Hyaluronic acid synthesis in fibroblasts of pretibial myxedema

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Hyaluronic acid production by fibroblasts from skin lesions of pretibial myxedema (PTM) and also from normal control skin was studied in tissue culture. Normal control fibroblasts produced increased amounts of hyaluronic acid when serum from patients with PTM was used to supplement the tissue culture medium. Further increases in hyaluronic acid synthesis were noted when PTM fibroblasts were incubated with normal human serum, and when PTM fibroblasts were incubated with serum from patients with PTM. These results suggest that a serum factor is operative in patients with PTM, and that this serum factor can influence the production of hyaluronic acid in both normal controls and PTM fibroblasts. This increased production of hyaluronic acid by fibroblasts may be a major factor in the pathogenesis of PTM.

Index terms: Hyaluronic acid • Skin, diseases

The dermatological manifestation of Graves' disease usually affects the dorsum of the leg and has been called localized or pretibial myxedema (PTM). PTM is a component of Graves' disease that also includes diffuse thyrotoxic goiter and exophthalmos. Fifty percent of cases of PTM occur during active hyperthyroidism, and 50% after treatment of hyperthyroidism. PTM occurs in 0.5% to 4.3% of cases of Graves' disease, and also has been seen in Hashimoto's thyroiditis and primary hypothyroidism.

The three clinical variants of PTM include (1) the common sharply circumscribed type with nodules on the dorsum of the legs and toes, (2) the diffuse type with nonpitting edema on the dorsum of the legs and feet, and (3) the rare
elephantiasic type with verrucous nodules and extreme enlargement of the affected extremities.\textsuperscript{5,6}

The skin lesions of PTM are yellow-brown or erythematous plaques and nodules, usually on the dorsum of the legs and rarely on the thighs, abdomen, and upper extremities. PTM may resolve totally or continue indefinitely with exacerbations and remissions.

Skin biopsy specimens from PTM lesions show a thickened dermis with replacement of the mid-dermal collagen by a band of amorphous basophilic material.\textsuperscript{8} The material stains positively with the colloidal iron method and digests with hyaluronidase, which identifies the material as acid mucopolysaccharide (glycosaminoglycans).

Sisson\textsuperscript{9} has reported that plaques of PTM contain 6-16 times more hyaluronic acid than normal control skin. Cheung et al\textsuperscript{10} have noted increased hyaluronic acid synthesis in tissue culture by fibroblasts from the pretibial areas of patients with PTM and also in normal control pretibial skin.

This study was designed to evaluate the effects of a serum factor on hyaluronic acid synthesis by PTM fibroblasts and by normal fibroblasts in tissue culture.

**Materials**

*Subjects:* Skin biopsy specimens were obtained from the leg lesions of two patients with PTM. Patient 1, a 44-year-old white man, had a six-year history of Graves' disease, severe exophthalmos, and severe PTM. The long-acting thyroid stimulator (LATS) showed a 1750% level (normal, 80% to 120%) by the mouse bioassay method.\textsuperscript{11,12}

Patient 2, a 54-year-old woman, had a three-year history of Graves' disease, mild periorbital edema, and moderate PTM. The serum LATS level was 1307% by the mouse bioassay method.

Skin biopsy specimens from the PTM lesions in both patients showed a bandlike deposition of gray-white material and increased numbers of fibroblasts in the mid-dermis on staining with hematoxylin and eosin. Colloidal iron stains and digestion by hyaluronidase identified the material as hyaluronic acid.

*Control fibroblasts:* Skin biopsy specimens were obtained from the pretibial areas of 3 healthy male patients aged 29, 36, and 41 years, respectively.

*Control serum:* Control serum was obtained from normal healthy individuals without evidence of acute or chronic skin disease.

*Pretibial myxedema serum:* PTM serum was obtained from patients 1 and 2 described in this report.

**Methods**

The skin biopsy specimens from PTM and control patients were collected aseptically, washed with sterile phosphate-buffered saline,
minced into 1-mm\(^2\) pieces and cultured in 75-
cm\(^2\) Falcon flasks. The tissue was fed L15L me-
dium supplemented with 10% fetal calf serum and
incubated at 37\(^\circ\)C in an atmosphere of 5% car-
bon dioxide and 95% air.\(^{13}\) Fibroblasts began
to sprout from the explants approximately 10
days after explantation.

When the monolayer reached confluency, the
cells were removed with trypsin and passed into
clean 75-cm\(^2\) flasks. Normal control fibroblasts
from the scalp and PTM fibroblasts were grown
in parallel. Fibroblasts from the 6th to the 10th
passage were placed in 100-mm Petri dishes. Hy-
aluronic acid synthesis was determined with the
modified carbazol method\(^{14}\) from fibroblasts
grown in separate cultures with 10% normal
serum, 10% fetal calf serum, and 10% PTM
serum.

