

## PROGRESSIVE LIPO-LYMPHEDEMA ASSOCIATED WITH INCREASED ACTIVITY OF DERMAL FIBROBLASTS IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE: IS THERE A CAUSAL RELATIONSHIP?

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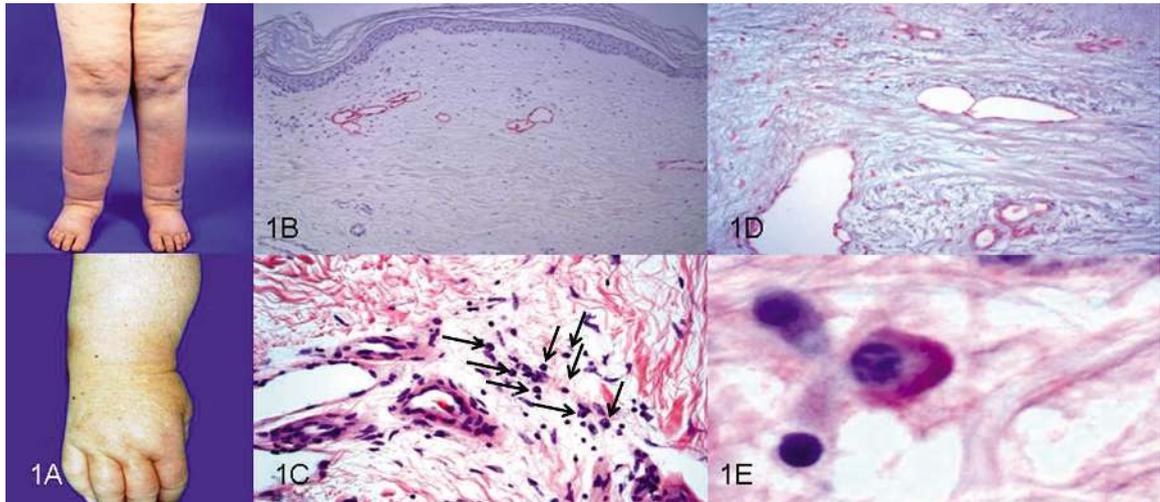
### ABSTRACT

*The pathophysiology of skin diseases associated with monoclonal gammopathies is generally unknown. Our aim was to investigate whether a monoclonal gammopathy could be a causal factor in progressive lymphedema. We describe a 75 year old patient with a rapidly progressive lipo-lymphedema and a monoclonal gammopathy of unknown significance (MGUS) suspected as a key etiological factor. Dermal fibroblasts were cultured from lesional lower leg skin and non-lesional abdominal skin and compared to healthy control fibroblasts. We found 10-fold elevated basic fibroblast growth factor 2 (FGF-2) in the patient's serum and significantly increased basal FGF-2 production of lesional and non-lesional fibroblasts compared to healthy controls. Upon restimulation with patient or healthy control serum, lesional fibroblasts showed significantly increased proliferation rates and FGF-2 production in vitro. Non-lesional abdominal fibroblasts showed an intermediate phenotype between lesional and control fibroblasts. Our findings provide the first evidence that lesional dermal fibroblasts from lipo-lymphedema with plasma cell infiltration show increased proliferation and FGF-2 production and that both local tissue*

*factors and altered FGF-2 serum levels associated with monoclonal gammopathies might contribute to this phenotype. Thus we propose a possible pathophysiologic link between the gammopathy-associated factors and the generation of lymphedema with initial fibrogenesis aggravating pre-existing lipedema.*

**Keywords:** fibroblast activity, lipo-lymphedema, monoclonal gammopathy, FGF-2, dermal fibrosis, immunoglobulins

Numerous skin diseases are associated with monoclonal gammopathies and originate from a direct proliferation of plasma cells and the deposition of pathologic immunoglobulins within the dermis (1-3). Fibrotic disorders frequently associated with monoclonal gammopathies are scleromyxedema and scleroderma, though pathogenetic mechanisms of these diseases are poorly understood (4). In monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma, elevated serum concentrations of profibrotic cytokines like TGF- $\beta_1$  (transforming growth factor- $\beta_1$ ) or FGF-2 (fibroblast growth factor-2) correlate with the severity of the disease and may play a role in its etiology (5,6). Elevated expression of FGF-2 can also be linked to other



