

# Tenascin immunoreactivity in normal and pathological bone marrow

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## Abstract

**Aims:** To determine the distribution of tenascin in normal and pathological bone marrow.

**Methods:** 48 different bone marrow lesions were studied immunohistochemically using a monoclonal antibody to tenascin.

**Results:** Tenascin immunoreactivity was found in lesions with increased fibrosis and high numbers of reticular fibres. The strongest immunoreactivity was found in myelofibrosis. Bone marrow from acute and chronic myeloid and lymphatic leukaemias showed weak or moderate immunoreactivity. In hyperplasias inconsistent reticular tenascin immunoreactivity was found; in normal bone marrow, only a few scattered positive fibres were occasionally seen.

**Conclusions:** Tenascin was generally observed in conditions in which megakaryocytic hyperplasia was a feature. This is in line with the notion that tenascin synthesis in bone marrow fibroblasts is stimulated by TGF- $\beta$  which is synthesised by the megakaryocytic lineage. Tenascin also contains EGF-like repeats. It might therefore function as a growth promoter and in this way could also stimulate synthesis of other matrix components. On the other hand, tenascin could function as an adhesive molecule to some cells of the bone marrow. The presence of tenascin in many pathological states of the bone marrow suggests that it may have a role in their pathogenesis and that it also could be a potential marker of disease.

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Tenascin is an extracellular matrix protein which was originally isolated from embryonic tissues.<sup>1-4</sup> It is also present in adult tissues in smooth muscle cells, endothelial cells, myotendinous tissue, cartilage and in healing wounds.<sup>5-7</sup> Tenascin is synthesised by fibroblasts and glial cells.<sup>5</sup> It is a hexameric molecule with a total molecular weight of 1900 kilodaltons.<sup>5</sup> There are three isoforms of the molecule with molecular weights of 190, 200, and 230 kilodaltons and a variable tissue distribution.<sup>8</sup> Tenascin contains epidermal growth factor-like and fibronectin-like repeats.<sup>9</sup> Because of the epidermal growth factor-like repeats, it has been suggested that tenascin has growth promoting properties.<sup>10</sup>

Tenascin has been found in the stroma of various tumours, especially malignant ones.<sup>5 7 11-14</sup> In tumour tissue it is thought to be synthesised by stromal fibroblasts that are induced by cytokines, especially TGF- $\beta$ .<sup>5 15 16</sup> Synthesis of tenascin has also been reported in embryonic bronchial epithelial cells,<sup>17</sup> in chondroblasts, smooth muscle cells, and in some other mesenchymal cells.<sup>18</sup> Moreover, intracytoplasmic tenascin immunoreactivity has been detected in malignant melanomas<sup>14</sup> and in lung carcinomas.<sup>19</sup>

In haematolymphoid tissues the distribution of tenascin has been studied in lymph nodes and in spleen,<sup>20 21</sup> but there are no reports on the distribution of tenascin in bone marrow tissue. In splenic tissue tenascin immunoreactivity is closely associated with the reticular fibres.<sup>21</sup> Similarly, in reactive and neoplastic lymph node tissue (except for the nodular sclerosing variant of Hodgkin's lymphoma) tenascin was seen as a reticular network, similar to that detected by the reticulin stain.<sup>20</sup> In the nodular sclerosing variant of Hodgkin's lymphoma the entire fibrotic stromal tissue stains strongly for tenascin.<sup>20</sup>

In a previous study we introduced a novel monoclonal tenascin antibody (143DB7): this functions in formalin fixed and paraffin wax embedded material.<sup>19 22</sup> The antibody recognises all three isoforms of tenascin.<sup>19 22</sup> Using this antibody, we have now examined the distribution of tenascin in 48 bone marrow biopsy specimens to elucidate the role of tenascin in a variety of different neoplastic and reactive conditions of the bone marrow.

