Virchows Arch [Pathol Anat] (1985) 406:67–74

Virchows Archiv A

Immunohistochemical demonstration of lactoferrin in follicular adenomas and thyroid carcinomas

G. Tuccari and G. Barresi

Istituto di Anatomia Patologica, Cattedra di Tecnica e Diagnostica Istopatologica, Università di Messina, Policlinico "G. Martino", 98100 Messina, Italia

Summary. By immunohistochemistry, the presence of lactoferrin was investigated in follicular adenomas (10 cases) and carcinomas of the thyroid gland (23 cases). Normal thyroid tissue was also tested as control.

Follicular adenomas showed a consistant negativity, whereas follicular and papillary carcinomas exhibited various degrees of positivity for lactoferrin. Incorporated organoid structures observed in anaplastic carcinomas were strongly stained; the spindle cell parts of these cancers were always negative for this iron-binding protein. Medullary carcinomas were also unstained.

These findings are discussed in relation to the distribution pattern of thyroglobulin.

The authors emphasize the possibility that lactoferrin may be useful in clarifying some diagnostic problems in neoplastic thyroid pathology.

Key words: Lactoferrin – Follicular adenoma – Thyroid carcinomas – Immunohistochemistry

Introduction

Lactoferrin is an iron-binding protein initially discovered in milk but also present in many other biological fluids and tissues (Masson and Heremans 1966; Mason and Taylor 1978; Isobe et al. 1979; Reitamo et al. 1980; Korsrud and Brandtzaeg 1982; Parmley et al. 1982).

Recently, using immunoperoxidase procedures, lactoferrin has been detected in adenocarcinomas of the parotid gland (Caselitz et al. 1981) and in well differentiated prostatic carcinomas (Barresi and Tuccari 1984); in such neoplastic conditions it has been suggested that lactoferrin may be interpreted as a "marker" of glandular or acinar differentiation (Caselitz et al. 1981; Barresi and Tuccari 1984). Furthermore, the absence of lactofer-

Offprint requests to: G. Barresi at the above address

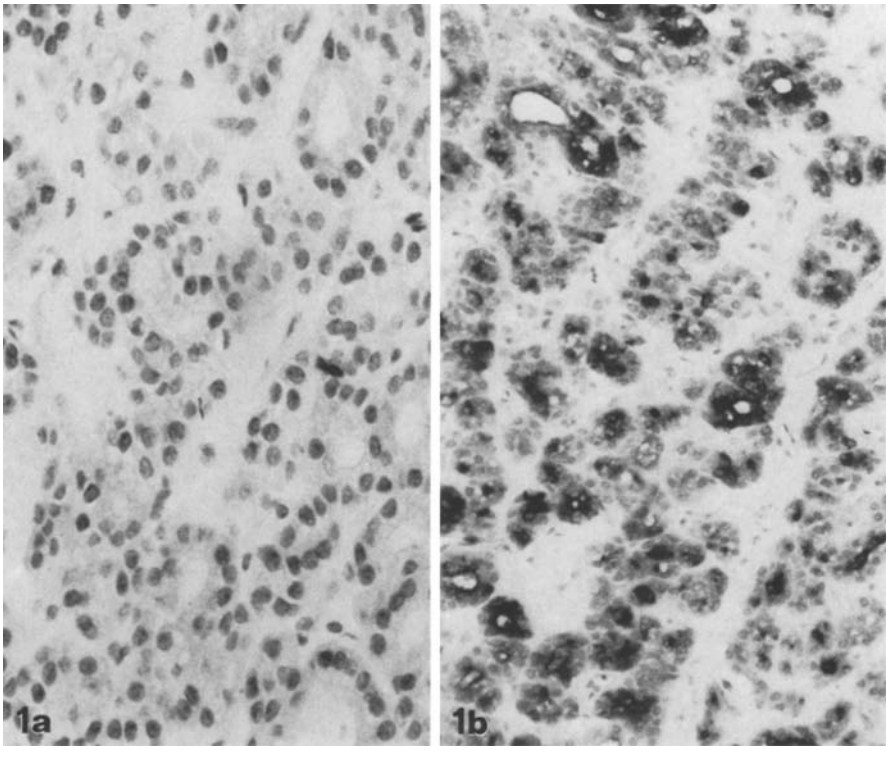

Fig. 1 a, b. Follicular adenoma. No reaction for lactoferrin is observed (**a**; x140); intense staining of the cytoplasm of the thyrocytes and occasionally of lumina with thyroglobulin antiserum (**b**; × 80). Immunoperoxidase, Mayer's Haemalaum counterstain

rin staining has been reported in breast carcinomas, whereas its presence has been emphasized as a potential "marker" of benign breast proliferative lesions (Rossiello et al. 1984).

