

Follicular thyroid cells of autoimmune thyroiditis may coexpress ICAM-1 (CD54) and its natural ligand LFA-1 (CD11a/CD18)

Marcello Bagnasco, MD,^a Giampaola P. Pesce, BS,^a
Antonio Caretto, MD,^b Francesca Paolieri, MD,^a Caterina Pronzato, MD,^a
Barbara Villaggio, BS,^c Carla Giordano, MD,^d Corrado Betterle, MD,^b
and G. W. Canonica, MD^a Genoa, Padoa, and Palermo, Italy

The leukocyte functional associated antigen-1 (LFA-1)-intercellular adhesion molecule-1 (CD11a-CD18/CD54) intercellular adhesion system plays a crucial role in several immunologic phenomena, including adhesion between lymphocytes and epithelial cells. In previous studies evidence for CD54 expression on thyroid follicular cells in Hashimoto's thyroiditis was provided. In this study we evaluated the possible expression of CD11a and CD18 antigens on thyrocytes of patients with Hashimoto's thyroiditis and on thyrocytes of patients with Graves' disease and simple goiter as controls; we used both alkaline phosphatase immunostaining and indirect immunofluorescence on cryostatic tissue sections. The results showed that LFA-1 (both CD11a and CD18) positivity on thyroid follicles may occur in glands of patients with Hashimoto's disease, with a pattern very similar to that of CD54: this was observed in five of seven specimens. Conversely, no positivity was observed in tissues from patients with Graves' disease or goiter: notably, isolated follicular cells from Graves' goiter tissues are induced in culture to express CD54, but not LFA-1. Using double-staining techniques, we were able to show that in specimens from patients with Hashimoto's disease, the same follicular structures coexpressed LFA-1 and CD54. Such a coexpression of the two ligands further emphasizes the possible role of this adhesion system in the pathogenesis of epithelial damage, through bidirectional interactions between thyroid epithelial cells and infiltrating LFA-1 or CD54-positive mononuclear cells. (J ALLERGY CLIN IMMUNOL 1995;95:1036-43.)

Key words: ICAM-1, LFA-1, thyroid autoimmunity, epithelial cells, cell adhesion

During the last 10 years, much evidence has been provided for "cross-talking" between thyroid

From ^aServizio di Allergologia ed Immunologia Clinica e Centro di Immunoendocrinologia, Dipartimento di Medicina Interna, University of Genoa; ^bIstituto Semeiotica Medica, University of Padoa; ^cCattedra di Nefrologia, Dipartimento di Medicina Interna, University of Genoa; and ^dCattedra di Endocrinologia, Clinica Medica, University of Palermo.

Supported in part by Consiglio Nazionale delle Ricerche P.F. Fattori di Malattia SP2 grant to G. W. Canonica, MD, no. 5710, Consiglio Nazionale delle Ricerche P.F. "Ingegneria genetica" grant no. 91.00018, to M. Bagnasco, and by Associazione Ricerca Malattie Immunologiche e Allergiche foundation. Caterina Pronzato is the recipient of an Associazione Italiana per la Ricerca sul Cancro fellowship.

Received for publication May 27, 1993; revised Aug. 9, 1994; accepted for publication Oct. 3, 1994.

Reprint requests: Marcello Bagnasco, MD, Servizio di Allergologia e Immunologia Clinica, D.J.M.I.—University of Genoa, Viale Benedetto XV, 6, 16132 Genoa, Italy.

Copyright © 1995 by Mosby-Year Book, Inc.
0091-6749/95 \$3.00 + 0 1/1/62173

Abbreviations used

APAAP:	Alkaline phosphatase-monoclonal anti-alkaline phosphatase complex
FITC:	Fluorescein isothiocyanate
GD:	Graves' disease
HT:	Hashimoto's thyroiditis
ICAM-1:	Intercellular adhesion molecule-1
LFA-1:	Leukocyte functional associated antigen-1
mAb:	Monoclonal antibody
SG:	Simple goiter
TBS:	Tris-buffered saline
TPO:	Thyroid peroxidase

follicular cells and infiltrating mononuclear cells in autoimmune diseases. Namely, the autoimmune thyrocyte is able to express major histocompatibility class II antigens,¹ in addition to large amounts

