidal T cells from patients with GD in response to lectin, IL-2 or autologous thyroid cells, and has a direct inhibitory effect on autoantigen expression by thyroid epithelial cells [34]. In this regard, up-regulated endoglin expression may help to control the autoimmune process through the inhibition of autoantigen expression.

In conclusion, we have found in AITD an up-regulated intrathyroidal expression of the different vascular adhesion molecules studied, with no specific differences between GD and HT. Nevertheless, previous therapies with anti-thyroid drugs and thryoxine therapy may have modified the expression of these molecules. Although differences between thyroid specimens were clear using semiquantitative methods, quantification of the expression using confocal laser scanning microscopy could add further evidence to demonstrate this issue more accurately.

Fig. 2. (left) Intrathyroidal expression pattern of platelet/endothelial cell adhesion molecule (PECAM)/CD31 by immunohistochemistry. Strong CD31 reactivity in both perifollicular endothelial cells (EC), small and large vessel Graves' disease (GD) (a) glands. (b) Lesser number of $CD31^+$ EC in normal thyroid tissue.



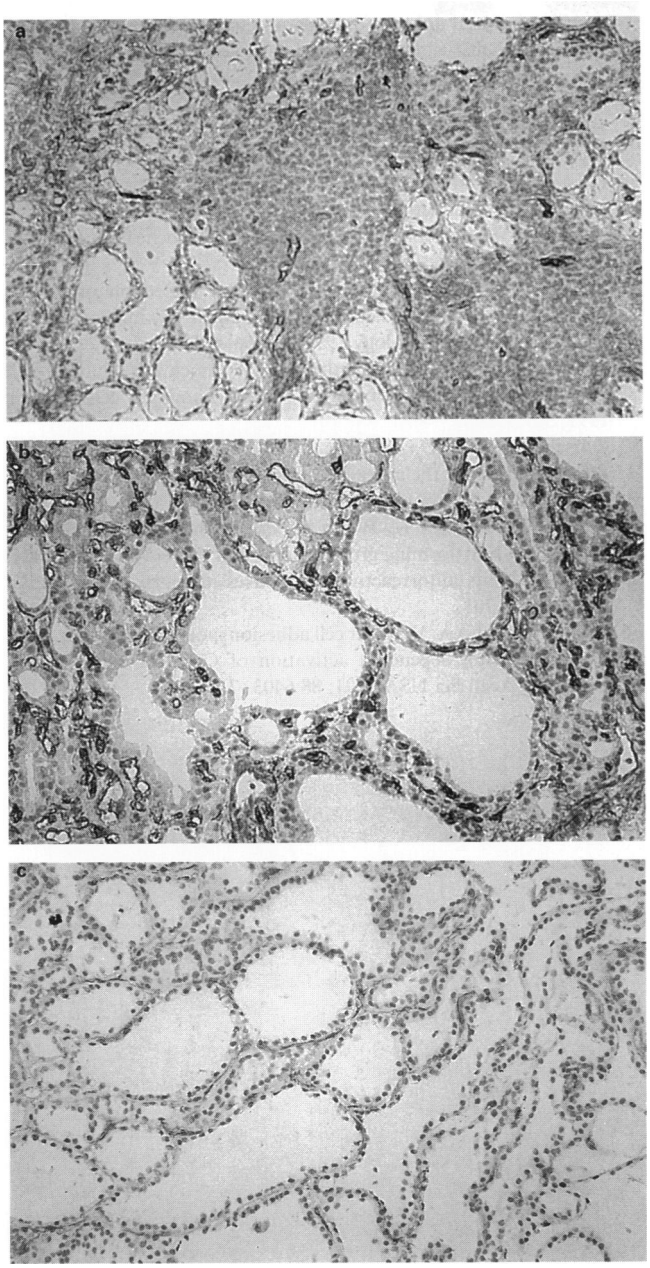


Fig. 4. Intrathyroidal endoglin expression pattern. (a,b) Endoglin was markedly expressed on inflamed vessels as well as on adjacent macrophages in Hashimoto's thyroiditis (HT) (a) and Graves' disease (GD) (b) thyroids. (c) In normal thyroid tissue, a lower number of endoglin-positive cells was observed in perifollicular and large vessel endothelial cells (EC). Magnifications were ×125 (a) and ×250 (b,c).