In view of an increased interest in neoplastic pathology for this iron-binding protein, we have investigated the morphological distribution of lactoferrin in benign and malignant tumours of the thyroid gland, which in normal conditions is negative for lactoferrin (Mason and Taylor 1978). Moreover, we have thought it would be of interest to correlate the presence of lactoferrin, substance unrelated to thyroid function, with the pattern of a well-known functional marker as thyroglobulin.

Materials and methods

Surgical specimens of primary thyroid tumours obtained from 33 patients were studied. Using the criteria of Franssila (1973), Hedinger and Sobin (1974), Tscholl-Ducommun and Hedinger (1982), the histopathological diagnosis was: trabeculo-follicular adenoma ("cold nodules"; 10 cases), follicular carcinomax (11 cases), papillary carcinoma (4 cases), anaplastic spindle cells carcinoma (4 cases). Four specimens of medullary carcinoma, in which the diagnosis was supported by immunohistochemical demonstration of calcitonin, were also investigated.

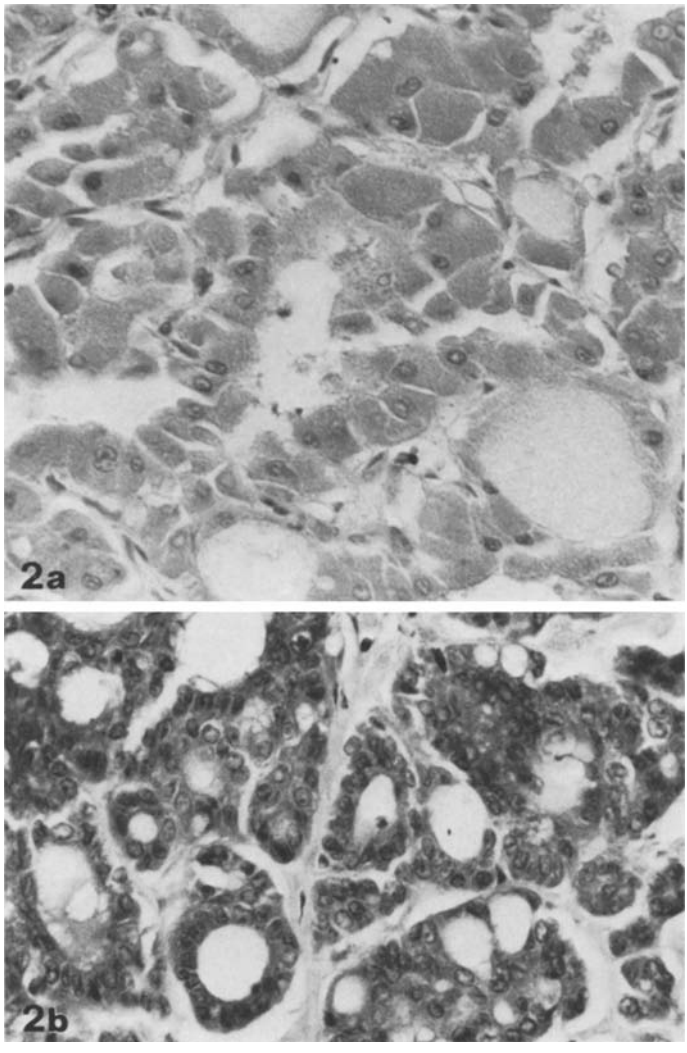

Fig. 2a, b. Follicular carcinoma. A clear cytoplasmatic positivity for lactoferrin is noted in follicular neoplastic cells (**a**; ×200); prominent cytoplasmatic staining for thyroglobulin (**b**; ×160); Immunoperoxidase, Mayer's Haemalum counterstain

Normal portions of thyroids surrounding adenomas were also tested as controls.