of class I antigens, to efficiently present exogenous processed peptides² and to elicit the proliferation of specific autoreactive CD4⁺ T-cell clones.³ It is likely that, especially in autoimmune thyroiditis, follicular cells may interact with CD4⁺ regulatory or CD8⁺ (or even CD4⁺) cytotoxic cells through surface major histocompatibility complex molecules, and such a mechanism is crucial in the pathophysiology of the disease. Moreover, it has been demonstrated in different laboratories, including our own, that CD54, or intercellular adhesion molecule-1 (ICAM-1), an intercellular adhesion molecule belonging to the immunoglobulin superfamily, can be induced on thyrocytes in culture⁴⁻⁷ and is constitutively expressed on autoimmune thyrocytes, notably in Hashimoto's thyroiditis (HT).^{4,8,9} Such a molecule, by interacting with its physiologic ligand, the leukocyte integrin leukocyte functional associated antigen-1 (LFA-1) (CD11a/CD18), plays a crucial role in most immune reactions requiring cell-to-cell contact.¹⁰⁻¹³ Thereafter, expression of CD54 has been demonstrated on other types of epithelial cells during the course of an inflammatory reaction, including allergic inflammation.¹⁴⁻¹⁹

In this study we show that thyroid epithelial cells from patients with HT may bear on their surface, together with CD54 antigen, its physiologic ligand LFA-1, which is not present on cultured thyrocytes and has been previously described only on leukocytes.

METHODS

Tissue specimens

We examined surgically removed thyroid tissue from seven patients with HT, five with Graves' disease (GD), and three with simple goiter (SG) (all women, aged 22 to 64 years). The diagnosis was established on the basis of commonly accepted clinical and laboratory parameters (including free thyroxine and triiodothyronine radioimmunoassays, thyroid-stimulating hormone immunoradiometric assay, technetium 99 scintiscan, thyroid ultrasonography, ELISA of antithyroglobulin and anti-TPO antibodies) and typical histologic features. The patients with HT underwent surgery because of suspected thyroid lymphoma on the basis of cytologic examination results after fine needle biopsy was performed. At the time of histologic examination (hematoxylin-eosin staining of paraffin-embedded sections), specimens from patients with GD showed mild to moderate presence of lymphoid cells, in some instances grouped in little areas; however, no important mononuclear cell accumulation, as observed in HT (focal thyroiditis), was observed.

HT specimens invariably showed large to very large

accumulation of lymphoid cells, with oxyphil metaplasia of variable extent. Fibrosis was present in one specimen.

After removal, thyroid specimens were cut into small blocks, snap-frozen in 2-methylbutane, and then stored at -80°C until they were sectioned. In some cases fresh sterile tissue specimens were immediately carried to the laboratory in cold phosphate-buffered saline and processed as described below for follicular cell isolation.

Monoclonal antibodies

The following mouse monoclonal antibodies (mAbs) were used:

CD54 (or ICAM-1): 84H10 (Immunotech, Marseilles, France), IgG₁

CD11a (α -chain of LFA-1): 25.3.1, IgG₁, and 8F2.7, IgG₁

CD18 (β -2 chain of LFA-1): 41.23.2, IgG₁

(The latter three were generous gifts from Dr. Daniel Olive, INSERM U119, Marseilles).^{11, 20, 21}

Other IgG₁ mAbs were also tested as controls in parallel experiments: anti-very late activation antigen-1 (Act-T-set; T Cell Diagnostics, Cambridge, Mass.) and the anti-natural killer cell subsets, mAbs GL183 and EB6 (a generous gift of Prof. Allesandro Moretta, Genoa). The anti-thyroglobulin mAbs DAK-IgG (IgG 2a, Dakopatts A/S, Copenhagen, Denmark) were used in other control experiments.

Immunohistochemistry and double staining

Four-micrometer-thick tissue sections were cut with a Reichert-Jung cryostat (Leica Instruments GmbH, Nussloch, Germany), placed on alcohol-cleaned glass slides, air-dried, and stored at -80°C until use. Just before staining, the sections were allowed to equilibrate to room temperature, then exposed to absolute acetone for 10 minutes, followed by air-drying for another 10 minutes. An immunoenzymatic alkaline phosphatase monoclonal anti-alkaline phosphatase complex (APAAP) procedure was used throughout, according to previously described methods.^{22, 23} Briefly, the sections were submitted to appropriate dilutions of each mAb. After washing in Tris-buffered saline (TBS) pH 7.6, the sections were incubated with rabbit anti-mouse immunoglobulins, followed by APAAP complex: the two last steps were repeated to enhance the sensitivity. Finally, the sections were incubated in substrate solution, containing basic new fuchsin, naphthol AS-BI phosphate, and levamisole (as an inhibitor of endogenous alkaline phosphatase) (all reagents from Sigma Chemical Co., St. Louis, Mo.). Control sections were set up with irrelevant isotype-matched mAbs or by omitting anti-mouse immunoglobulins. The preparations were counterstained with Carazzi's hematoxylin.

