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REVIEW

CD30+ cells in regressing keratoacanthoma and in non-keratoacanthomatous squamous cell carcinoma

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Abstract: In a previous report, we demonstrated how a percentage of CD30+ cells was nearly constant in the inflammatory infiltrate that accompanies keratoacanthoma (KA), but we saw a lack of CD30 cells when KA was regressing. In the current study we investigated the presence of CD30+ cells in the inflammatory infiltrate of regressing KA as well as in well-differentiated squamous cell carcinomas of non-keratoacanthomatous type. We examined 80 keratoacanthomas from our archives, and selected those with the pattern of advanced regression. We also examined 14 well-differentiated non-KA type SCCs from our archives. In all the cases, we performed a immunohistochemical study for CD30. Of the 80 KAs, 6 cases (7.5 %) showed the pattern of regression. While the mean of the percentages of CD30+ cells in the infiltrate was 0.58 for the regressing KAs, it was 1.77 for SCCs. While cells with the paranuclear-dot pattern of immunostaining plus membranous pattern of immunostaining could be easily found in SCC cases, cells only with membranous expression of the marker were the rule in KA. We conclude that CD30 positive cells might play a role in KA regression *(Tab. 2, Fig. 4, Ref. 24)*. Full Text (Free, PDF) *www.bmj.sk*.
Key words: squamous cell carcinoma, CD30, keratoacanthoma, regression, imiquimod.

In a previous report, we demonstrated how a percentage of CD30+ cells was nearly constant in inflammatory infiltrate that accompanies keratoacanthoma (KA) (1). To our surprise, the case that showed the smallest percentage of CD30+ cells in the inflammatory infiltrate had the pattern of regressing keratoacanthoma. We then hypothesized that CD30 might play a role in the regression of KA. The current study has two main purposes. On one hand, we have studied only cases of KA with the regressing morphology for the evidence of CD30+ cells in their inflammatory infiltrate. We also investigated the same cells in cases of well-differentiated squamous cell carcinomas of non-keratoacanthomatous type. We evaluated the quantity and percentage of CD30+ cells in the inflammatory infiltrate of each lesion.

Materials and methods

We examined 80 keratoacanthomas from our archives, and selected those with the pattern of regression according to the morphology of the evolution of the lesions that has been beautifully described in some texts (2), or reports (3). Only lesions that have been described as in advanced regression (2) or with marked regressive changes (3), were selected. We also examined 14 well-differentiated non-KA type SCCs from our archives.

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In all these cases (KAs and SCCs), we performed an immunohistochemical study for CD30 expression in the dermic inflammatory infiltrate, with the monoclonal mouse anti-human CD30 antibody of DakoCytomation (Clone Ber-H2; code N1558), and with the Dako REAL EnVision detection system.

To evaluate the immunostaining, we used the same scheme we had used in our previous report (1): 0=no CD30+ cells; 1= occasional CD30+ cells; 2=CD30+ cells which are more than occasional but still appear non-grouped; 3=CD30+ cells in groups of 3 or less cells; 4=CD30+ cells in groups of more than 3 cells. Again, when two or more patterns of immunostaining were observed (other than 0), only that most frequent in the biopsy (not that with the highest value) was the one considered. Moreover, we have estimated the percentage of CD30+ cells in the infiltrate, following the same semiquantitative method that has been described by Cepeda et al (4). We have also re-evaluated our cases of KA that were published in our previous report, (1) and quantified the percentage of CD30+ cells, using this same semiquantitative approach.

Results

Of the 80 KAs, 6 cases (7.5 %) were found showed the pattern of regression (Fig. 1). Table 1 shows the details concerning the genders, ages, location and size of the lesions in these 6 cases.

Table 2 shows the details of all the SCCs that were studied, including the genders, ages, location of the lesions, stage and grade. All the cases were reviewed in order to confirm the diagnosis of SCC (Fig. 2). None of them was of the KA-type.

Tab. 1. Clinical data of the patients from whom keratoacanthomas in a regressing stage were studied.

Case	Gender	Age	Location of the lesion	Size of the lesion (cm)	CD30+ cells, pattern*	Percentage of CD30+ cells
1	Female	71	face	0.6	2	0.82
2	Male	68	shoulder	0.8	1	0.12
3	Female	83	left ear	2.5	1	0.19
4	Male	71	forehead	1	3	1.74
5	Female	64	upper lip	1.3	3	0.37
6	Female	92	chest	1	1 (**)	0.25

* The CD30 immunostaining pattern evaluated was as follows: 0=no CD30+ cells; 1=occasional CD30+ cells; 2=CD30+ cells which are more than occasional but still appear non-grouped; 3=CD30+ cells in groups of 3 or less cells each; 4=CD30+ cells in groups of more than 3 cells each.

