

## Cyclin A Expression in Chronic Leg Ulcers

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Sir,

Normal wound repair involves an initial inflammatory phase characterized by neutrophil infiltration and excessive protease activity, which is followed by macrophage migration, angiogenesis and keratinocyte proliferation and migration. In contrast to acute wounds, chronic venous ulcers fail to follow this timely pattern of events and persist in a chronic inflammatory stage involving high protease levels, bacterial influence, endothelial cell activation and high oxidative stress. Epidermal proliferation is a prerequisite for proper epithelial closure during the acute wound healing process, and various markers (such as Ki67 and cyclins) have been extensively studied (1–5). Furthermore, overexpression of cyclins has been reported in association with tumour formation and in psoriasis (6). Cyclins, intranuclear subunits of cyclin-dependent kinases, are regulatory proteins essential for passage through specific stages in the cell cycle. A number of different cyclinproteins, each associated with a certain stage of the cell cycle, have been described (7). Cyclin D is associated with G1 phase chromosome replication, cyclins A and E regulate the S phase DNA synthesis stage, whereas cyclin B1 controls the G2 phase (the premitotic phase). Mitosis is regulated by cyclin A and B. The purpose of this study was to assess whether cyclin A expression could be utilized as a novel marker for epidermal proliferation in chronic leg ulcers. Furthermore, we investigated whether expression of this regulator was down-regulated in chronic ulcers when compared with normally healing wounds 3 days after injury.

### PATIENTS AND METHODS

Eleven patients with chronic venous leg ulcers (duration >6 months) were included in this study. The venous insufficiency was routinely determined either by a hand-held Doppler or colour duplex examination. Systolic ankle index was >0.8. The patients had no diabetes or signs of general infection, and no signs of local infection or immunological disease. The research project was approved by the Ethics Committee, Lund University Hospital (LU509-01).

Punch biopsies (4 mm) were taken from the wound edges of chronic ulcers. As control material, biopsies were taken from four patients from the intact skin of the thigh. Three days later a new biopsy was taken from the wound edge of the healing biopsy wound. The tissues were fixed overnight in 4% paraformaldehyde, embedded in paraffin by routine procedures, and sections (5 µm) were incubated with mouse monoclonal antibodies (dilution 1:80) specific for cyclin A (Novocastra Laboratories Ltd) and subsequently developed (Vectastain ABC, Vector Laboratories, Burlingame, CA, USA). The slides were

examined in a light microscope and basal cells with positive signal and staining pattern were counted throughout the whole 4-mm biopsy. Statistical analysis was performed using the Mann-Whitney rank sum test.

### RESULTS AND DISCUSSION

Immunohistochemical analysis demonstrated cyclin A-positive keratinocytes in the basal layers, and no background staining was detected. In normal skin there was a positive signal for cyclin A in ~5% of the basal epidermal cells (Fig. 1). In the chronic wound margins from the group of 11 patients, the percentage of cyclin A-positive cells increased to ~25%, and this difference was statistically significant when compared with normal skin. When compared with the chronic ulcers, the acute wound margins displayed a similar staining pattern. Thus, the finding that the S phase marker cyclin A was up-regulated approximately fivefold in chronic ulcer margins, when compared with normal skin, suggests that basal epidermal cells at the wound margins of chronic leg ulcers are able to proliferate. In this context, our data correspond well with previous studies on the expression of proliferation-associated markers and cell cycle regulators in the margins of chronic diabetic and venous ulcers. For example, cytokeratin 16 and 17, as well as proliferation-associated nuclear antigens (PCNA and Ki67) were found to be up-regulated in the wound margins of these ulcers (5, 8). In the immediate leading edges of both the chronic and acute wounds, no cyclin A-positive cells were detected, indicating that these cells are non-proliferating

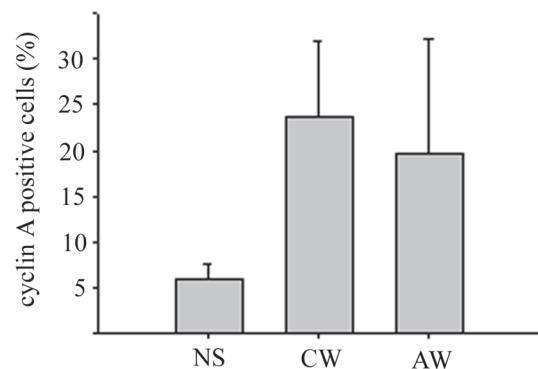


Fig. 1. Statistical analysis shows significant increase ( $p < 0.001$ ) of cyclin A-positive cells in chronic wound margins (CW) when compared with normal skin (NS). In acute wound margins (AW) the signal pattern showed the same trend as in chronic wounds. Whiskers=SD

and originate from basal stem cells beneath the leading edge. Interestingly, similar findings have been reported from studies on healing mucosal wounds (9, 10). Thus, our study is well in line with previous reports showing that epidermal proliferation is indeed present in the wound edges of chronic venous ulcers, suggesting that defective proliferation is not a major pathogenetic factor. What is then the major cause of venous ulcerations? An increasing amount of data indicates that high levels of proinflammatory factors (11, 12), high oxidative stress (11, 13), as well as proteolysis induced by various bacteria in chronic ulcers (14, 15), resulting in excessive proteolysis and endothelial activation, contribute to the non-healing of venous ulcers. Taken together, these findings reinforce the view that future therapeutic approaches directed at venous ulcers should target inflammatory mechanisms, excessive bacterial influence and epidermal migration.

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