For double staining, streptavidin-peroxidase and streptavidin-AP were used in combination.²⁴ To this end, the sections were pretreated with H₂O₂ and methanol to inhibit endogenous peroxidase activity and then washed by immersion in TBS. Then they were incubated with the first mAb at room temperature for 30 minutes,

followed by repeated TBS washing. Then, link biotinylated anti-mouse immunoglobulins (Biogenex Laboratories, San Ramon, Calif.) at saturating concentrations were added. After a 10-minute incubation and repeated washing, the sections were incubated with horseradish peroxidase-labeled streptavidin (Biogenex) for another 10 minutes. After washing, the preparations were stained with diaminobenzidine (Sigma) and H_2O_2 at room temperature for 15 minutes, followed by 5-minute washing in distilled water. The samples were then incubated with excess avidin (Sigma) for 7 minutes, the second mAb was added, and incubation was carried out for 30 minutes as described above. After washing in TBS, the sections were again added to link biotinylated anti-mouse immunoglobulins for 10 minutes.

After washing with phosphate-buffered saline, the samples were incubated with alkaline phosphatase-labeled streptavidin (Biogenex) for 10 minutes. After further washing, the sections were stained with APAAP substrate solution as described above, and hematoxylin counterstaining (30 seconds) was performed. Control samples were set up by omitting either the first or second mAb or both. The preparations were mounted in buffered glycerol and examined by means of a Leitz Laborlux microscope (Wild Microscopes, Rockleigh, N.J.) equipped for microphotography.

Indirect immunofluorescence staining

Immunofluorescence was performed as previously described.⁸ Briefly, the sections were incubated for 60 minutes with the appropriate mAbs; after washing, they were stained with biotinylated rabbit anti-mouse immunoglobulin antiserum for 30 minutes, followed by fluorescein isothiocyanate (FITC)-conjugated avidin (Sigma) for an additional 30 minutes. Parallel sections were set up with the control mAbs. Double immunofluorescence control staining was performed with a human serum strongly positive for anti-peroxidase autoantibodies and CD11a mAb in combination. The section was first incubated for 30 minutes with the human serum (optimal dilution 1:40, tested by previous titration, compared with a TPO-negative serum) for 30 minutes, washed, then incubated with biotinylated anti-human IgG F(ab)2 goat antiserum (Dakopatts) for 30 minutes. After appropriate washing, sequential incubations were carried out with CD11a mAb and appropriately diluted phycoerythrin-labeled F(ab)2 rabbit anti-mouse immunoglobulin antiserum (Dakopatts). The fluorescence pattern was evaluated with a fluorescence-equipped microscope.

Follicular cell isolation and staining

Follicular cell isolation and staining were performed as previously described.²³ Briefly, tissues were minced in small pieces, extensively teased in RPMI 1640 culture medium containing L-glutamine (Gibco, Grand Island, N.Y.) and antibiotics supplemented with 10% fetal calf serum, washed, and digested by type IV collagenase (2

mg/ml in culture medium). The resulting suspension was filtered through a 60-mesh screen and submitted to density gradient centrifugation on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). After washing, the cells were resuspended in culture medium and kept in tissue culture flasks for 2 to 4 days in humidified air containing 5% CO_2 . At the appropriate time, the isolated cells were stained by indirect immunofluorescence with the above-mentioned mAbs and an FITC-labeled anti-mouse immunoglobulin antiserum (Organon Teknika Corp., Durham, N.C.). Control staining was performed with a human serum strongly positive for both anti-thyroglobulin and anti-peroxidase antibodies and an FITC-labeled anti-human immunoglobulin antiserum (Dakopatts).

Cytofluorimetric reading was performed by an Epics ProFile II (Coulter Corp., Miami, Fla.) or a Becton-Dickinson FacScan cytofluorimeter (Becton Dickinson, Mountain View, Calif.).

RESULTS

We stained thin cryostatic sections of thyroid tissue from patients with HT, or tissue from patients with GD and SG for control, with CD54 and CD11a (LFA-1 α -chain) mAbs with both immunohistochemistry and indirect immunofluorescence. No thyroid follicular cell staining was observed in GD with both immunofluorescence and APAAP methods: in this disease LFA-1 mAb stained only infiltrating mononuclear cells, as expected (not shown). No staining was observed in tissues from patients with SG.

However, when thyroid follicular cells from patients with SG ($n = 3$) or patients with GD ($n = 2$) were isolated after collagenase digestion and cultured for 2 to 4 days, even without any stimulus, a spontaneous CD54 expression was noted on a variable proportion of thyrocytes (12% to 65%) (not shown). This confirms the results obtained in other laboratories.^{5,6} In the same conditions, no positivity for CD11a was observed.

