Expression of tenascin in lymphocytic autoimmune thyroiditis

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Abstract

Aims—To study the distribution of tenascin by immunocytochemistry in autoimmune diseases of the thyroid.

Methods—Thyroids from patients with inflammatory lesions of the thyroid (lymphocytic thyroiditis Hashimoto, Grave's disease, thyroiditis DeQuervain) were studied by immunocytochemistry using antibodies against tenascin, collagen III, and collagen IV.

Results—In autoimmune lymphocytic thyroiditis Hashimoto there was a characteristic corona-like staining pattern of tenascin around all activated lymph follicles with germinal centres. This staining pattern contrasted with the immunoreactions for collagen III and IV, which were not enhanced in the perilymphofollicular interstitium. In cases of thyroiditis DeQuervain the areas of early and ongoing fibrosis showed some diffuse staining for tenascin and for collagen III. Enhanced diffuse immunostaining for collagen IV in the perivascular and interfollicular interstitium was present in cases of Grave's disease. In Grave's disease no characteristic immunoreaction was detectable for tenascin.

Conclusions—The corona-like expression of tenascin around lymphofollicular infiltrates is distinctive of cases of lymphocytic thyroiditis. A similar staining pattern for tenascin has been reported in lymphoid hyperplasia of the thymus associated with

myasthenia gravis, another autoimmune-logical disorder. There are good arguments that the activation and infiltration of lymph follicles in the thyroid during the course of autoimmune diseases lead to stimulation and activation of the surrounding mesenchyme producing tenascin as part of the extracellular matrix. (*J Clin Pathol* 1997;50:863–866)

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Figure 1 Case of lymphocytic thyroiditis Hashimoto. Characteristic corona-like immunostaining for tenascin around activated lymph follicle with germinal centre (alkaline phosphatase detected with Fast Red; original magnification $\times 120$).

Tenascin is a major matrix glycoprotein with spatially and temporally restricted distribution in embryonic and adult tissues.^{1,2} Genetically this multifunctional matrix protein has been highly preserved during evolution and is present in many tissues and organs during development.³ Tenascin is a relatively large hexameric glycoprotein consisting of six identical protein arms and has been shown to have mainly antiadhesive properties for cultured cells in vitro.^{4,5} A substantial re-expression of tenascin occurs in the mesenchyme during regeneration and in neoplasia.^{6–8} In vitro experiments have shown that transforming growth factor β (TGF- β) can induce the expression of tenascin in fibroblast cell cultures.⁹ In lymphatic tissues tenascin can be demonstrated in T dependent zones and in some types of granulomatous lymphadenitis and lymphoma.^{10–12} The antiadhesive effect of tenascin under experimental conditions suggests a role in the remodelling of tissue especially in tissue composed of mobile cells such as lymphoid cells.¹³ Based on these data we investigated the expression of tenascin in autoimmune and other inflammatory lesions of the thyroid to demonstrate differences in the morphological distribution of tenascin among these disorders of the thyroid, and to determine whether autoimmune diseases of the thyroid correlate with a particular distribution pattern of tenascin. Moreover we compared the expression of tenascin with the expression of collagen III and collagen IV.

Material and methods

Forty cases of inflammatory thyroid lesions were studied using paraffin wax embedded tissue of surgical resection specimens of the thyroid. The cases had been thoroughly documented clinically and endocrinologically before surgery. These 40 cases comprised 15 cases of lymphocytic thyroiditis Hashimoto, 10 of Grave's disease, 10 of focal lymphocytic thyroiditis, and five of granulomatous thyroiditis

Figure 2 Case of granulomatous thyroiditis DeQuervain. Weak, diffuse immunostaining for tenascin in areas of early fibrosis and around small blood vessels (alkaline phosphatase detected with Fast Red; original magnification $\times 60$).

Figure 3 Pronounced diffuse staining of the interstitium for collagen IV in Grave's disease (alkaline phosphatase detected with Fast Red; original magnification $\times 90$).

Figure 4 Grave's disease. Negative immunostaining of the interstitium for tenascin (alkaline phosphatase detected with Fast Red; original magnification $\times 90$).