## Methods

The material consisted of eight cases of myelofibrosis (three primary, five secondary), six chronic myeloid leukaemias, one acute myeloid leukaemia, 13 hyperplastic bone marrows (five myeloid, three megakaryocytic, two erythroid, two diffuse, one megakaryocytic and erythroid), seven normal bone marrows, four aplasias, three chronic lymphatic leukaemias, two acute lymphatic leukaemias, one hypoplastic bone marrow, one non-specific fibrosis, one atypical lymphatic infiltrate and one metastatic adenocarcinoma. The material was collected from the files of the Department of Pathology, Oulu University Hospital, between 1980-1984. The diagnosis of the cases was based on a light microscopic evaluation of the slides stained with haematoxylin and eosin, periodic acid Schiff, Gomori's reticulin stain, the Giemsa and Herovici stains. Bone marrow

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## Results of immunohistochemical staining

Diagnosis	Number of positive cases/total number of cases	Quantification of immunoreactivity
Myelofibrosis	8/8	+++ / +++ / +++ / +++
Chronic myeloid leukaemia	4/6	+++ / +++ / +++ / +
Acute myeloid leukaemia	0/1	-
Chronic lymphatic leukaemia	3/3	++ / + / +
Acute lymphatic leukaemia	2/2	+ / +
Hyperplasia		
Myeloid	2/5	+ / + / - / - / -
Megakaryocytic	3/3	+++ / + / (+)
Erythroid	0/2	- / -
Erythroid and megakaryocytic	1/1	+
Diffuse	2/2	+ / (+)
Normal	2/7	(+) / (+) / - / - / - / - / -
Aplasia and hypoplasia	0/5	- / - / - / -
Fibrosis	1/1	+
Metastasis	1/1	+
Atypical lymphoid infiltrate	1/1	+

- negative  
 (+) occasional positive fibres  
 + weakly positive  
 ++ moderately positive  
 +++ strongly positive.

biopsy specimens from the iliac crest were decalcified in EDTA for 36 hours and fixed in 10% buffered formalin and embedded in paraffin wax. Case histories of all the patients and their additional bone marrow biopsy specimens were reviewed and re-examined.

## IMMUNOHISTOCHEMICAL STAINING

Sections (5µm) were dewaxed in xylene and rehydrated in graded alcohol. The endogenous peroxidase was blocked by treating with 0.3% hydrogen peroxide in absolute methanol for 30 minutes. Before immunostaining, the sections were treated with 0.4% pepsin (Merck, Darmstadt) at 37°C for 30 minutes. The avidin-biotin complex (ABC) method was used for immunostaining.<sup>23</sup>

Monoclonal mouse antibody to human tenascin (143DB7) was used as the primary antibody. The sections were incubated with the primary antibody at 4°C overnight, followed

by the biotinylated rabbit anti-mouse antibody and ABC (Dakopatts, Glostrup, Denmark). The colour was developed with diaminobenzidine, after which the sections were mounted in an aqueous medium. The sections were counterstained with a light haematoxylin stain. Negative controls consisted of substituting phosphate buffered saline (140 ml NaCl, 0.01M phosphate buffer, pH 7.2) and normal mouse serum for the primary antibody.

The staining reaction was evaluated quantitatively as follows: - negative; (+) occasional positive fibres; + weak reactivity, ++ moderate reactivity; and +++ strong reactivity.

## Results

The results are summarised in the table.

Tenascin was found in all bone marrow samples with increased fibrosis or increased amount of reticular fibres. The strongest immunoreactivity was observed in the cases with myelofibrosis; in most cases the immunoreactivity was very strong (fig 1), with only three cases showing moderate or weak staining. In fibrotic areas the staining was linear; in the more cellular areas it was mainly reticular. The staining pattern was similar to that obtained by reticulin staining of the same biopsy specimens. Immunoreactivity was also found in the walls of the dilated sinusoids.

Tenascin immunoreactivity could also be seen in all samples from acute and chronic lymphatic leukaemias. The reactivity was, however, weaker than in myelofibrosis. The staining pattern was reticular. Similar immunoreactivity was also seen in the positive cases of myeloid leukaemias.

Inconsistent reticular staining was seen in the hyperplastic bone marrow. Positive staining was observed in cases with myeloid or megakaryocytic hyperplasia (fig 2), while in erythroid hyperplasia there was no tenascin immunoreactivity.