Confirming previous results, five of six specimens from patients with HT exhibited CD54 positivity on follicular cells (Fig. 1). This was apparent with both immunofluorescence and APAAP methods. As previously described, entire follicular structures or follicular portions expressed CD54: the positivities were often (but not invariably) near mononuclear cell infiltration areas.

As far as LFA-1 is concerned, follicular cell staining, similar to that of CD54, was apparent in four specimens. As shown in Fig. 2 (APAAP) and Fig. 3 (immunofluorescence), CD11a mAb stained whole follicles or follicular portions with a pattern quite similar to that of CD54 mAb. Quite similar LFA-1 staining (mainly pericellular) was observed

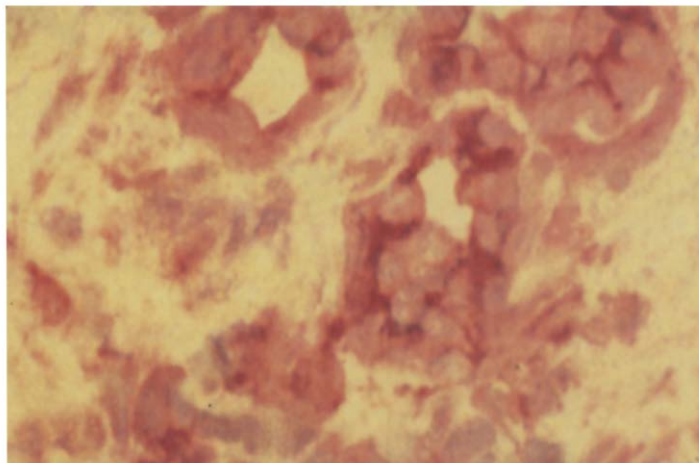


FIG. 1. Follicular APAAP staining (red) by the CD54 mAb 84H10 in a patient with HT (original magnification, $\times 500$).

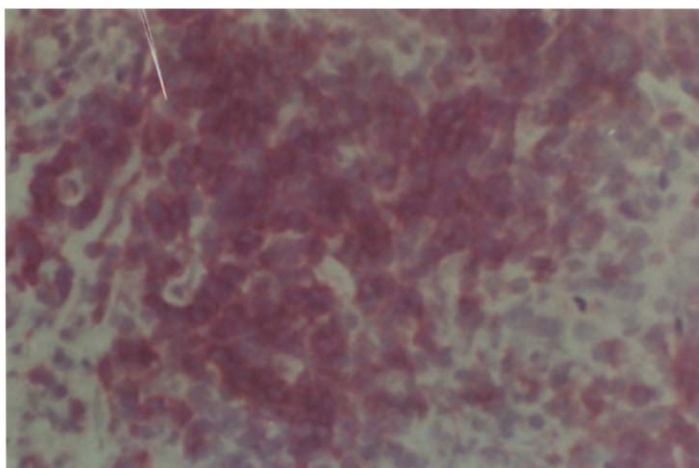


FIG. 2. Follicular APAAP staining by the CD11a mAb 25.3.1 in a Hashimoto's specimen. Identical results were obtained with the other CD11a mAb (8F2.7) (original magnification, $\times 500$).

on follicular structures and infiltrating mononuclear cells. Similar results were obtained when a CD18 mAb (recognizing the invariant β_2 chain of leukocyte integrins) was used (not shown). The specificity of LFA-1 follicular staining was confirmed by the lack of staining with other isotype-matched mAbs tested as controls, such as anti-very late activation antigen-1 and anti-natural killer cells—GL183 and EB6. That LFA-1 stained follicular structures was confirmed by double immunofluorescence experiments, in which thyroid follicular cells were stained by using (simultaneously) a human serum with high titers of anti-peroxidase antibodies, a specific FITC-labeled antiserum, and the CD11a mAb, followed by specific phycoerythrin-labeled antiserum. As shown in Fig. 4, double staining of

follicular portions (orange fluorescence) is apparent. Similar results were obtained by double immunoenzymatic staining by using CD11a and anti-thyroglobulin mAbs simultaneously (not shown)

Of note, CD11a (or CD18) positivity on follicular cells was observed only in subjects whose follicular cells were also CD54-positive. Moreover, staining of serial sections with CD11a and CD54 suggested that the same follicular structures were stained by the two mAbs. To verify this possibility, we performed double immunoenzymatic staining experiments. As shown in Fig. 5, some follicular structures were double-stained, as indicated by the amaranth color (resulting from the fusion of dark brown produced by diaminobenzidine-CD54 staining and red produced by fuchsin-LFA-1 staining).