DeQuervain. The cases of lymphocytic thyroiditis Hashimoto had serological evidence of thyroid autoantibody production and exhibited at least latent or manifest hypothyroidism. The cases of Grave's disease had endocrine hyperfunction, the cases of focal lymphocytic thyroiditis presented with normal thyroid function, and the cases of granulomatous thyroiditis DeQuervain showed variable endocrine disturbances of thyroid function. Ten cases of ordinary colloidal goiter with normal thyroid function served as controls.

Representative paraffin wax blocks were taken from the files of the department of pathology to perform immunohistochemical investigations with antibodies against tenascin (Dako, Glostrup, Denmark), collagen type III (Biogenesis, Poole, England), and collagen type IV (Camon, Wiesbaden, Germany). Antibodies against TGF- $\beta 3$ (Oncogene Sci, Cambridge, USA) were also used. A three step immunohistochemical procedure was applied according to the avidin-biotin labelled method with alkaline phosphatase visualised by Fast Red as chromogen (Zymed, San Francisco, USA). Dewaxed paraffin sections were optionally digested with trypsin before incubation with antibodies to tenascin, collagen III or collagen IV, or treated with microwave heating for 10 minutes in citrate buffer before incubation with antibodies to TGF- $\beta 3$. The staining intensity and the number of lymph follicles were evaluated with a four point semiquantitative scoring system from 0 (virtually no immunostaining or no lymph follicles) to 3 (most prominent parameters).

Results

Immunohistologically a characteristic corona-like expression of tenascin around activated lymph follicles was detected in cases of lymphocytic thyroiditis Hashimoto (fig 1). This finely reticular staining pattern of tenascin around activated lymph follicles was restricted to the direct surroundings of the activated lymph follicles and was most prominent in cases of lymphocytic thyroiditis Hashimoto. There was also a weak corona-like immunoreaction for tenascin around intrathyroidal lymph follicles in focal lymphocytic thyroiditis. Lesions of granulomatous thyroiditis DeQuervain show a diffuse staining for tenascin in areas of early tissue fibrosis in conjunction with an inflammatory destruction of thyroid follicles (fig 2). Immunostaining for collagen IV showed a diffuse perivascular and intense interstitial staining pattern in the thyroid parenchyma of Grave's disease (fig 3). There was no comparable staining for collagen IV in cases of lymphocytic thyroiditis Hashimoto. In Grave's disease there was no corona-like tenascin reactivity in the thyroid except for a very weak tenascin rim around small blood vessels in the interstitium (fig 4).

As demonstrated by semiquantitative evaluation the immunohistological staining score for tenascin had the highest values in cases of lymphocytic thyroiditis Hashimoto (table 1). There was also a correlation of immunoreactivity for tenascin with the number of

Table 1 Mean values of semiquantitative scores (four point scoring system from 0 to 3) for the immunoreaction of tenascin and for the degree of lymph follicle infiltration

	Lymphocytic thyroiditis Hashimoto	Focal lymphocytic thyroiditis	Grave's disease	Thyroiditis DeQuervain
Number of cases	15	10	10	5
Tenascin score	2.4	1.1	1.2	1.6
Lymph follicle score	2.1	1.0	0.6	1.0

Figure 5 Immunostaining for TGF-β3 in an activated germinal centre (lymphocytic thyroiditis Hashimoto) (alkaline phosphatase detected with Fast Red; original magnification ×120).

intrathyroidal lymph follicles. Using antibodies against TGF-β3, a reaction product could be demonstrated by immunohistochemistry in activated germinal centres of the thyroidal lymphatic tissue in lymphocytic thyroiditis Hashimoto (fig 5). Normal thyroid tissue showed no reactivity for tenascin. In normal lymphatic tissues (tonsils, lymph nodes) used as controls, germinal centres of activated lymph follicles exhibited no perifollicular immunostaining for tenascin or detectable immunostaining for TGF-β3.

