Journal of Dermatological Science (2006) 44, 109—112

ELSEVIER

Journal of Dermatological SCIENCE

www.intl.elsevierhealth.com/journals/jods

LETTER TO THE EDITOR

A comparative study of granzyme B expression in keratoacanthoma and squamous cell carcinoma

KEYWORDS
Granzyme B;
Keratoacanthoma;
Squamous cell carcinoma

Keratoacanthoma (KA) is a cutaneous neoplasm characterized by initial intensive growth and usually spontaneous regression [1]. Squamous cell carcinoma (SCC) is a malignant proliferation of the epithelial cells of the skin and mucous membranes characterized by locally destructive growth and tendency to metastases [1]. The most important feature that separates these closely related entities is a tendency of KA to regress but causes and detailed mechanism of this regression are still not completely elucidated.

Recent studies suggest that tumor regression depends mainly on immune response mediated by cytotoxic CD8+ T lymphocytes (CTLs) supported by CD4+ T cells [2]. CTLs can kill tumor cells and mediate tumor regression *in vivo* through two distinct molecular mechanisms: (1) direct exocytosis of granules containing granzyme B and perforin; (2) binding of the CD95 receptor on target cells [2,3]. Granzyme B/perforin pathway has been shown to efficiently kill tumor cells *in vitro*, induce apoptosis in multiple-drug-resistant and death-receptor resistant cell lines [3] and could be involved in tumor regression process.

One hundred and fifty skin specimens were obtained from 82 male and 68 female patients. Average age of the patients was 69.10 (±11.39), 67.87 (±11.64), 75.50 (±10.20), 76.57 (±9.74) and 65.86 years (±14.84) for proliferative keratoacanthoma (pKA), regressing keratoacanthoma (rKA), well differentiated SCC (wdSCC), poorly differentiated SCC (pdSCC) and healthy controls, respectively. The specimens included 30 cases of each: normal skin (NS), pKA, rKA, wdSCC and pdSCC. Tumors were predominately located on photo-exposed skin (80% of pKA, rKA and wdSCC; 85% of pdSCC). SCCs were classified according to Broders' grading into two categories, well (Broders' grade I) and poorly (Broders' grade III) differentiated type while KAs were classified according to previously used criteria to designate pKA and rKA [4].

Four micrometers thick sections of formalin fixed paraffin embedded tissue were stained with hematoxylin-eosin and two pathologists examined each slide independently. Specific antibodies were used to recognize CD8 (1:50, DAKO A/S, Glostrup, DK), CD3 (1:25, DAKO A/S, Glostrup, DK), CD4 (1:25, NOVOCASTRA, Newcastle upon Tyne, UK) and granzyme B (prediluted, NOVOCASTRA, Newcastle upon Tyne, UK). Immunostaining results for CD8, CD4, CD3 and granzyme B were quantified by expressing the number of positive cells/mm^2 of the lesion.

Expression of granzyme B was significantly increased in all skin tumors examined as compared to NS ($p < 0.0001$). Median values and ranges of granzyme B, CD3, CD8 and CD4 expression are shown in Fig. 1. Granzyme B expression was significantly increased in KAs as compared to pdSCC and had the highest values in rKA. Localization of granzyme B immunoreactivity in examined tumors is shown in Fig. 2. Granzyme B was detected in a part of peritumoral and intra-tumoral lymphocytes as sparsely granular pattern. Majority of granzyme B positive cells was found at the interface between lymphocytic areas and tumor cells in the dermis and tumor stromal septa. In KAs, granzyme B positive T cells were scattered between malignant keratinocytes and weak positive staining was also observed in some of the malignant keratinocytes.

Tumors were variably infiltrated with CD3+, CD8+ and CD4+ T cells with significantly higher density of the infiltrate comparing to NS ($p < 0.0001$). We have detected significantly higher density of CD3+ and CD4+ T cells in KAs as compared to SCCs (Fig. 1). Majority of the tumor infiltrating cells in KAs and SCCs were CD8+. There was a significant increase of