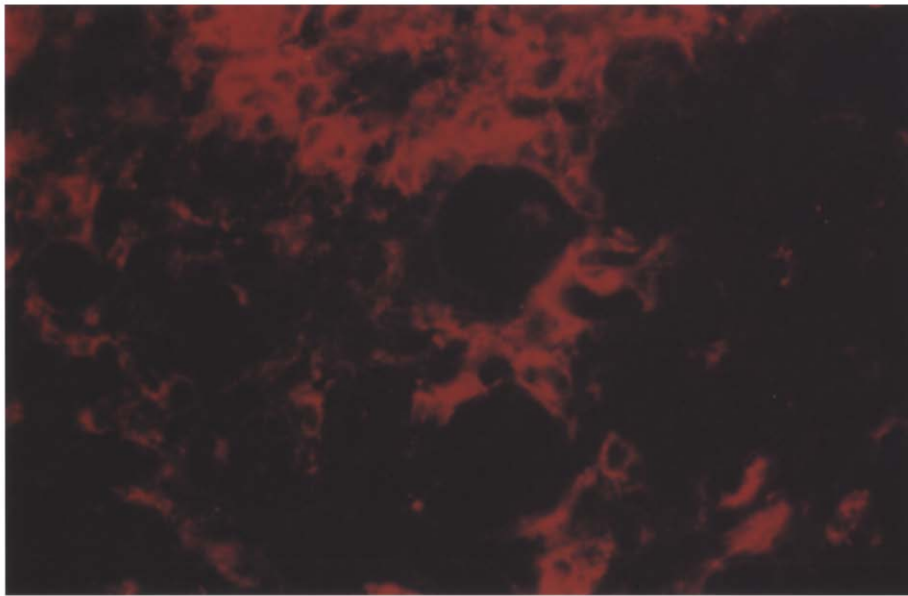


FIG. 3. Follicular CD11a (25.3.1) staining in HT (indirect immunofluorescence-phycoerythrin) (original magnification, $\times 500$).

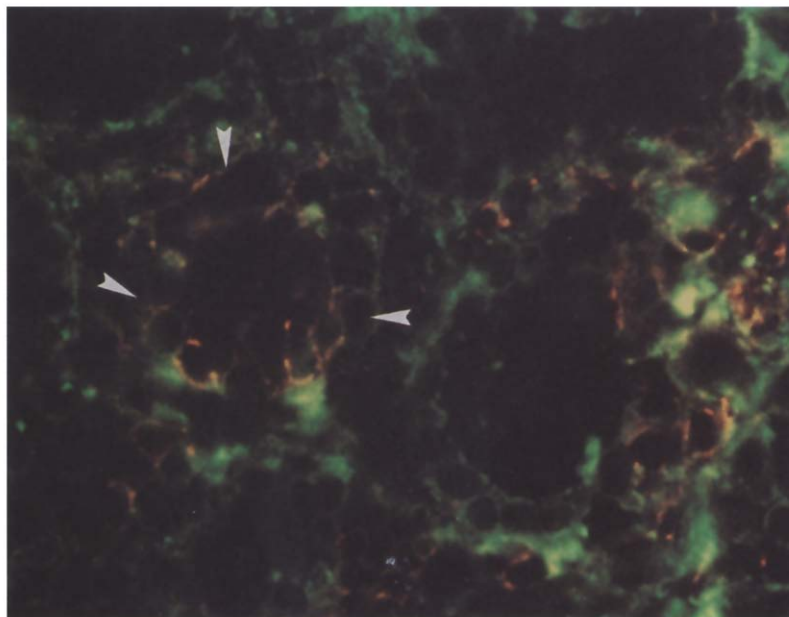


FIG. 4. Double immunofluorescence with anti-TPO-positive human serum and CD11a (8F2.7) mAb in a section of HT tissue. Diffuse follicular green fluorescence caused by TPO is apparent; some follicular portions are double-stained (*orange fluorescence*), that is, LFA-1-positive (*arrows*).

DISCUSSION

There is increasing evidence that intercellular adhesion mechanisms play a crucial role in several immunologic responses, either related or unrelated to specific antigenic recognition.^{12, 13, 19} Notably, the role of such mechanisms in immunologic events related to acute or chronic inflammation

has been emphasized in regard to both leukocyte-endothelium and leukocyte-epithelial cell interaction. Among the pairs of ligands mediating intercellular adhesion involved in inflammation, the LFA-1/ICAM-1 system (CD11a/CD18-CD54) has received special attention.