Tab. 2. Details about the cases of SCC studied. *According to the Broder criteria.

Case	Gender	Age	Location	Grade*	Staging**	CD30 cells pattern***	Percentage of CD30+ cells****
1	Male	64	Nose	2	pT1b	4	1.87
2	Male	85	Left cheek	1	pT1b	4	1.74
3	Female	90	Forehead	1	pT1b	4	1.62
4	Female	82	Head	1	pT1c	4	1.87
5	Male	71	Trunk	1	pT1b	4	3.00
6	Male	78	Right Cheek	2	pT1a	4	1.12
7	Male	75	Abdomen	1	pT1b	4	2.00
8	Female	73	Concha		pT1b		
9						4	3.37
10	Female	48	Left hip	1	pT1a	4	1.87
11	Male	82	Right ear	2	pT1b	4	2.12
12	Male	84	Right ear	1	pT1c	4	1.50
13	Male	90	Head	2	pT1b	4	1.37
14	Male	80	Concha	1	pTX	4	1.37

** TNM classification. *** The CD30 immunostaining pattern evaluated was as follows: 0=no CD30+ cells; 1=occasional CD30+ cells; 2=CD30+ cells which are more than occasional but still appear non-grouped; 3=CD30+ cells in groups of 3 or less cells each; 4=CD30+ cells in groups of more than 3 cells each. **** The percentage was calculated according to the semiquantitative method used by Cepeda et al. (Reference number 2)

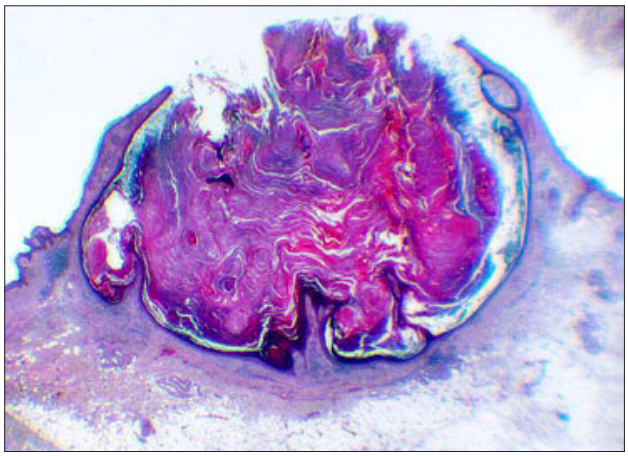

Fig. 1. Low power view of one of the KA that was studied (case 1).

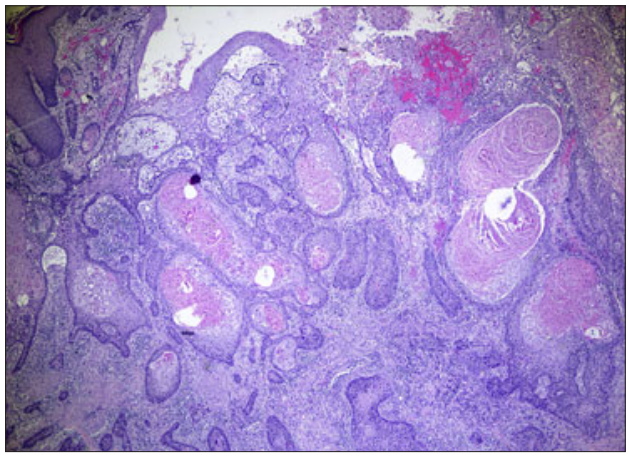

Fig. 2. One of the examples of SCC that was investigated (case 12).

Both tables also show the results of the evaluation of CD30+ cells population, in terms of the grading system, as well as the percentage. Figure 3 (top) shows an example of the immunostaining that we observed in KA with scattered cells in the inflammatory infiltrate. The immunoexpression of CD30 by the cells in the infiltrate was membranous alone, and a close detail of the cytologic morphology of the lymphocytes expressing the CD30 in KA showed that they were mostly non atypical cells with a small or inconspicuous nucleolus (Fig. 3 bottom).

