Pachydermoperiostosis (primary hypertrophic osteoarthropathy): in vitro evidence for abnormal fibroblast proliferation


Institute of Internal Medicine IV, Institute of Gerontology, and Institute of Dermatology, University of Florence, Italy; Institute of Physical Rehabilitation, University of Zagreb, Croatia.

ABSTRACT. Pachydermoperiostosis (PDP) is a disease characterized by the presence of pachydermia, periostosis and finger clubbing. Evidence that the skin and soft tissues are involved in the disease prompted the in vitro investigation of the behaviour of fibroblasts obtained from cutaneous biopsies of involved and apparently uninvolved PDP skin.

PDP fibroblasts from affected skin demonstrated an abnormal proliferation, very rapid and tumultuous when compared to the growth of fibroblasts derived from apparently uninvolved skin and fibroblasts from the skin of healthy subjects. This characteristic was confirmed by the rate of thymidine incorporation, which was increased in PDP-affected fibroblasts (1152 dpm) compared to apparently non-PDP involved fibroblasts (273 dpm) and controls (262 dpm).

Ultracentrifuged and non-centrifuged conditioned medium (CM) of fibroblasts affected or apparently not affected with PDP were used to evaluate the effect on the proliferation of healthy skin fibroblasts, compared to the effect of CM derived from healthy fibroblasts and from healthy fibroblasts incubated with 10% and 1% foetal calf serum. The CM of non-centrifuged PDP fibroblasts resulted in a statistically significant stimulation of fibroblast growth when compared to that expressed by ultracentrifuged PDP CM, healthy fibroblast CM and 10% stimulated CM.

These data show that PDP fibroblasts maintain in vitro the capacity to proliferate at a higher rate than healthy fibroblasts and that in the CM residual cells and/or their debris may be present, inducing the abnormal growth of healthy fibroblasts. This evidence suggests that fibroblasts in PDP may play a role in the development of the disease.

Key words: pachydermoperiostosis, finger clubbing, fibroblast growth.

Introduction

Pachydermoperiostosis (PDP) is a disease involving several organs and systems: the skin (seborrhea, pachydermia, cutis verrucosis gyrata), the bones (periostosis,acroosteolysis), the joints (arthritis or arthralgia), and acral soft connective tissues (finger clubbing) (1,2). The aetiology-genesis of PDP and hypertrophic osteoarthropathy is still unknown (3).

Recently two interesting hypotheses have been proposed to explain the tissue modifications. The first concerns the role that platelets and their products, in particular growth factors, may play in the development of tissue modification (4, 5). The second suggests that endotheliocytes may be involved in the pathogenesis of PDP (6).

The most striking feature of the disease consists in a peculiar cutaneous involvement characterized by skin hypertrophy reaching the highest degree with pachydermia (1, 2, 7). This clinical observation led us to investigate in vitro the behaviour of skin fibroblasts derived from PDP and their capacity to proliferate.
Materials and methods

Three male patients affected with PDP were selected; all three presented finger clubbing, periostitis and pachydermia. Patients were informed of the aim of the study and all the procedures were performed after consent was obtained.

Patients underwent skin biopsies of the periungual zone of the second finger of the left hand and of apparently healthy skin from the left forearm. Biopsies from the periungual zone were also taken from 3 healthy subjects matched for sex and age, who were undergoing finger surgery. Lidocaine 5% was injected subcutaneously and skin sections were obtained with a scalpel.

The biopsy samples were immersed in culture medium MEM (GIBCO) with penicillin and streptomycin (100 µg/mL), gentamycin (50 mg/L), NaHCO₃ (2.2 g/L) and 20% fetal calf serum (FCS) (FLOW) in a humidified atmosphere with 5% CO₂ at 37°C, and allowed to adhere to the surface of 60 mm plastic tissue culture dishes (Nunc). The cells were then subcultivated in flasks with 10% FCS. At the 3rd, 6th, and 10th passages, cells were seeded 2 x 10⁶/mL in six-well dishes, resuspended by treatment with trypsin in PBS, pH 7.4, and then counted using a hemocytometer on the 1st, 5th, 9th, 12th, 15th, 18th, 21st, 24th, 27th, 33rd and 37th days to evaluate their growth. The experiments were performed in triplicate.

5 x 10⁵ cells/well were also seeded in 96-well plates, and after three days were incubated for 18 hours with 20 µCi³H-thymidine/well (25 µCi/mL, Amersham). The radioactivity was counted with a liquid scintillation counter (Beckman, USA). After 3 days from the 3rd passage, the conditioned medium (CM) was sampled from flasks containing healthy (5 x 10⁵) and affected cells (1 x 10⁵). A part of the CM was then ultracentrifuged at 12,000 g for 5 minutes and the supernatant stored together with the whole CM.