The heterodimer CD11a/CD18, or LFA-1, is a

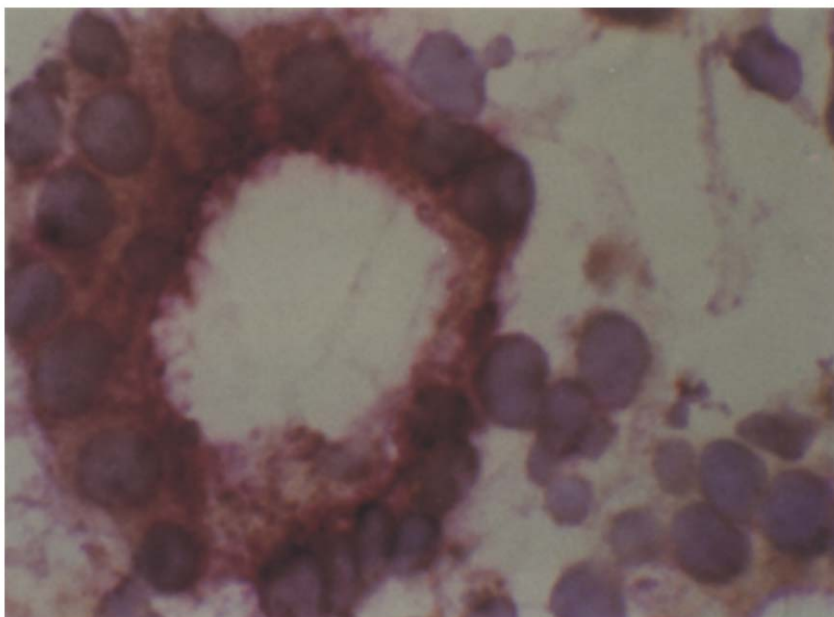


FIG. 5. Double staining of a follicular portion with CD11a (25.3.1) and CD54 staining (as indicated by amaranth color) in HT (original magnification, $\times 1000$).

β_2 -integrin that is widely expressed on leukocytes, and it appears to be involved in most immune reactions requiring cell-to-cell adhesion.^{10, 12, 13} LFA-1-mediated adhesion is strictly temperature-dependent and requires divalent cations.²⁵⁻²⁷ LFA-1 also participates in T-cell signalling in synergy with CD3-TcR^{28, 29} or CD2²¹; moreover, CD3 triggering proved to enhance the ability of LFA-1 to interact with its physiologic ligands through induction of conformational changes.³⁰

At least three physiologic ligands for LFA-1 exist: ICAM-1, ICAM-2, and ICAM-3.^{11, 31, 32} They pertain to the immunoglobulin supergene family: the most extensively studied among them, ICAM-1 or CD54, is not only expressed on activated T cells, B cells, and monocytes but also on epithelial cells in inflammatory processes.¹⁴⁻¹⁹ Its expression is upregulated by proinflammatory cytokines.¹³ Its presence on thyroid epithelial cells has been demonstrated in autoimmune thyroiditis by some groups, including our own.^{4, 8, 9} However, at least three groups (including ours) failed to demonstrate CD54 expression on follicular cells derived from thyroids of patients with GD, despite remarkable CD54 positivity on endothelium.^{6, 8, 34, 35}

To our knowledge, as yet no data have been published concerning the expression of CD11a or CD18 molecules on epithelial cells, either on tissue specimens or in primary cultures.

In this study we provide evidence, by means of indirect immunofluorescence and single or double

immunostaining, that follicular thyroid cells in HT may bear on their surfaces the β_2 integrin LFA-1, together with its ligand CD54. Note that the latter is spontaneously expressed by nonautoimmune thyrocytes or GD thyrocytes in culture, whereas there is no evidence for LFA-1 expression in the same condition. There was no evidence of follicular LFA-1 expression in histologic samples from patients with GD or SG.

In a broad sense, such a phenomenon can be presumed to be related to lymphocyte infiltration, because infiltrates are more represented in HT than in GD and absent in SG specimens. However, it was not possible to establish a clear relationship between infiltrate amount and follicular LFA-1 expression among HT specimens.

The mechanisms by which LFA-1 is expressed by epithelial cells in HT are actually unknown. One could hypothesize that synergic stimulation by proinflammatory cytokines (as for ICAM-1 expression) is responsible for the phenomenon: as a matter of fact, ICAM-1 and LFA-1 follicular expression closely correlate. However, in preliminary experiments we attempted to elicit LFA-1 expression on nonautoimmune thyrocytes (three SG specimens) by culturing them for up to 5 days in the presence of interferon- γ (500 U/ml), tumor necrosis factor- α (50 U/ml), interleukin-1 β (10 U/ml). None of these stimuli, alone or in combination, were effective (data not shown). Thus it is possible that synergic stimulation by other cyto-

kines or other molecular interactions is involved. We are currently investigating this possibility.