The mean of the percentages of CD30+ cells in the infiltrate

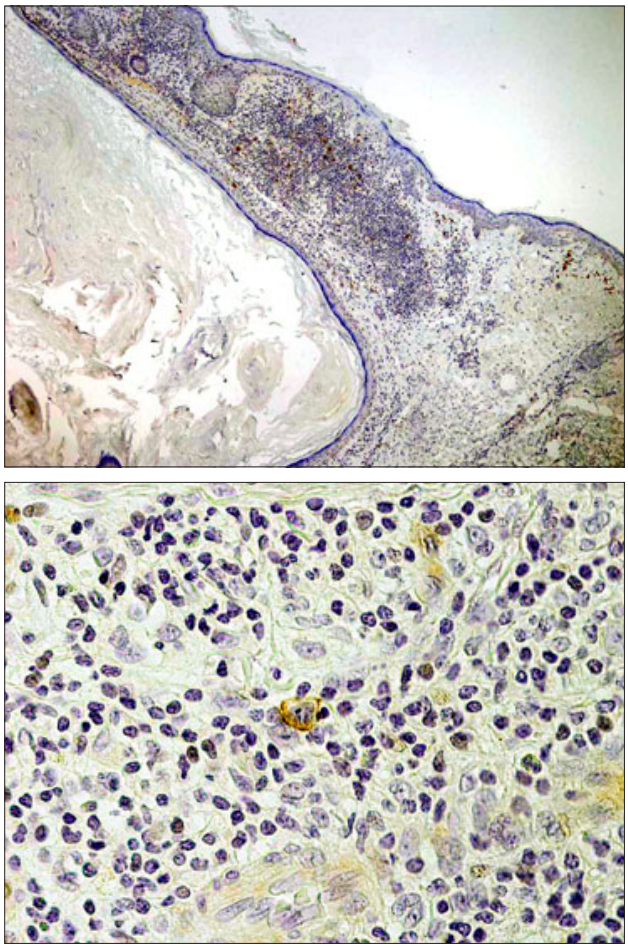

Fig. 3. Top: Case number 1 of the regressing KA showing an immunostaining for CD30 (X 4). Even at this low magnification the positive CD30 cells stand out clearly as scattered in the inflammatory infiltrate.

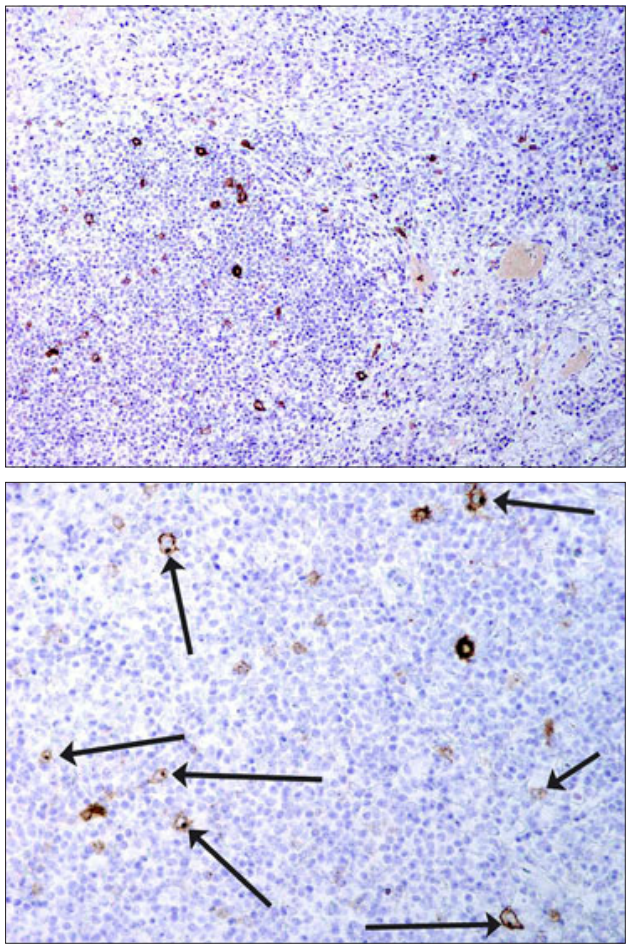

Fig. 4. Top: Case number 2 of the SCCs in which many CD30 positive cells can be seen in the infiltrate (top). With a higher magnification, it can be seen how many of the cells present a paranuclear immunostaining (bottom).

was 0.58 for the regressing KAs, while it was 1.77 For the SCCs. CD30 positive cells were not only more numerous in SCC cases, but also, the immunostaining pattern was different: in KA the CD30 immunostaining was membranous alone, while in SCC some cells with membranous plus paranuclear immunostaining were easily found (Fig. 4).

Discussion

In a previous report, we investigated the CD30+ cell population in keratoacanthoma (KA), with curious results (1). Nearly all the cases showed an inflammatory infiltrate with obvious CD30+ cells. It caught our attention that the case that showed the lowest percentage of CD30+ cells was a KA already in regression. This fact led us to the design of this current study and brought about one obvious conclusion: no matter what the role of these CD30+ cells is during the life of KA, they seem to disappear already during the process of KA regression. Nevertheless, they are present in invasive non-KA type SCC.

