Do All Epidermal Keratinocytes Contain Parathyroid Hormone Related Protein (PTHrP)?

To the Editor:

In our studies [1,2] and those of others [7,8] parathyroid-hormone-related protein (PTHrP) has been localized by immunohistochemistry to the spinous keratinocyte layer of normal skin. The work of Atillasoy et al (J Invest Dermatol 96:277, 1991) disputes this, reporting that PTHrP is present throughout the viable portion of the epidermis and in adnexal epithelial cells. They suggest that the polyclonal antisera we have used are not affinity purified and therefore contain antibodies to keratin that give rise to the pattern of staining we have reported. In support of this they quote a paper [6] as showing that "anti-keratin antibodies are known to contaminate many rabbit polyclonal antisera." That paper is misquoted and its title incorrectly cited. Gordon et al [6] routinely screened rabbits before any immunization, and found only one of their large colony to possess a spontaneous anti-intermediate filament antibody. In work over the last 4 years with eight rabbit antisera and four sheep antisera against PTHrP peptides, we have uniformly shown specific staining of the spinous keratinocyte layer with the immunoperoxidase method. If Atillasoy et al are correct, it implies that all of the sera we used contained spontaneous antibodies, which had persisted throughout our immunization program, to give us an identical false result in all cases. We should add that we have made clear the controls that we use, and we have demonstrated in the original publication of PTHrP immunohistochemistry [1] that affinity purification of the antiserum made no difference to the staining pattern. This has been confirmed with other of our antisera. The antisera have been extensively characterized by Western blot, ELISA, and blockade of biologic activity [1–5]. No adequate data were provided by Atillasoy et al on characterization of their antisera.

For many technical reasons it cannot be concluded that the pattern of staining we have observed is in any way related to keratin. Most anti-keratin antibodies must be used with frozen sections, or if paraffin-embedded tissue is used, trypsinization must be undertaken first, to allow penetration of the antibody. These were not done or required for PTHrP immunohistochemistry. We have previously found that the staining patterns for keratin and cytokeratin were totally different from that of PTHrP [1], and we have reproduced our results with several different antisera.

We agree that demonstration by immunohistochemistry of PTHrP in the spinous keratinocyte layer tells us nothing of synthesis or function. This will require in situ hybridization and other studies. All that we have suggested [2] based on our own data and that of others is that PTHrP could have a role in squamous differentiation. We did not claim, as alleged by Atillasoy et al, that the presence of PTHrP is a marker or a result of keratinocyte differentiation. Their report fails to question seriously the data indicating PTHrP localization in the spinous acanthocyte layer of skin.

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REPLY

Danks et al have taken umbrage with our demonstration that all viable epidermal keratinocytes, including basal keratinocytes, contain PTHrP. They do not, however, present any data to dispute this finding. We did not question the observation that PTHrP is present in differentiating keratinocytes; we only disputed the assertion that it is absent from undifferentiated keratinocytes.

Danks et al have stated that "detection of PTHrP . . . could provide a useful diagnostic marker for squamous differentiation" [1]. Other investigators have now demonstrated PTHrP [2,3] and its mRNA [4] in non-epithelial as well as epithelial tissues and in non-keratinizing as well as keratinizing epithelia. In contrast to the results reported by Danks et al, we observe staining in basal cell carcinomas as well as in squamous cell carcinomas (unpublished).

We never stated that the staining observed by Danks was due to an antikeratin antibody. We stated that antibodies that gave the suprabasal pattern of staining in *our* crude rabbit antiserum to PTHrP could not be adsorbed to a PTHrP affinity column, but could be eliminated by preincubation with keratin. We regret the incorrect title to citation #27 and thank Danks et al for calling it to our attention. Nonetheless, our concern stands: antikeratin antibodies in rabbits can arise spontaneously and, more frequently, after sham immunization.

We describe our antibody (R6) in Table I and in [8]. Ab-1 and Ab-2 are commercially available and have been described in a recent publication [2].

We think that our disagreement with Danks et al may be largely a signal-to-noise issue. In some tissues we have noticed accentuation of staining in one or another of the epidermal layers. For example, in psoriasis, in hair follicles, and in some squamous carcinomas, we often notice accentuation in the suprabasal spinous layers. However, in cultured epithelia we often notice accentuation in the basal layer. Dilution of the antisera in these cases could then cause loss of stain-

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