Whatever the underlying mechanisms, the finding that LFA-1 is present on HT thyrocytes is relevant, in our opinion, for a number of reasons. First, this is the first evidence to suggest that β_2 (or "leukocyte") integrins are expressed on the epithelial cell surface. Such evidence is based only on immunohistologic findings. One might hypothesize that the presence of LFA-1 on thyrocytes is due to uptake of soluble LFA-1 shed by inflammatory cells. We think that the direct demonstration of CD11a and CD18 mRNA in epithelial cells is necessary to ultimately clarify this point. However, preliminary evidence obtained in our laboratory indicates that LFA-1 can be directly expressed by epithelial cells. In fact, continuously cultured differentiated epithelial cell lines can express LFA-1 under nonspecific activation stimuli, such as phorbol esters or γ -interferon.³⁶ Moreover, at variance with other adhesion molecules expressed on leukocytes of the immunoglobulin (e.g., ICAM-1, vascular cell adhesion molecule-1) or selectin families, evidence for a soluble form of LFA-1, or other integrins, has not been provided.³⁷ Second, this finding further stresses the role of epithelial cells as active participants in the immune reaction in some forms of organ-specific autoimmunity and possibly in other inflammatory immune reactions. The LFA-1/CD54 system could mediate "bidirectional" interactions between, for example, activated T cells and follicular cells, both bearing the two ligands. Finally, this finding underlines the possible major pathogenetic role of adhesion-dependent direct cytolytic effector mechanisms in HT.

We thank Dr. Rina Miriello for skillful help in manuscript preparation and Dr. Daniel Olive (Marseille) and Dr. Alessandro Moretta (Genoa) for providing monoclonal antibodies.

REFERENCES

- Hanafusa T, Pujol-Borrel R, Chiovato L, Russel RCG, Doniach D, Bottazzo GF. Aberrant expression of HLA-DR antigen on thyrocytes in Graves' disease: relevance for autoimmunity. *Lancet* 1983;2:1111-5.
- Londei M, Lamb JR, Bottazzo GF, Feldmann M. Epithelial cells expressing aberrant MHC class II determinants can present antigen to cloned human T cells. *Nature* 1984;312:639-41.
- Londei M, Bottazzo GF, Feldmann M. Human T-cell clones from autoimmune thyroid glands: specific recognition of autologous thyroid cells. *Science* 1985;228:85-9.
- Weetman AP, Cohen S, Magkoba MW, Borysiewicz LK. Expression of an intracellular adhesion molecule, ICAM-1, by human thyroid cells. *J Endocrinol* 1989;122:185-91.
- Tolosa E, Roura C, Marti M, Belfiore A, Pujol-Borrell R. Induction of intercellular adhesion molecule-1 but not of lymphocyte function-associated antigen-3 in thyroid follicular cells. *J Autoimmunity* 1992;5:119-35.
- Ciampolillo A, Mirakian R, Giorgino R, Bottazzo GF. Expression of intercellular adhesion molecules (LFA-1 and ICAM-1) in thyrocytes of patients with Graves' disease. *J Endocrinol Invest* 1990;1:177-7.
- 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.
- Bagnasco M, Caretto A, Olive D, Pedini B, Canonica GW, Betterle 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;83:309-13.
- Zheng RQH, Abney ER, Grübeck-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 diseases. *J Autoimmunity* 1990;3:727-36.
- Krensky AM, Robbins E, Springer TA, Burakoff SJ. LFA-1, LFA-2, and LFA-3 antigens are involved in CTL-target conjugation. *J Immunol* 1984;132:2180-5.
- Magkoba MW, Sanders ME, Luce GEG, et al. ICAM-1: definition by multiple antibodies of a ligand for LFA-1 dependent adhesion of B, T and myeloid cells. *Nature* 1988;331:86-8.
- Springer TA, Dustin ML, Kishimoto TK, Marlin SD. The lymphocyte function-associated LFA-1, CD2 and LFA-3 molecules: cell adhesion receptors of the immune system. *Annu Rev Immunol* 1987;5:223-52.
- Dustin ML, Springer TA. Role of lymphocyte adhesion receptors in transient interactions and cell locomotion. *Annu Rev Immunol* 1991;9:27-66.
- Volpes R, Van Den Oord JJ, Desmet VJ. Immunohistochemical study of adhesion molecules in liver inflammation. *Hepatology* 1990;12:59-64.
- Sobel RA, Mitchell ME, Fondren G. Intercellular adhesion molecule-1 (ICAM-1) in cellular immune reactions in the human central nervous system. *Am J Pathol* 1990;136:1309-14.
- Editorial. Adhesion molecules in diagnosis and treatment of inflammatory diseases. *Lancet* 1990;336:1351-3.
- Wegner CD, Gundel RH, Reilly P, Haynes N, Letts LG, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science* 1990;247:456-9.
- Ciprandi G, Buscaglia S, Pesce GP, Villaggio B, Bagnasco M, Canonica GW. Allergic subjects express intercellular adhesion molecule-1 (ICAM-1 or CD54) on epithelial cells of conjunctiva after allergen challenge. *J ALLERGY CLIN IMMUNOL* 1993;91:783-91.
- Bagnasco M, Buscaglia S, Pronzato C, et al. Adhesion molecules: functional and clinical features. *Semin Clin Immunol* 1992;4:19-33.
- Olive D, Charnot D, Dubreuil P. Human lymphocyte functional antigens. In: Feldmann M, ed. *Human T cell clones. A new approach to immunoregulation*. Clifton, New Jersey: Humana Press, 1989:173-96.
- Cerdan C, Lipcey C, Lopez M, et al. Monoclonal antibodies against LFA-1 or its ligand ICAM-1 accelerate CD2 (T11.1 + T11.2)-mediated T cell proliferation. *Cell Immunol* 1989;123:44-9.