**Fig. 1.** A) Upper part: Asymmetric lipo-lymphedema of legs with preponderance on the right side. Lower part: Edema of the forearm and fingers. B-E) Histopathology of a 6 mm punch biopsy from the lower leg. B) Immunohistochemistry using a lymphatic endothelial marker (mAb; D2-40; Hiss Diagnostic) shows a relatively thin epidermis and an enlarged edematous dermis with slightly augmented and dilated lymph vessels, but no increased angioplasia. Magnification: 100x. C) Hematoxylin-Eosin staining showing an accentuated plasma cell infiltrate (designated by black arrows →) in an edematous region with fibroblasts surrounded by flimsy fibers indicating collagen synthesis without manifest fibrosis. D) Myxedema was excluded by Alcian blue staining showing minor deposits of mucopolysaccharides within the physiological range around dilated lymphatic vessels. E) Single plasma cell from the lower leg showing positive staining for anti-human kappa light chains (mAb, clone R10-21-F3; DAKO), magnification: 400x.

hematological malignancies (7,8). No such correlation has been established in lymphedema patients. Here, we report the case of a 75 year old female patient with rapidly progressive lipo-lymphedema of the upper and lower extremities with a newly diagnosed MGUS suspected as a leading etiological factor. We hypothesize that the MGUS-associated perivascular plasma cell proliferations within the dermis and serum fibrogenic factors contribute to altered fibroblast activity of lesional skin, linking gammopathy and progressive lymphedema contributing to pre-existing lipedema.

The patient was a female obese (BMI 32) Caucasian with a history of slowly progressive edema of both lower legs over 20 years developing despite regular use of compression stockings for chronic venous insufficiency. According to CEAP classification, the grade was C4bE<sub>p</sub>A<sub>s</sub>2P<sub>R</sub> of the right leg. During

the 6 months preceding the presentation in our clinic, a rapid progression of the edema was observed including edema of buttock and both forearms as well as numbness and paresthesias of both upper and lower extremities (Fig. 1A). Lymphoscintigraphy showed delayed lymphatic drainage of the right leg with complete retention of Tc-99m-nanocolloid in the digital application area of the right foot after 20 min and only dim visualization of popliteal and parailliac lymph nodes after 180 min. Edema of the right leg was more prominent and showed mild signs of epidermal involvement (papillomatosis in the ankle and interdigital region). On both sides, Stemmer's sign was positive and edema was pitting, involving also the dorsum of the feet. MGUS had been diagnosed one month before hospitalization based on a monoclonal band (M-spike) in the serum protein electrophoresis. According to the diagnostic criteria

of the International Myeloma Working Group (2003), the laboratory findings of monoclonal bone marrow plasma cells of 12% and serum monoclonal protein level (IgG kappa type) of 35 g/l without hypercalcemia, osteolysis, significant anemia or renal dysfunction, the patient was classified as “smouldering myeloma” representing a transitional stage between MGUS and multiple myeloma. Immunofixation of the urine was negative. Dermatohistopathology including immunohistochemistry were compatible with lipo-lymphedema and showed a thin epidermis, an enlarged dermis with mildly enlarged lymph vessels (*Fig. 1B,D*), an accentuated plasma cell infiltrate among perivascular lymphocytes (*Fig. 1C*), and a discretely enhanced fibrogenesis, denoted by fibroblasts surrounded by flimsy fibers indicating collagen synthesis without manifest fibrosis (*Fig. 1C*). Lack of pathologically increased mucin deposition or angioplasia (*Fig. 1D*) excluded the differential diagnosis of obesity-associated lymphedematous mucinosis (9). Infections with *Borrelia burgdorferi* and *Treponema pallidum* were ruled out serologically.

## METHODS

Dermal fibroblasts were cultivated by explant technique from 6 mm punch biopsies of lesional skin from the lower leg and non-lesional abdominal patient skin. Skin fibroblasts from healthy donors were used as controls. Cells of passages 3-6 were used for the experiments and maintained in serum-free Keratinocyte growth medium (Promocell). Lesional patient fibroblasts (LF), abdominal patient fibroblasts (AF) and control fibroblasts (CF) from a healthy donor were incubated for 24 h *in vitro* with either 5% patient serum or 5% control serum from a healthy donor. Proliferation was measured by [<sup>3</sup>H]-thymidine incorporation using liquid scintillation counting. Cytokine levels of TGF- $\beta_1$  and FGF-2 were measured after 24 h of serum incubation according to the

manufacturer's instructions (R&D Systems). To determine abnormalities in serum cytokine levels of the patient, 10 different patient serum samples were analyzed in comparison to ten control sera from healthy donors. This study was conducted according to the Principles of the Declaration of Helsinki. Informed consent was obtained before collection of all clinical samples.