A possible model to explain tissue infiltration in AITD could be an adhesion cascade consisting of a coordinated series of receptor–ligand interactions, which include: (i) initial tenuous adhesion or tethering mediated by the selectin family [1]; (ii) triggering; CD31 is an excellent candidate for an adhesion amplifier in T cell–EC interactions, giving its adhesion-inducing capacity [17]; (iii) integrin-mediated strong adhesion, most probably mediated by the integrins LFA-1 and VLA-4 as previously reported [25]. Altogether, these data could help in an understanding of the possible role of adhesion molecules in directing leucocyte traffic to the thyroid gland, not only involving its localization, but also their activation and proliferation, as these molecules can also deliver proliferative signals to T cells [35]. It is likely that interference of these mechanisms could provide new approaches in the therapy of AITD.

ACKNOWLEDGMENTS

This work was supported by grants from INSALUD (FIS 94/1695).

REFERENCES

1 Shimizu Y, Newman W, Tanaka Y, Shaw S. Lymphocyte interactions with endotheial cells. Immunol Today 1992; **13**:106–12.
2 Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 1994; **71**:301–14.
3 Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. Blood 1994; **84**:2068–101.
4 Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 1992; **69**:11–25.
5 McEver RP. Leukocyte–endothelial cell interactions. Curr Opinion Cell Biol 1992; **4**:840–9.
6 Bevilacqua M, Nelson RM. Selectins. J Clin Invest 1993; **91**:379–87.
7 Feizi T. Oligosaccharides that mediate mammalian cell–cell adhesion. Curr Opinion Struct Biol 1993; **3**:701–10.
8 Phillips ML, Nudelman E, Gaeta FCA *et al.* ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Le^x. Science 1990; **250**:1130–2.
9 Sako D, Chang X-J, Barone KM *et al.* Expression cloning of a functional glycoprotein ligand for P-selectin. Cell 1993; **75**:1179–85.
10 Berg EL, Yoshino T, Rott LS *et al.* The cutaneous lymphocyte antigen is a skin lymphocyte homing receptor for the vascular lectin endothelial cell-leukocyte adhesion molecule 1. J Exp Med 1991; **174**:1461–6.
11 Larsen E, Palabrica T, Sajer S *et al.* PADGEM-dependent adhesion of platelets to monocytes and neutrophils is mediated by a lineage-specific carbohydrate, LNF III (CD15). Cell 1990; **63**:467–74.
12 Picker LJ, Kishimoto TK, Smith CW, Warnock RA, Butcher EC. ELAM-1 is an adhesion molecule for skin-homing T cells. Nature 1991; **349**:796–9.
13 Postigo AA, Garcia-Vicuña R, Diaz-Gonzalez F *et al.* Increase of synovial T lymphocytes from rheumatoid arthritis to endothelial-leukocyte adhesion molecule-1 (ELAM-1) and vascular cell adhesion molecule-1 (VCAM-1). J Clin Invest 1992; **89**:1445–52.
14 Postigo AA, Marazuela M, Sánchez-Madrid F, Landázuri MO. B lymphocyte binding to E- and P-selectins is mediated through *de novo* expression of carbohydrates on *in vitro* and *in vivo* activated human B cells. J Clin Invest 1994; **94**:1585–96.
15 DeLisser HM, Newman PJ, Albelda SM. Molecular and functional aspects of PECAM-1/CD31. Immunol Today 1994; **15**:490–5.
16 Newman PJ, Berndt MC, Gorski J *et al.* PECAM-1 (CD31) cloning and relation to adhesion molecules of the immunglobulin gene superfamily. Science 1990; **247**:1219–22.
17 Tanaka Y, Albelda SM, Horgan KJ *et al.* CD31 expressed on distinctive T cell subsets is a preferential amplifier of β1 integrin-mediated adhesion. J Exp Med 1992; **176**:245–53.
18 Ashman LK, Aylatt GW. Expression of CD31 epitopes on human lymphocytes: CD31 monoclonal antibodies differentiate between naive (CD45RA$^+$) and memory (CD45RA$^-$) CD4-positive T cells. Tissue Antigens 1991; **38**:208–12.
19 Muller WA, Berman ME, Newman PJ, DeLisser HM, Albelda SM. A heterophilic adhesion molecule for platelet/endothelial cell adhesion molecule-1 (CD31). J Exp Med 1992; **175**:1401–4.
20 Lastres P, Bellón T, Cabañas C *et al.* Regulated expression on human macrophages of endoglin, an Arg-Gly Asp-containing surface antigen. Eur J Immunol 1992; **22**:393–7.