doi:10.1016/j.jdermsci.2006.07.003

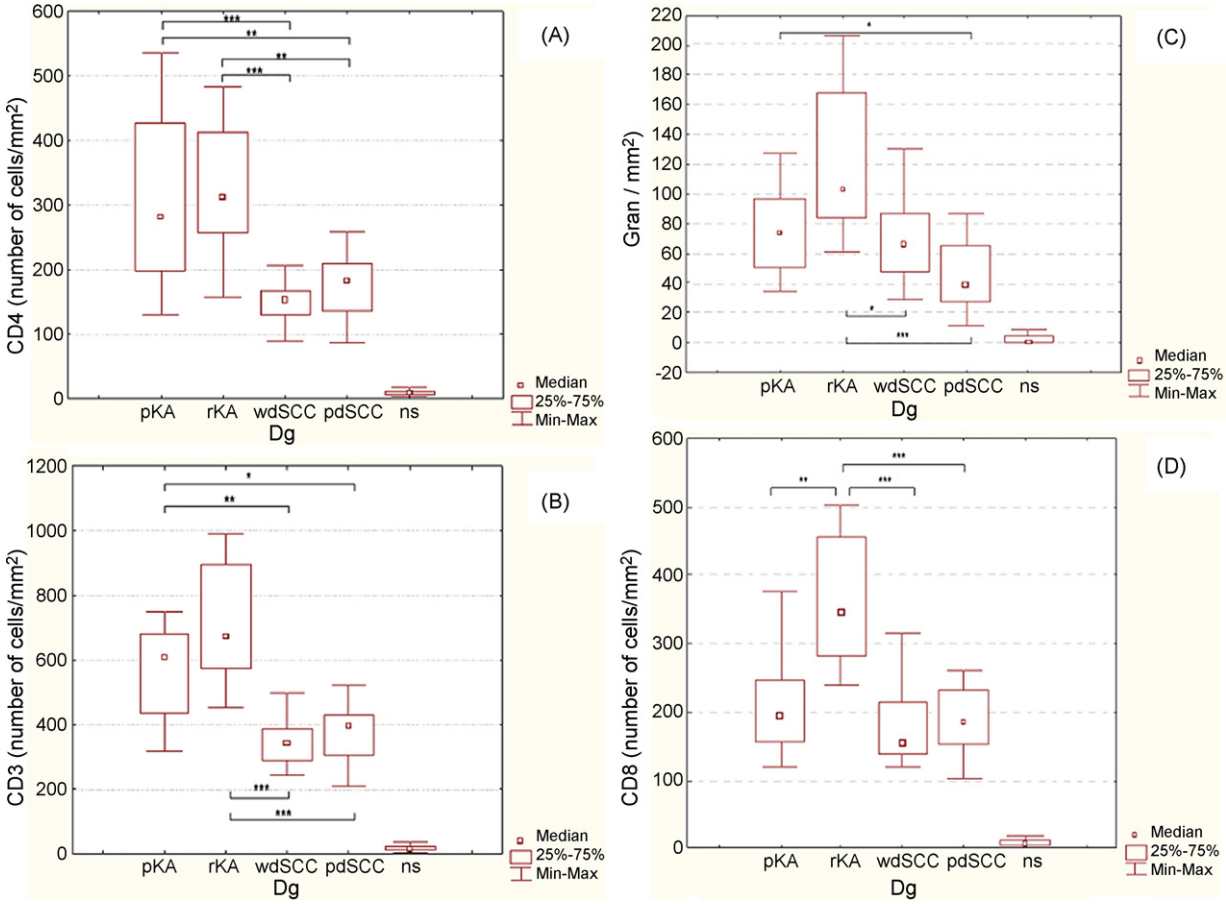

Fig. 1 Expression of CD4, CD3, CD8 and granzyme B in keratoacanthoma and squamous cell carcinoma (statistical significance: $^{*}p < 0.05$; $^{**}p < 0.001$; $^{***}p < 0.0001$).

CD8+ T cells density in rKA as compared to pKA and SCCs (Fig. 1).

The highest density of CD3+, CD8+ and CD4+ T cells was found in rKA with significant change in CD4/CD8 ratio (1.66 in pKA to 0.9 in rKA) due to increased density of CD8+ cells (density of CD4+ T cells did not change in KAs). While CD4/CD8 ratio in rKA and SCCs (0.9 in wdSCC; 0.94 in pdSCC) was similar, density of inflammatory infiltrate was much lower in SCCs. CD3+ T cells infiltrate was detected along tumor—host interface and dermis or tumor septa. Peri-tumoral infiltrate was composed of both CD8+ and CD4+ cells and intra-tumoral cells were mainly CD8+, as described previously [5].

Expression of granzyme B correlated positively with density of CD4+ T cells in pKA ($p = 0.037$, $r = 0.383$) and pdSCC ($p = 0.026$, $r = 0.406$). However, in wdSCC expression of granzyme B correlated with density of CD8+ T cells ($p < 0.0001$, $r = 853$) and in rKA with both, CD8+ ($p < 0.0001$, $r = 726$) and CD4+ ($p = 0.001$, $r = 0.566$) T cells. These findings suggest discordant expression of granzyme B in human lymphocyte subsets and the possibility of granzyme B and/or other granzymes to be produced by a different subset of T cells in immune response mounted against different tumors, what might influence the effectiveness of host immune response [6]. Our results also imply the presence of adequately stimulated CD8+ T cells in rKA and indicate that both CD8+ and CD4+ T lymphocytes contribute to immunosurveillance against skin cancer as suggested recently [2].

We have detected a decrease in granzyme B expression in tumor and NS samples from sun exposed areas ($p < 0.05$) suggesting inhibition of cytotoxic activity. Modulation of cytotoxic activity by UV-radiation could contribute to the suppression of anti-tumor immune response stimulating promotion and cancer progression.

Presence of significantly lower density of CD3+, CD8+ and CD4+ T-cell infiltrate in SCCs (especially noted when comparing rKA and pdSCC), suggests insufficient host immune response mounted against SCCs. Besides the highest total inflammatory infiltrate in rKA, we have detected a significant increase in CD8+ T cells in rKA as compared to pKA accompanied by intense cytotoxic activity. These data confirm previously suggested significance of the