All tissues, fixed in 10% neutral formalin for 12–24 h at room temperature, were embedded in paraffin at 55° C and cut into thin sections by the routine histological procedure.

4–5 μ thick serial sections were treated for 30 min each time in: 1. 0.1% H_2O_2 in methanol to block the intrinsic peroxidase activity (Streefkerk 1972); 2. with normal sheep serum 1–3% to prevent unspecific adherence of serum proteins; 3. with rabbit anti-human lactoferrin, thyroglobulin and calcitonin (purchased from Dakopatts, Copenhagen, Denmark; dilution 1:200, 1:400, 1:300 respectively); 4. with sheep anti-rabbit globulin antiserum (Behring Institute; dilution 1:20); 5. with rabbit anti-horseradish PAP complexes (Dakopatts; dilution 1:25). For the demonstration of peroxidase activity, the sections were incubated in darkness (Weir et al. 1974) for 10 min with 3-3′ diaminobenzidine tetrahydrochloride (Sigma Chemical CO.,

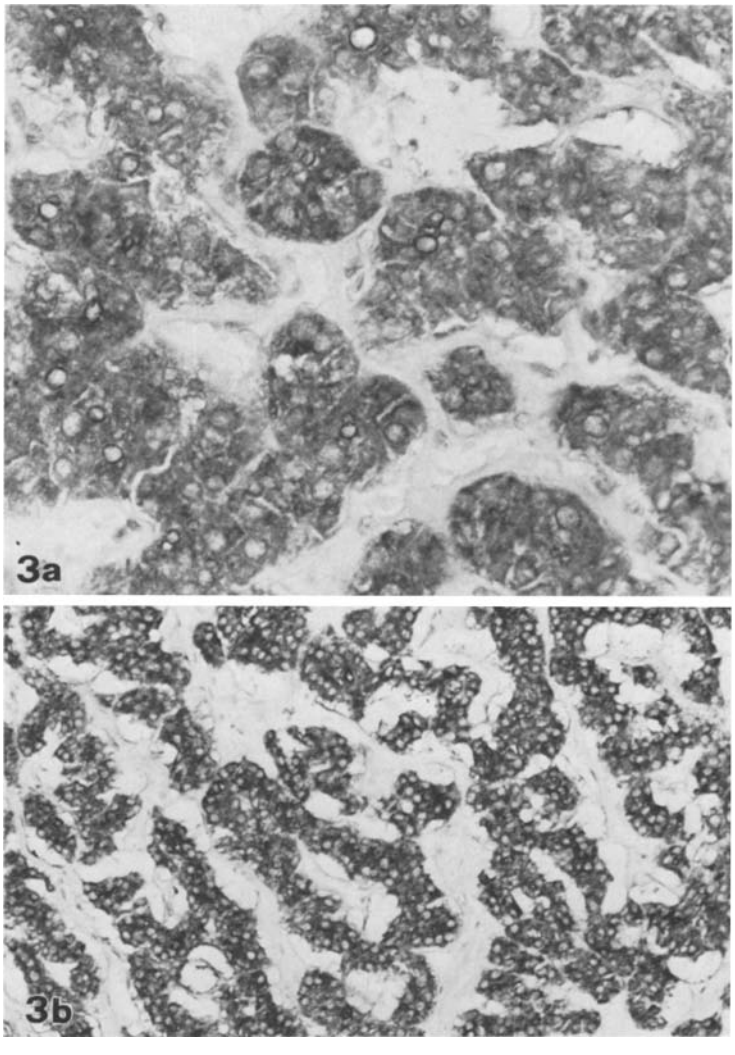

Fig. 3a, b. Follicular carcinoma. Lactoferrin is evident in tubular neoplastic cords (**a**; ×200); note the intense cytoplasmatic staining of thyrocytes with thyroglobulin antiserum (**b**; ×120); Immunoperoxidase, Mayer's Haemalum counterstain

St. Louis, MO, USA). To test the specificity of lactoferrin, thyroglobulin and calcitonin stainings (Heyderman 1979), each specific antiserum was replaced by either phosphate buffered saline, normal rabbit serum or absorbed with excess of purified human lactoferrin, human thyroglobulin and human synthetic calcitonin (Sigma Chemical Co., MO, USA): negative results were obtained.

