

THE STIMULATING EFFECT OF HUMAN SKIN TISSUE FLUID/LYMPH ON CULTURED KERATINOCYTES.

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Our previous studies revealed presence of a number of growth factors and cytokines in skin tissue fluid/lymph of patients with lymphedema. Lymphedematous skin is characterized by hyperkeratotic epidermis. The question arises whether lymph cytokines have an effect on proliferation of keratinocytes (KC) or the proliferative drive is stimulated by stretching of skin/epidermis by increasing edema.

Aim: to study the effect of human lymph obtained from patients with obstructive lymphedema on human keratinocytes obtained from foot, calf and thigh, areas representing different physiological tempo of KC growth.

Methods: KC were isolated using enzymatic digestion. Cells were cultured in various concentrations of tissue fluid/lymph. Lymph contained IL1, IL6, TNFalpha, KGF, EGF, VEGF, TIMP1,2 at higher levels than serum. Control cell culture were conducted in RPMI with of 5% FCS. To identify cytokines responsible for KC proliferation IL6, TNFalpha, KGF and VEGF were blocked with specific antibodies. After 7 days culture phenotypes were defined using moAbs against p63 (stem cells), CD29 (transient daughter cells) and markers of differentiation: Ki67 and PCNA. Additionally, incorporation of BrdU was checked.

Results: seven day culture in lymph showed increased percentage of p63 and CD29 positive cells compared with control culture. Moreover, there was increased number of dividing cells in lymph. Antibodies KGF, IL6 and VEGF partially blocked proliferation.

Conclusion: Cells cultured in lymph obtained from patient with lymphedema showed increased KC proliferation rate. These cells expressed stem cell markers. Lymph cytokines participate in KC proliferation. Drugs downregulating KC cytokine production should be tried in culture as an initial step before clinical trial.