The studies were conducted with the use of
100-mm Petri dishes, each with inoculum densi-
ties of 400,000 cells. Normal control serum or
PTM serum was added to the fibroblast cultures
after the third passage. Hyaluronic acid synthesis
was measured on the 4th day when the cultures
became confluent.

Results

Normal control fibroblasts that were cultured
in media supplemented with normal control
serum demonstrated hyaluronic acid production
in the 6.6–7.6 \(\mu\)g/\(\mu\)g DNA range (Figure). These
values were considered the baseline normal val-
ues for the study. When PTM serum was substi-
tuted for normal control serum, hyaluronic acid
synthesis increased. When PTM fibroblasts were
used in place of the normal control fibroblasts
and normal control serum was added to the me-
dia, the hyaluronic acid synthesis increased fur-
ther. The highest values for hyaluronic acid syn-
thesis were obtained when PTM serum was added
to the media in which PTM fibroblasts were being grown.

Comment

Results of our experiments revealed that pre-
tibial fibroblasts from PTM patients produced
increased amounts of hyaluronic acid when in-
cubated with either PTM serum or normal con-
trol serum. Fibroblasts from the pretibial area of
normal control patients produced increased
amounts of hyaluronic acid when incubated with
PTM serum, but not with normal serum. These
results confirm that a “fibroblast-stimulating fac-
tor” is present in the serum of patients with PTM.

Hyaluronic acid synthesis by normal control fi-
broblasts as well as PTM fibroblasts can be stim-
ulated by the “fibroblast-stimulating factor.”

Cheung et al\(^{10}\) reported increased hyaluronic
acid synthesis when fibroblasts from the pretibial
areas of both PTM patients and normal controls
were incubated with PTM serum in tissue cul-
ture. Fibroblasts from other areas (normal-ap-
pearing shoulder skin of PTM patients and nor-
mal controls; normal-appearing foreskin and skin
from the backs of normal controls), under identi-
tical conditions, demonstrated no increased hy-
aluronic acid synthesis. In addition, pretibial fi-
broblasts from PTM patients showed no in-
creased hyaluronic acid synthesis when incubated
with normal human serum. These results were
suggestive of a “fibroblast-stimulating factor” in
the serum of PTM patients. Fibroblasts from
different regions of the body of PTM patients
responded differently to the serum “fibroblast-
stimulating factor.”

LATS is a circulating immunoglobulin with
thyroid-stimulating effects that are produced by
lymphocytes in Graves’ disease.\(^{15}\) LATS has been
detected in the sera of most patients with
PTM.\(^{16–18}\) This association may or may not indi-
cate a pathogenetic role for LATS in PTM. Mc-
Kenzie et al\(^{19}\) believed that LATS was a sig-
nificant causative factor in Graves’ disease and
might act by influencing cell-mediated immune
mechanisms.\(^{20,21}\)

The LATS antigen has not been precisely loc-
bated, but is thought to be within the thyroid
gland.\(^{22}\) Thyroid injury caused by radioiodine
treatment may result in the release of LATS
antigen and the subsequent development of
LATS antibody. This hypothesis is consistent
with the observation that half the cases of PTM
occur after treatment of Graves’ disease.\(^{1}\) Fur-
thermore, spontaneous remission or resolution
of PTM has been associated with decreased levels
of LATS in the serum.\(^{22}\) Thus, LATS may
be the fibroblast-stimulating factor. However,
LATS is a 7S gamma globulin; Cheung et al\(^{10}\)
found that their fibroblast-stimulating factor was
not a 7S gamma globulin. Further studies on
PTM sera are necessary to determine the nature
of the serum factor involved.

Studies are also needed to determine whether
fibroblasts from certain areas of the body are
possibly more susceptible to activation by PTM
serum, and to determine normal variations in
hyaluronic acid synthesis by normal fibroblasts in
different body areas of normal individuals.
Schmer et al\(^{18}\) proposed that venous stasis, varicosities, focal extravasation of blood, and local trauma might account for the focal accumulations of hyaluronic acid in the pretibial areas of PTM patients. The results of our study and also those of Cheung et al\(^{10}\) suggest that pretibial fibroblast stimulation by serum factors may be a more direct cause of the pretibial hyaluronic acid accumulations than these previously proposed hypotheses.

Lichen myxedematosus is a disease similar to PTM, in that it is associated with a serum factor and also results in the deposition of hyaluronic acid in skin lesions. Harper and Rispler recently reported that a factor in lichen myxedematosus serum can stimulate DNA synthesis and cell proliferation by normal control fibroblasts. Further studies may show that the same serum factor also stimulates fibroblast hyaluronic acid synthesis in these patients.

We are currently conducting laboratory studies of hyaluronic acid synthesis in fibroblasts from many normal-appearing skin areas in patients with PTM.

References