Results

Follicular adenomas. In main cell type adenomas, follicular cells were constantly negative for lactoferrin (Fig. 1a); follicular lumina were also unstained (Fig. 1a). A varied pattern of positivity for thyroglobulin, mainly

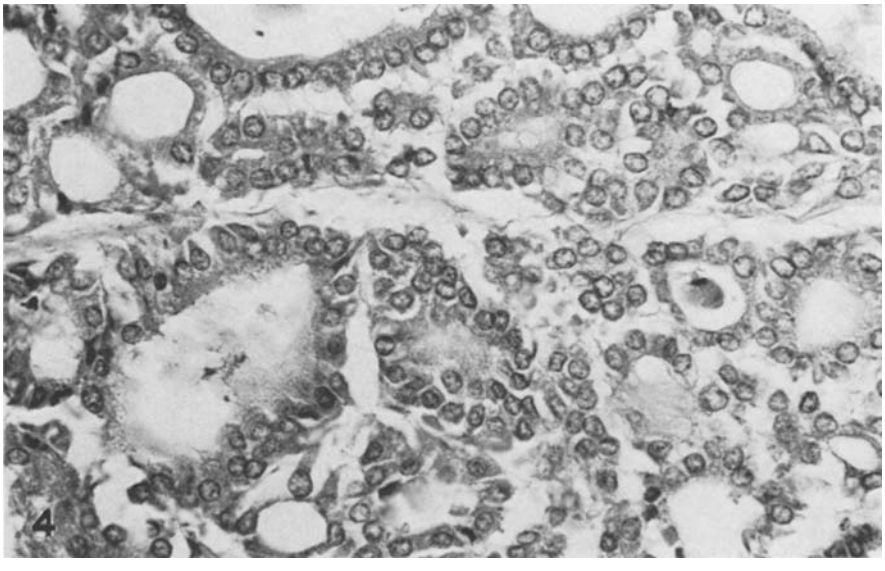

Fig. 4. Papillary carcinoma. A cytoplasmatic positivity of neoplastic cells with some luminal reaction is seen in so-called follicular variety (Lindsay's tumour; × 300); Anti-lactoferrin immunoperoxidase, Mayer's Haemalaum counterstain

localized at the cytoplasmatic apical border, was observed in all follicular adenomas with a lesser extent within the lumina (Fig. 1 b).

Thyroid carcinomas. In follicular carcinomas, a diffuse cytoplasmatic positivity for lactoferrin was observed (Fig. 2a and 3a); the follicular lumina were mostly devoid of this antigen. In same specimens, a strong positive staining for thyroglobulin was seen in the cytoplasm of neoplastic cells (Fig. 2b and 3b); occasionally the follicular lumina showed the presence of this antigen (Fig. 2b).

Papillary carcinomas showed positivity for lactoferrin, which could be visualized in both papillary and follicular areas; one well differentiated papillary carcinoma, wholly organized in follicles with characteristic "ground glass" nuclei, exhibited also a positive cytoplasmatic staining (Fig. 4). With thyroglobulin antiserum, papillary carcinomas were not so strongly stained as follicular carcinomas; some cells present in neoplastic papillae exhibited an intense staining throughout the cytoplasm, whereas others were stained only along the apical surface.

Spindle cell areas of anaplastic carcinomas manifested a negative reaction for lactoferrin, whereas incorporated follicular structures were strongly stained (Fig. 5); epithelial positive cells arranged in an organoid manner were found in direct contact with rarely negative ones (Fig. 5). A negative thyroglobulin staining was encountered in the spindle cell parts of anaplastic tumours; thyroglobulin was demonstrable only in the epithelial cells of occasional incorporated follicles.