22. Cordell JL, Falini B, Erber WN, et al. Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 1984;32:219-29.
23. Zeromski J, Bagnasco M, Paolieri F, Dworacki G. Expression of CD56 (NKH-1) differentiation antigen in human thyroid epithelium. *Clin Exp Immunol* 1992;89:474-8.
24. Cutolo M, Accardo S, Villaggio B, Clerico P, Indiveri F, Carruba G. Androgen receptors in the synovial tissue of rheumatoid arthritis patients and healthy controls. *Arthritis Rheumatol* 1992;35:1007-15.
25. Shaw S, Luce GEG, Quinones R, Gress RE, Springer TA, Sanders ME. Two antigen-independent adhesion pathways used by human cytotoxic T cell clones. *Nature* 1986;323:262-4.
26. Marlin SD, Springer TA. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen-1 (LFA-1). *Cell* 1987;51:813-9.
27. Shaw S, Magkoba MW, Shimizu Y. Antigen-independent adhesion: a critical process in human cytotoxic T cell recognition. In: Springer TA, Anderson DC, Rosenthal AS, Rothlein R, eds. *Leukocyte adhesion molecules: structure, function and regulation*. New York: Springer-Verlag, 1990: 236-43.
28. Pircher H, Groscurth P, Baumhutter S, Aguet S, Zinkernagel RM, Hengartner H. A monoclonal antibody against altered LFA-1 induces proliferation and lymphocytes' release of cloned T cell. *Eur J Immunol* 1986;16:172-81.
29. Wacholtz MC, Patel SS, Lipski PE. Leucocyte function-associated antigen I is an activation molecule for human T cells. *J Exp Med* 1989;170:431-40.
30. Dustin ML, Springer TA. T cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature* 1989;341:619-24.
31. Staunton DE, Dustin ML, Springer TA. Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. *Nature* 1989;339:61-4.
32. De Fourgerolles AR, Springer TA. Intercellular adhesion molecule-3, a third adhesion counter-receptor for lymphocyte function-associated molecule-1 on resting lymphocytes. *J Exp Med* 1992;175:185-94.
33. Paolieri F, Pesce GP, Torre GC, Canonica GW, Bagnasco M. Expression of very late activation antigen-1 on intra-thyroid lymphocytes in autoimmune thyroid disease. *J Endocrinol Invest* 1992;15:63-6.
34. Miyazaki A, Mirakian R, Bottazzo 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;89:52-7.
35. Tolosa E, Roura C, Catalfamo M, et al. Expression of intercellular adhesion molecule-1 in thyroid follicular cells in autoimmune, nonautoimmune and neoplastic diseases of the thyroid gland: discordance with HLA. *J Autoimmunity* 1992;5:107-18.
36. Canonica GW, Parodi MN, Boero F, Jing G, Pesce GP, Bagnasco M. Effects of cetirizine and deflazacort on cell adhesion molecule expression on lymphocytes and epithelial cells [Abstract]. *Schweiz Med Wochenschr* 1991;40:38.
37. Gearing AJH, Newman W. Circulating adhesion molecules in disease. *Immunol Today* 1993;14:506-12.