In normal bone marrow staining was mostly negative. In some cases, however, a few positive fibres could be seen. In the bone trabeculae positivity could be seen in the periosteal region and in osteocytes (fig 3). The walls of the blood vessels also stained positive.

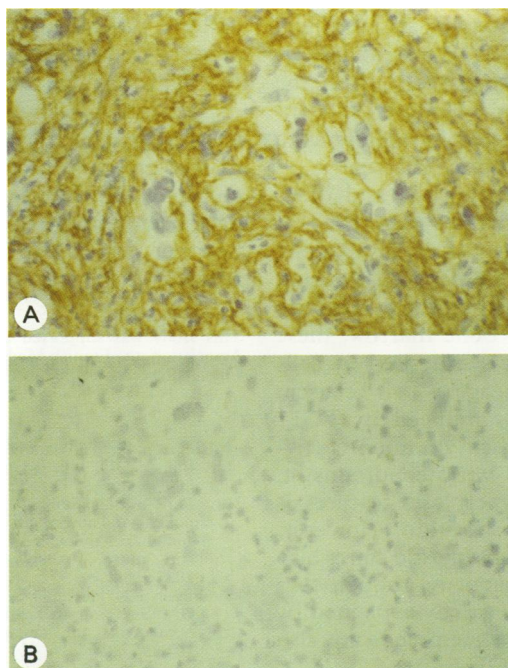
No tenascin could be observed in the aplastic bone marrow. In cases with fibrosis, metastatic adenocarcinoma, and atypical lymphoid infiltrate weak tenascin immunoreactivity was observed.

There was no clear correlation between the prognosis of myelofibrosis or leukaemias and tenascin immunoreactivity (data not shown). No difference was observed in tenascin immunoreactivity between the primary and secondary myelofibrosis.

## Discussion

The occurrence of tenascin in bone marrow correlated with the presence of reticular fibres and fibrosis, thus displaying a pattern of expression similar to that found in lymphatic tissues and neoplasias. Normal lymph nodes and splenic tissue displayed only a faint tenas-

Figure 1 Case of myelofibrosis: strong reticular tenascin immunoreactivity can be seen in the bone marrow (A). In the negative control (B) (normal mouse serum) no reactivity is present (immunoperoxidase stain)



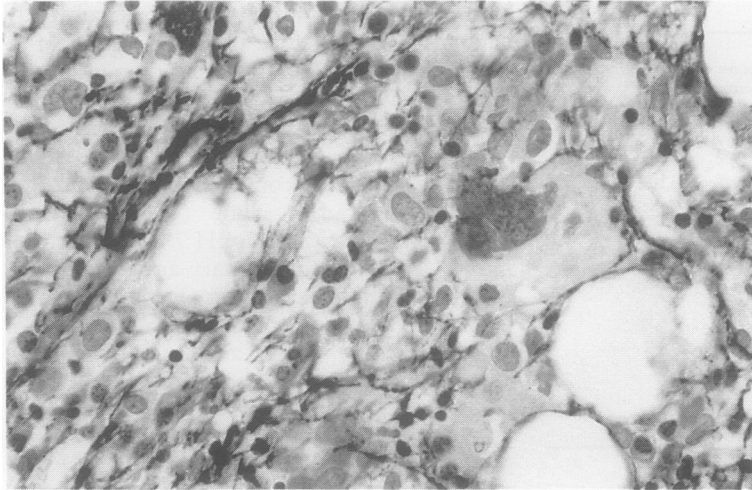


Figure 2 Megakaryocytic hyperplasia: slender reticular tenascin immunoreactivity can be seen (immunoperoxidase stain)