To test the effect of the ultracentrifuged and non-centrifuged CM on the proliferation of healthy skin fibroblasts, 2 x 10⁵ cells/mL obtained as described above were seeded in four 24-well dishes. Cells were incubated with a medium containing 10% and 1% FCS, respectively, and with non-centrifuged and ultracentrifuged CM obtained from healthy and affected cells. Two dishes were cultured for 3 days and the other two after 5 days. All the experiments were performed in triplicate. To verify the growth capacity of PDP-affected fibroblasts, in comparison to healthy fibroblasts, they were seeded at 2 x 10⁵ cells/mL in a culture medium supplemented with 1% and 10% FCS, respectively.

The cell count was performed after 3 and 5 days.

A statistical analysis of the results of the experiments with ultracentrifuged and non-centrifuged CM was carried out using the unpaired Student's t-test.

Results

Fibroblast growth

The study of fibroblast growth shows (Fig. 1) that the proliferation of PDP fibroblasts derived from involved skin was enormously rapid and tumultuous: The cell rate had almost doubled by the 5th day and increased progressively up to the 37th day. The PDP cells of the apparently uninvolved skin demonstrated a growth that was always higher than that of the controls, but which did not reach the growth rate expressed by the affected skin. It is worth noting that the PDP cells showed a distinctive and characteristic growth curve. They reach confluence, but the surprising growth did not stop afterwards as it did for the other two cell lines.

The increased growth rate of PDP cells was confirmed by the thymidine incorporation rate (1152 dpm), which was greater in the fibroblasts of the periungual zone than in the cells derived from apparently uninvolved skin (273 dpm) and from the controls (202 dpm).

The cell count demonstrated a striking growth of PDP affected cells not only at 10% FCS (3rd day: 9.2 ± 0.4; 5th day: 12.5 ± 0.2), but also at 1% FCS (3rd day: 8.8 ± 0.5; 5th day: 12 ± 0.8), when the control lines resulted in a resting state (see Table 1).

Effects of conditioned medium on fibroblast growth

The evidence of abnormal PDP cell growth prompted the second experiment, because we thought that these PDP cells might be producing substances capable of maintaining such a striking growth rate.

It was evident that the medium with 10% FCS stimulated cell growth, but a relevant statistical difference was seen both at the 3rd and 5th days of incubation, when it was compared to the growth of healthy cells stimulated with non-centrifuged CM derived from PDP cells (see Table 1). When the growth with 10% FCS was compared to that of healthy non-centrifuged CM, no statistical difference was found.

It was evident that the ultracentrifuged CM derived from PDP cells resulted in reduced growth, both at the 3rd
and 5th days (p < 0.001), when compared to that obtained with 10% FCS and with the non-centrifuged CM of PDP cells (Table I).

### Discussion

Pachydermoperiostosis is often characterized by enormous skin and tissue growth, expressed mainly by pachydermia and finger clubbing (1, 2). The pathogenesis of hypertrophic osteoarthropathy is still uncertain. In recent years attention has been focused on the potential role that platelets (4, 5, 8) and the endothelium (9) may play in the genesis of the disease. It seems that platelet-derived growth factors could contribute to the activation of endothelium and to fibroblast growth.

Cobral et al. (10) suggested that humoral growth-promoting factors for fibroblasts, present in the sera of patients with primary and secondary hypertrophic osteoarthropathy, might contribute to the pathogenesis of the disease. In fact, he found that the plasma and sera of primary and secondary hypertrophic osteoarthropathy stimulated healthy skin fibroblasts more than controls. The hypothesis that the growth hormone might be involved in the pathogenesis of tissue hypertrophy has recently been definitively abandoned, however (11).

In previous works (6, 7) we described the modification of the connective tissue which occurs in PDP, in particular changes in the collagen fibres which appeared packed but in a disorderly rather than periodic fashion (Luse bodies). This evidence focused attention on the role that fibroblasts may play in the development of the disease. Our results here clearly show that PDP fibroblasts, obtained from the periungual zone, have a very high growth rate when compared to healthy fibroblasts. Indeed, the CM of these cells resulted in a very potent stimulation of healthy fibroblasts; this effect was abolished when the cells were stimulated only with the supernatant of the centrifuged CM. All these data show that: (a) PDP fibroblasts maintain in vitro the capacity to proliferate at a higher rate than healthy fibroblasts, which seems to indicate that PDP fibroblasts may undergo autocrine proliferation, assuming a growth behaviour very similar to that of malignant virally-transformed cells; and (b) in the non-centrifuged CM, cells and/or their debris are present that may modify the growth of healthy fibroblasts at a higher rate.

Our results seem to indicate that fibroblasts may also play a role in the development of the disease. It is controversial whether fibroblasts are cardiac cells in the pathogenesis of the disease, or just the target of a process that takes place in the circulatory system, involving at the same time platelets and endothelial cells.

We believe that further investigations are needed to ascertain if factors exist in PDP which are capable of stimulating the growth of fibroblasts, and what is the real role of these cells in the pathogenesis of the disease.

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### References

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