21 Cheifetz S, Bellón T, Calés C *et al.* Endoglin is a component of the transforming growth factor-β receptor system in human endothelial cells. J Biol Chem 1992; **267**:19027–30.
22 Moses HL, Yang EY, Pietenpol JA. TGF-β stimulation and inhibition of cell proliferation: new mechanistic insights. Cell 1990; **63**:245–7.
23 García-Monzón C, Sánchez-Madrid F, García-Buey L, García-Arroyo A, García-Sánchez A, Moreno-Otero R. Vascular adhesion molecule expression in viral chronic hepatitis: evidence of neoangiogenesis in portal tracts. Gastroenterology 1995; **108**:231–41.
24 Champion BR, Cooke A, Rayner DC. Thyroid autoimmunity. Curr Opinion Immunol 1992; **4**:770–8.
25 Marazuela M, Postigo AA, Acevedo A, Díaz-González F, Sánchez-Madrid F, Landázuri MO. Adhesion molecules from the LFA-1/ICAM.1, 3 and VLA-4/VCAM-1 pathways on T lymphocytes and vascular endothelium in Graves' and Hashimoto's thyroid glands. Eur J Immunol 1994; **24**:2483–90.
26 Miyazaki A, Mirakian R, Botazzo GF. Adhesion molecule expression in Graves' thyroid glands: potential relevance 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.
27 Cotran RS, Gimbrone MA, Bevilacqua MP, Mendrick DL, Pober JS. Induction and detection of a human endothelial activation antigen *in vivo*. J Exp Med 1986; **164**:661–6.
28 Carlos T, Kovach N, Swartz B *et al.* Human monocytes bind to two cytokine-induced adhesive ligands on cultured human endothelial cells: endothelial-leukocyte adhesion molecule-1 and vascular cell adhesion molecule-1. Blood 1991; **77**:2266–71.
29 Iwatani Y, Gerstein HC, Iitaka M, Row VV, Volpé R. Thyrocyte HLA-DR expression and interferon-γ production in autoimmune thyroid disease. J Clin Endocrinol Metab 1986; **63**:695–708.
30 Zheng RQH, Abney E, Chu CQ *et al.* Detection of interleukin-6 and interleukin-1 production in human thyroid epithelial cells by non-radioactive *in situ* hybridization and immunohistochemical methods. Clin Exp Immunol 1991; **83**:314–9.
31 Zheng RQH, Abney ER, Chu CQ *et al.* Detection of *in vivo* production of tumour necrosis factor-alpha by human thyroid epithelial cells. Immunology 1992; **75**:456–62.
32 Picker LJ, Kishimoto TK, Smith CW, Warnoch RA, Butcher EC. ELAM-1 is an adhesion molecule for skin-homing T cells. Nature 1991; **349**:796–9.
33 Gotsch U, Jäger U. Dominis M, Vestweber D. Expression of P-selectin on endothelial cells is upregulated by LPS and TNF-α *in vivo*. Cell Adh Commun 1994; **2**:7–14.
34 Widder J, Dorfinger K, Wilfing A *et al.* The immunoregulatory influence of transforming growth factor β in thyroid autoimmunity: TGF β inhibits autorreactivity in Graves' disease. J Autoimmun 1991; **4**:689–701.
35 Damle N, Aruffo A. Vascular cell adhesion molecule 1 induces T cell antigen receptor-dependent activation of $CD4^+$ T lymphocytes. Proc Natl Acad Sci USA 1991; **88**:6403–7.