This latter fact leads us to hypothesize that there must be a breaking point in the life of KA, which determines the regression of the lesion through apoptosis. Even more important, if some conditions are not accomplished, KA could progress into deeply infiltrating and metastasizing squamous cell carcinoma (SCC). This approach conciliates the two opinions on KA being either a benign condition or a good prognosis-type of SCC.

The possible role of CD30+ lymphocytes in the regression of KA may in our opinion be of therapeutic use.

The idea of inducing an anti-tumor lymphocytic response is not new and it has been explored in medicine in the last decades with different techniques: they varied from the administration of interleukin-2 (5), of CD8 anti-tumoral monoclonal T-cells (6–8) or in the last years of imiquimod, for instance (9, 10). Some have even qualified the changes induced by imiquimod and the ones of spontaneous regression in KA as “identical” (11).

Some groups have investigated the nature of the inflammatory infiltrate during the treatment with imiquimod (12). Furthermore, some have even studied such an infiltrate before, dur-

ing and after the treatment with imiquimod of malignant lesions such as SCC (13). They all emphasize the increase in T-cells, dendritic cells (12) and cytotoxic cells in the infiltrate (14). But while for some groups the infiltrate is rich in CD4+ lymphocytes (15), with "a few" CD8+ cells, and CD68+ macrophages (11) others have found "an increased population of T-lymphocytes positive for CD3, CD4 and CD8" (13). While some only found scattered cytotoxic cells (11), others concluded that imiquimod induced "an enhanced cytotoxic T-cell-mediated immune response" (13). These apparent contradictions can probably be explained if one considers the dynamism of the inflammatory infiltrate, in which probably the number of cytotoxic CD8 increases during the evolution of the lesion.

In a way, this mimics what happens during the spontaneous regression of KA when CD8:CD4 cell ratio increases with the regression till it reaches its highest point with late regression (16). Nevertheless, it is obvious that cytotoxic cells do not have to be the only phenomenon determining the regression. This is proven by the fact that a high quantity of CD8+ T cells is not enough to induce tumor regression (17).

In this context, our findings could be contributing in a way. Although CD30 is accepted as an activation marker, its role is not totally understood.

It has been concluded that IL2-bearing "activated" lymphocytes, for instance, play a crucial role in spontaneous tumoral regression (18, 19), but paradoxically this activation and regression could be negatively related.

In a very stimulating report, Muta et al studied the effect of CD30 signals on effector functions of natural killers (NK) and T-cells (20). They concluded that CD30 suppresses the cytotoxic pathways of cytolytic lymphocytes. Results pointing to a similar conclusion were communicated by Jürgen Eberle, from the Charité-Uniersitätsmedizin of Berlin at the 4th International Symposium on Biology and Immunology of Cutaneous Lymphomas, held in Berlin in January 2008. Although these latter results have not been published yet, as far as we know, he communicated his observations on how CD30 decreases the sensitivity for CD95 activity, decreases the activity of the caspases 8 and 3, and increases the expression of FLIPL.

What happens with the cytotoxic infiltrate in KA, and more specifically in regressing KA? Granzime B expression, for instance, is significantly increased in KA in comparison to SCC (21). Moreover, the highest values of granzyme were found in regressing KA (22). These latter findings enhance the possibilities that CD30 could act as an inhibitor of the regression in KA: only when CD30 action starts being inhibited in some way, KA regresses, and when the regression is nearly complete, CD30 expression is virtually nil.

From this, two conclusions seem to emerge: first, that more attention should be paid to CD30+ cell population in order to completely understand the process of regression. Second, that an inhibition of CD30 activation might perhaps enhance the possibilities of tumoral regression.

Since imiquimod is not effective in 100 % of cases, and since some recurrences of SCC have been described after the drug had been used (10), it might also be interesting for instance, to correlate the recurrences with the effectiveness of imiquimod (or any other future drug) and CD30 lymphocytic expression.

CD30+ cells could even be suggested as a morphological control to make sure that a drug for local induction of anti-tumoral inflammatory infiltrate is working.

Nevertheless, we would not like to put all the stress on the inflammatory infiltrate as having the main role in the regression of KA. Confident as we were in previous publications on such a role (23), we must recognize the beautiful and original approach that has recently been presented by Kossard et al (24). In it, KA is considered as the well-differentiated type of SCC of infundibulocystic origin, and its spontaneous involution is linked not only to the inflammatory infiltrate that accompanies KA, but also to the tendency to involution that is naturally presented by the hair follicle.

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