## RESULTS

The TGF- $\beta_1$  concentrations in the patient sera were comparable to control sera ( $35.6 \pm 7.3$  ng/ml vs.  $34.8 \pm 6.2$  ng/ml), whereas the FGF-2 concentrations were significantly elevated in the patient serum samples ( $9.2 \pm 1.5$  pg/ml) compared to control sera ( $0.5 \pm 0.5$  pg/ml);  $p < 0.001$ . The basal FGF-2-production of fibroblasts measured in the supernatant was also significantly increased in LF and AF compared to CF (*Fig. 2A*), whereas TGF- $\beta_1$  production was comparable in all experiments (not shown). After incubation with patient and control serum, the FGF-2 production of LF was significantly less suppressed as compared to CF upon incubation with both patient and control serum (*Fig. 2A*). Furthermore, AF produced significantly higher FGF-2 levels as compared to CF upon incubation with patient serum only, but not with control serum, indicating that disease-related circulating cytokines, such as a 10-fold increased serum FGF-2 concentration, may be involved in the alteration of fibroblast functions observed in this patient. Furthermore, we found a significantly increased stimulation of DNA synthesis of LF as compared to AF and CF independent of the type of serum used for the stimulation (*Fig. 2B*).

## DISCUSSION

The development of secondary lymphedema can occur as a result of longstanding preexisting lipedema of the skin, especially when no preventive measures are undertaken.