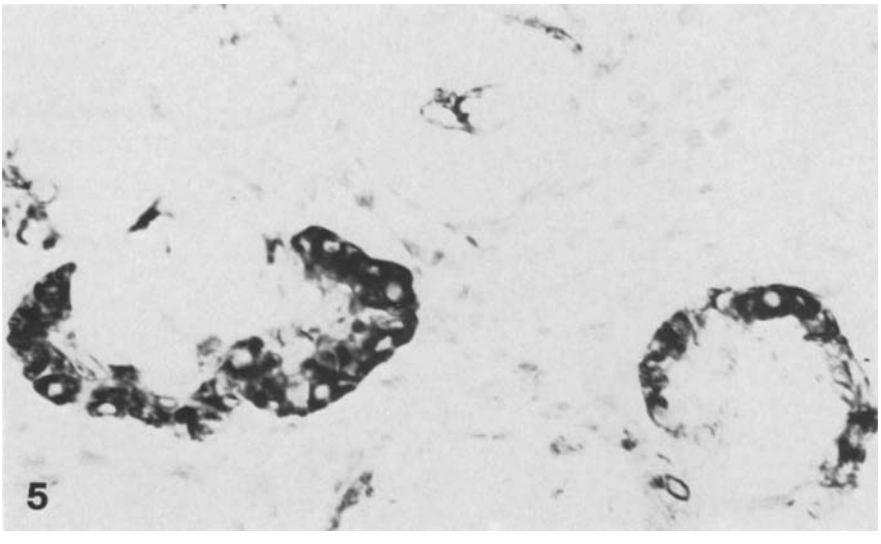

Fig. 5. Anaplastic carcinoma of spindle cell type. The anaplastic parts are completely devoid of lactoferrin; note the intense staining of some included follicles (×320; Anti-lactoferrin immunoperoxidase)

Medullary carcinomas were constantly negative for lactoferrin and thyroglobulin. Calcitonin was demonstrated as brown deposits within the cytoplasm of the tumour cells; there was a considerable variation in the intensity of the staining reaction.

In normal thyroid, follicular cells and colloid of the thyroid tissue surrounding adenomas failed to react with the lactoferrin antiserum. In the same specimens, small to moderate amounts of thyroglobulin with some luminal reaction were observed.

Discussion

Thyroid tumours have been shown to express functional properties correlated with hormone production and cytological differentiation (Bocker 1979; Kawaoi et al. 1981). By immunohistochemistry, thyroglobulin has been considered the most useful functional marker to standardize adenomas and carcinomas of the thyroid gland (Bocker et al. 1978; Burt and Goudie 1979; Bocker et al. 1980; Albores-Saavedra et al. 1983). Nevertheless, the distribution pattern of thyroglobulin is very similar in follicular adenomas of main cell type and follicular carcinomas (Dralle and Bocker 1977; Bocker et al. 1980); in fact, the differences consist only in a more heterogeneous thyroglobulin pattern and less luminal staining in follicular carcinomas (Bocker et al. 1980). In addition, the thyroglobulin positivity, exclusively observed in incorporated follicles of anaplastic carcinomas, do not allow us to distinguish whether these organoid structures represent normal thyroid tissue or highly differentiated areas of the carcinoma. In an attempt to settle

the above-mentioned diagnostic questions, new additional "markers" have been proposed (Kawaoi et al. 1981; Calmettes et al. 1982; Permanetter et al. 1982).

In the present study, we have encountered a constantly positive reaction for lactoferrin in follicular and papillary thyroid carcinomas. The spindle cell parts of anaplastic carcinomas were lacking in this staining, whereas included follicular structures were intensely stained. Therefore, it may be suggested that these organoid structures represent well differentiated areas of anaplastic carcinomas, since our specimens of normal thyroid were devoid of lactoferrin, as previously reported elsewhere (Mason and Taylor 1978).

In our cases, the comparative immunohistochemical analysis between lactoferrin and thyroglobulin showed a similar pattern in follicular, papillary and anaplastic carcinomas. Medullary carcinomas were always negative for lactoferrin and thyroglobulin. In contrast, in follicular adenomas the behaviour of these two substances exhibited an evident discrepancy since lactoferrin was constantly absent whereas thyroglobulin was always present.

The present observations suggest that lactoferrin is produced exclusively by differentiated carcinomas of follicular cell origin, although the biological significance of this iron-binding protein in relation to the functional neoplastic activity of thyrocytes is unknown. Hence it may be concluded that immunohistochemical demonstration of lactoferrin seems to be a new additional "marker" for the differential diagnosis between follicular adenomas, follicular carcinomas and follicular variety of papillary carcinomas.

Acknowledgements. Work supported by grants from M.P.I (Rome).