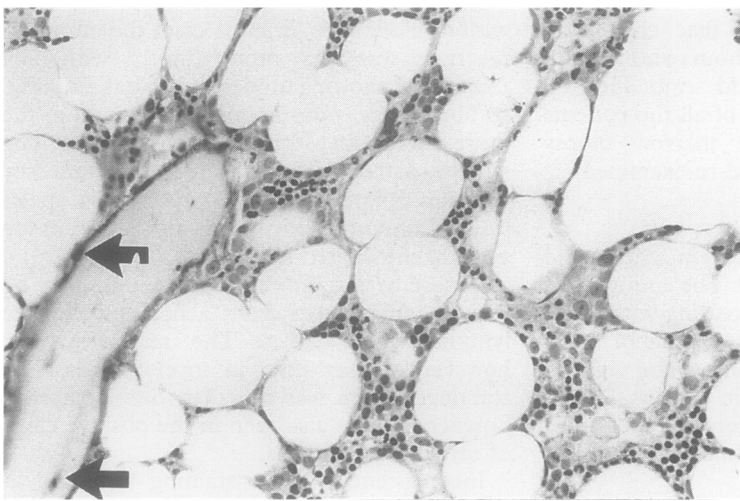


Figure 3 Normal bone marrow: only the periosteum of the bone and the osteocytes express tenascin (arrows) (immunoperoxidase stain)

cin immunoreactivity while in hyperplastic and neoplastic conditions stronger staining was observed.<sup>20</sup> Interestingly, in lymphatic lesions tenascin immunoreactivity was strong in the nodular sclerosing type Hodgkin's lymphoma<sup>20</sup> which, in this respect, is analogous to the myelofibrotic states of bone marrow.

The fibroplasia in myelofibrosis and other chronic myeloproliferative syndromes is induced by growth factors, such as platelet derived growth factor (PDGF), TGF- $\beta$ , and EGF: these stimulate fibroblasts to produce various collagens.<sup>24-26</sup> In bone marrow, these growth factors are mainly synthesised by cells of the megakaryocytic lineage.<sup>24, 26, 27</sup> Tenascin synthesis in fibroblasts is induced by TGF- $\beta$ .<sup>5, 15, 16</sup> The fact that tenascin immunoreactivity is mostly associated with conditions in which megakaryocytic hyperplasia features is well in line with this observation. However, neoplastic myeloid and hyperplastic states also show tenascin immunoreactivity, though to a lesser degree. This may be partly explained by the fact that bone marrow stem cells are also capable of synthesising TGF- $\beta$ .<sup>27</sup>

In bone marrow type III procollagen immunoreactivity closely resembles that of reticulin staining.<sup>25</sup> In myelofibrosis, the fibrous tissue also strongly stains for type III procollagen while laminin and type IV immunoreactivity can only be seen in the walls of the blood vessels and sinusoidal structures.<sup>25</sup> As tenascin follows the reticular staining pattern, its distribution is similar to that of type III procollagen. There are, however, no reports of any association between these two molecules. On the other hand, it is known that tenascin may be attached to fibronectin.<sup>8</sup> Interestingly, there is also a pronounced stromal deposition of fibronectin in myelofibrosis.<sup>26</sup>

The variability in tenascin immunoreactivity between different disease states of the bone marrow points to the fact that tenascin might have a role in their pathogenesis. It is known that tenascin contains EGF-like repeats.<sup>9</sup> As pointed out earlier, EGF have been implicated in the pathogenesis of bone marrow fibrosis.<sup>24</sup> Tenascin could therefore stimulate the fibrogenesis in bone marrow. Moreover, tenascin, via its EGF-like properties, might function as a growth promoting substance. In fact, it has been shown that EGF, along with several other growth stimulating substances, is capable of stimulating the proliferation of fibroblastic colony forming units in the bone marrow.<sup>28</sup> On the other hand, tenascin may function as an adhesive molecule to the cells of the bone marrow.

In normal bone marrow occasional tenascin positive fibres could be found, indicating that low amounts of tenascin are present in the reticular backbone of the marrow. In the bone tissue tenascin was seen in osteocytes and in the periosteum. Similar immunoreactivity in bone tissue has been reported earlier<sup>7</sup> and this also served as an internal positive control for the staining. No positivity was found in aplastic bone marrows which suggests that the matrix framework of the bone marrow is lost in aplasia.

In summary, the occurrence of tenascin in various pathological conditions of the bone marrow makes it a putative marker of disease. It could also be used as an adjunct in the diagnosis and follow up of myelofibrosis, as has been suggested for some other extracellular matrix proteins, such as laminin and type IV collagen. Derived protein fragments of these have been found in patients' serum.<sup>26</sup> To date, however, there are no serum markers for tenascin.

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