Discussion

Examinations of tissues from surgical and necropsy specimens of the thyroid gland showed that 20–50% of all thyroid glands, at least to a minor degree, contain lymphocytic aggregates.¹⁴ These lymphoid infiltrates in the thyroid gland often form lymph follicles. However, these minor lymphocytic and lymphofollicular infiltrates in the thyroid—referred to in the literature as focal lymphocytic thyroiditis—do not (yet) correlate with clinical and serological manifestations of thyroid dysfunction. Some investigators hypothesised that focal lymphocytic thyroiditis represents a mild form of ongoing autoimmune disease of the thyroid eventually resulting in hypothyroidism after a period of latency or after a period of acceleration of the autoimmune disease.^{15 16} As clinical studies of the natural course of focal lymphocytic thyroiditis are hampered by the fact that the histological diagnosis comes from resected thyroids, the presence of tenascin around activated lymph follicles in lymphocytic thyroiditis Hashimoto and to a minor degree in

focal lymphocytic thyroiditis is another argument for the pathogenetic link between both disorders.

If the diagnosis of thyroiditis is first made by histopathological examination of the resected thyroid gland, the postoperative determination of endocrinological parameters with regard to autoimmune disease will be of limited value. Therefore it is necessary to look for histological or immunohistochemical parameters to differentiate between mainly autoimmune diseases in the thyroid itself. With regard to the characteristic corona-like pattern of immunoreactivity for tenascin, immunostaining for tenascin could be an adjunct in the diagnosis of so far not detected or not suspected autoimmune lymphocytic thyroiditis. Other forms of inflammatory diseases can be distinguished by their negative or for instance diffuse immunostaining for tenascin in cases of thyroiditis DeQuervain. In our study Grave's disease did not show a prominent staining for tenascin but had a prominent perivascular and interstitial staining for collagen type IV.

On behalf of a distinct clinical setting a special subtype of thyroiditis with endocrine alterations has been separated—postpartum thyroiditis.^{17 18} By chance we could study one case of postpartum thyroiditis, which is in general not an indication for surgery if suspected preoperatively. Immunohistologically this case showed no corona-like staining for tenascin around lymph follicles. However, further studies on larger series of postpartum thyroiditis will have to clarify whether the expression of tenascin is different in this particular disease of the thyroid.

Immunohistochemically we found a weak reaction in germinal centres with an antibody to TGF-β3, which cross reacts with TGF-β2. On paraffin wax sections this relatively weak immunoreaction did not allow a sufficient differentiation of the cellular location in the germinal centres. As the stimulation of tenascin synthesis by TGF-β has been demonstrated in vitro⁹ this could be one possible mechanism for the re-expression of tenascin in lymphocytic thyroiditis Hashimoto. The presence of growth factors of the TGF-β family has previously been shown in activated lymphatic tissue by Andersson and coworkers.¹⁹ It is our hypothesis that this might be one pathogenetic explanation for the tenascin expression around activated lymph follicles in lymphocytic thyroiditis. Further investigations are necessary to provide more evidence for the role of TGF-β in thyroid disease.

The perifollicular deposits of tenascin in our opinion influence the mobility of lymphocytes and monocytes by their antiadhesive properties. The altered matrix composition could theoretically facilitate the expansion of immunologically activated lymphocytes in the thyroid by these antiadhesive properties of the tenascin sheaths. On the other hand, tenascin hinders T cell activation by soluble antigens as shown by in vitro studies.¹³ Possibly such a tenascin sheath around the lymph follicles protects the surrounding tissue of the thyroid from

a progression of autoreactive lymphocyte clones resulting in a deceleration of an autoimmune reaction. Most probably the effect of tenascin ambiguously effects both expansion and deceleration of lymphocytes depending on the setting of integrins and integrin receptors on effector cells and stromal cells. Therefore our findings argue for an immunomodulatory role of tenascin in lymphocytic thyroiditis, which clinically is known to have a prolonged and slowly progressive course.^{3, 20} Similarly lymphofollicular hyperplasia of the thymus in the setting of myasthenia gravis previously has been reported to show perifollicular tenascin deposition.¹⁰ Therefore the characteristic perifollicular accumulation of tenascin around activated lymph follicles can serve as an indicator of an autoimmunologically mediated tissue alteration at least in thyroid and thymic tissue.

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