References

Albores-Saavedra J, Nadji M, Civantos F, Morales AR (1983) Thyroglobulin in carcinoma of the thyroid: an immunohistochemical study. Hum Pathol 14:62–66

Barresi G, Tuccari G (1984) Lactoferrin in benign hypertrophy and carcinomas of the prostatic gland. Virchows Arch [Pathol Anat] 403:59–66

Bocker W, Dralle H, Koch G, de Heer K, Hageman J (1978) Immunohistochemical and electron microscopic analysis of adenomas of the thyroid gland. II. Adenomas with specific cytological differentiation. Virchows Arch [Pathol Anat] 380:205–220

Bocker W (1979) Funktionelle Pathmorphologie der menschlichen Schilldrusentumoren. Veroffentlichungen aus der Pathologie, vol. 110. Stuttgart New York, Gustav Fischer

Bocker W, Dralle H, Husselman H, Bay V, Brassow M (1980) Immunohistochemical analysis of thyroglobulin synthesis in thyroid carcinomas. Virchows Arch [Pathol Anat] 385:187–200

Burt A, Goudie RB (1979) Diagnosis of primary thyroid carcinoma by immunohistological demonstration of thyroglobulin. Histopathology 3:279–286

Calmettes C, Caillou B, Moukhtar MS, Milhaud G, Gerard-Marchant R (1982) Calcitonin and carcinoembryonic antigen in poorly differentiated follicular carcinoma. Cancer 49:2342–2348

Caselitz J, Jaup T, Seifert G (1981) Lactoferrin and lysozyme in carcinomas of the parotid gland. Virchows Arch [Pathol Anat] 394:61–73

Dralle H, Bocker W (1977) Immunohistochemical and electron microscope analysis of adenomas of the thyroid gland. I. A comparative investigation of hot and cold nodules. Virchows Arch [Pathol Anat] 374:285–301

Franssila KO (1973) Is the differentiation between papillary and follicular thyroid carcinoma valid? Cancer 32:853–864

Hedinger CE, Sobin LH (1974) Histological typing of thyroid tumours. International Histological Classification of tumours Nr. 11. World Health Organization, Geneve

Heyderman E (1979) Immunoperoxidase technique in histopathology: applications, methods and controls. J Clin Pathol 32:971–978

Isobe Y, Isobe K, Tabe T (1979) Localization of lactoferrin in the human gastrointestinal mucosa. Acta Histochem Cytochem 12:554

Kawaoi A, Okano T, Nemoto N, Shikata T (1981) Production of thyroxine (T_4) and triiodothyronine (T_3) in nontoxic thyroid tumors. Virchows Arch [Pathol Anat] 390:249–257

Korsrud FR, Brandtzaeg P (1982) Characterization of epithelial elements in human major salivary glands by functional markers: localization of amylase, lactoferrin, lysozyme, secretory component and secretory immunoglobulins by paired immunofluorescence staining. J Histochem Cytochem 30:657–666

Mason DY, Taylor CR (1978) Distribution of transferrin, ferritin and lactoferrin in human tissues. J Clin Pathol 31:316–327

Masson PL, Heremans JF (1966) Studies on lactoferrin, the iron binding protein of secretions. In: Peeters H (ed) Protides of biological fluids. Elsevier, Amsterdam 115–124

Parmley RT, Takagi M, Barton JC, Boxer LA, Austin RL (1982) Ultrastructural localization of lactoferrin and iron-binding proteins in human neutrophils and rabbit heterophils. Am J Pathol 109:343–358

Permanetter W, Nathrath WB, Lohrs U (1982) Keratin as marker in thyroid tumors. Hum Pathol 13:872

Reitamo S, Kontinnen YT, Segerber-Kontinnen M (1980) Distribution of lactoferrin in human salivary glands. Histochemistry 66:285–291

Rossiello R, Carriero MV, Giordano GG (1984) Distribution of ferritin, transferrin and lactoferrin in breast carcinoma tissue. J Clin Pathol 37:51–55

Tscholl-Ducommun J, Hedinger CE (1982) Papillary thyroid carcinomas. Virchows Arch [Pathol Anat] 396:19–39

Weir EE, Pretlow TG, Pitts A, Williams EE (1974) A more sensitive and specific histochemical peroxidase for the localization of cellular antigen by the enzyme-antibody conjugate method. J Histochem Cytochem 22:1135–1140

Accepted October 18, 1984