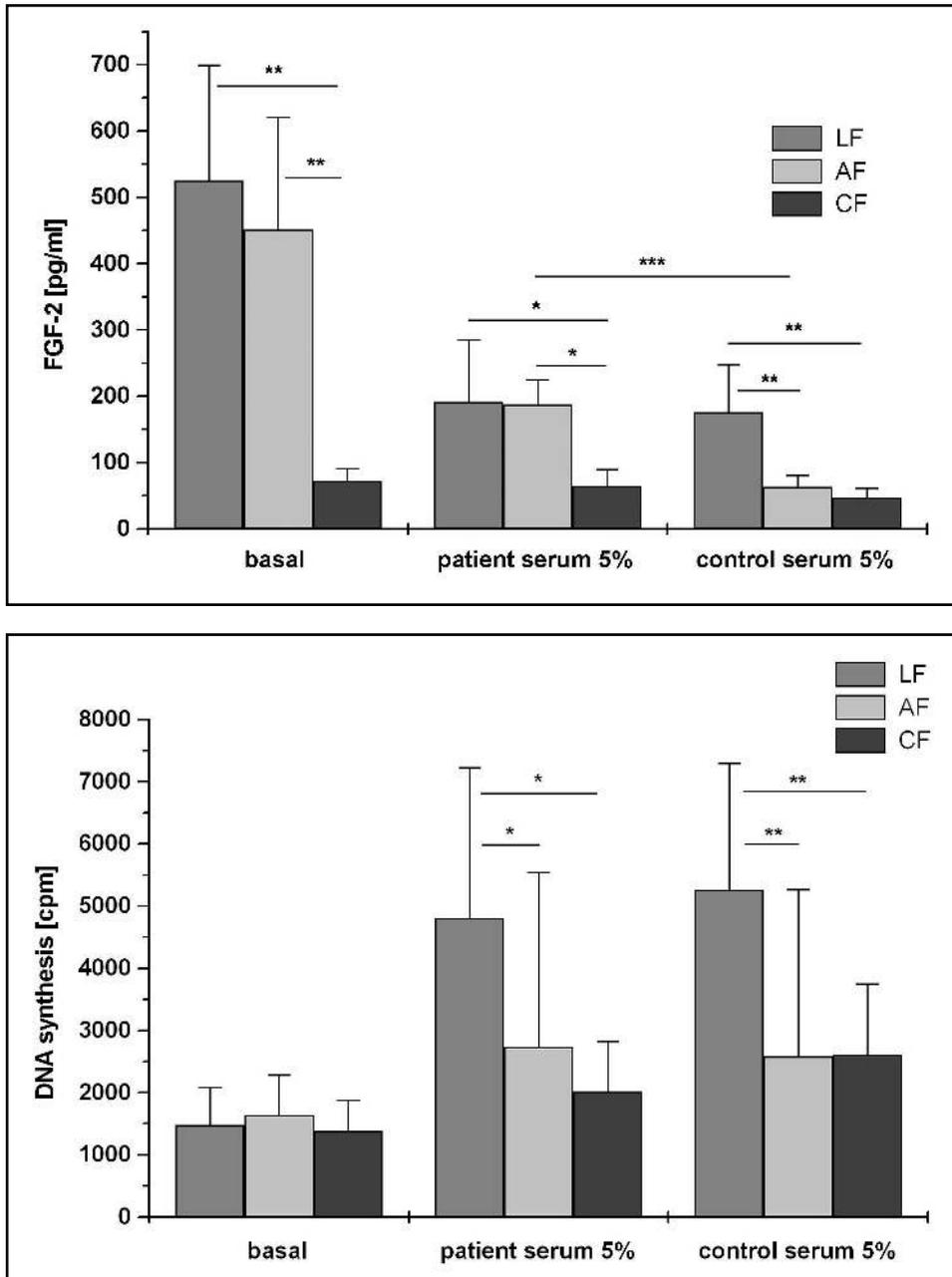


Fig. 2. A) Suppression of FGF-2-production of different fibroblasts by patient serum and control serum from healthy donors. Fibroblasts were seeded in a density of  $20 \times 10^4$  cells/ml and cytokine levels of FGF-2 were measured in supernatants after serum incubation for 24 h. Data represent the mean  $\pm$  SD of 4 independent experiments. Statistics were calculated by one-way-ANOVA ( $*p \leq 0.05$  and  $**p \leq 0.01$ ;  $***p \leq 0.001$ ; Tukey's multiple comparison test). B) Stimulation of DNA synthesis of different types of fibroblasts by patient serum and control serum from a healthy donor. Proliferation rates were detected via [ $^3$ H]-thymidine incorporation after serum incubation for 24h. The data represent the mean  $\pm$  SD of 4 independent experiments. Statistics were calculated by one-way-ANOVA ( $*p \leq 0.05$ , and  $**p \leq 0.01$ ; Tukey's comparison test.) LF = lesional fibroblasts, AF = abdominal fibroblasts, CF = control fibroblasts.

In our patient, the reported rapid symmetric progression of leg and forearm arm edema within a few months despite compression therapy gave reason to look for other systemic factors involved in this progression. The newly diagnosed MGUS was suspected to be a leading etiologic factor. Our data demonstrate for the first time that lesional fibroblasts derived from a region with pre-existing chronic lipedema in combination with early-stage lymphedema show an increased activity with elevated proliferation rates after stimulation as well as an increased production of fibrogenic cytokines like FGF-2 as compared to healthy control fibroblasts. Interestingly, non-lesional AF exhibited a phenotype partly comparable to LF and partly to CF, although they are subjected to the same individual fibrogenic stimuli of serum. This finding may indicate that both local factors derived from plasma cell infiltrates and serum factors associated with gammopathies, like FGF-2, probably contribute to the alteration of fibroblast functions. FGF stimulates lymphangiogenesis via a paracrine mechanism (10-13) and is an important factor to maintain fibrosis (14) together with other cytokines like TGF- $\beta_1$  (15) and CTGF (connective tissue growth factor). Interestingly, incubation with patient and control serum induced suppression of fibroblast FGF-2 production, which suggests a preponderance of suppressive cytokines or other serum factors regulating the production of this cytokine. Increased FGF-2 production of non-lesional abdominal fibroblasts stimulated with patient serum as compared to control serum may indicate that the patient's elevated FGF-2 levels probably activate the fibroblasts, whereas local factors related to pooling of plasma cells and cytokines in the edematous "stasis" region, such as mechanical forces stretching the connective tissue, alter the activity of lesional fibroblasts in a way that they react independently from the source of serum used for restimulation.

In conclusion, our data provide the first

evidence that increased fibroblast activity can be observed in fibroblasts derived from initial lymphedema with plasma cell infiltration and that increased serum levels of fibrogenic cytokines such as FGF-2 associated with monoclonal gammopathies might account partially for this altered phenotype of fibroblasts. Thus we propose for the first time a possible pathophysiologic link between the gammopathy-associated factors and the generation or acceleration of lymphedema with increased fibrogenesis, which should be corroborated in further studies.

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