RNA in situ hybridization permits evaluation of treatment relevant cytokine targets in routine skin biopsies of atopic dermatitis and psoriasis

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Background:
Eczema is a clinically and molecularly heterogeneous group of diseases that includes childhood and adult onset atopic dermatitis, nummular dermatitis, pompholyx, and others. While most cases of eczema show predominantly T helper 2 (Th2) polarization, some may also have evidence of Th1, Th17, and/or Th22 activation. Treatment of atopic dermatitis and other forms of eczema has been revolutionized by the introduction of dupilumab, which blocks the activity of both IL-4 and IL-13. However, while approximately 30% of patients with AD achieve complete or nearly complete clearance of their skin with dupilumab, approximately 70% do not. Many groups have proposed that this observation may relate to underlying molecular immunologic heterogeneity. In particular, patients with concomitant activation of Th1 (eg. IL-12), Th17 (e.g. IL-17A/F), and/or Th22 (e.g. IL-22) may respond sub optimally to dupilumab.

Detection of these different signals in routine biopsies for purposes of diagnosis and treatment selection has been difficult due to a lack of reliable immunohistochemical markers. Presently, such assays are only possible in research labs using RNA-based approaches and require specialized tissue processing, equipment, and expertise and are also time consuming and expensive.

Objective:
To determine whether a chromogen-based RNA in situ hybridization method, which has a work flow that is comparable to immunohistochemistry, could be used to accurately detect Th2 (IL-4, IL-13), Th17 (IL-17A, IL-23A), or Th1 (IL-12B) cytokine expression in routine formalin fixed, paraffin embedded biopsies specimens. These genes were selected as the proteins they encode are the targets of currently available targeted therapies in eczema and psoriasis.

Methods:
A retrospective histologic case series was assembled with cases of eczema (atopic dermatitis) (n=26), psoriasis (n=20), and normal skin (n=10) for initial evaluation of the approach and comparison of cytokine expression patterns. RNA in situ hybridization for *NOS2*, *IL4*, *IL12B*, *IL13*, *IL17A*, and *IL23A* was performed and quantified in each case. Patterns in atopic dermatitis were compared to those in psoriasis to validate the approach. Next, a series of 20 cases with overlapping clinical and histologic features (“psoriasiform dermatitis”) (n=20) was assembled. We asked if the approach could be used to molecularly classify these morphologically ambiguous cases.

Results:

We found that *NOS2*, *IL17A* and *IL13* staining patterns most readily distinguished cases of psoriasis from atopic dermatitis. 85% of atopic dermatitis cases showed staining for *IL13*. No cases of normal skin and only 18% of psoriasis cases had *IL13* staining, which was focal and weak. 100% of the psoriasis cases showed *NOS2* and *IL17A* expression. *IL17A* was rarely observed in the atopic dermatitis cases. *IL13* and *IL17A* staining was observed predominantly in lymphocytes within the epidermis. *IL4* staining was only occasionally observed in atopic dermatitis, but not normal skin nor psoriasis. *IL12B* and *IL23A* staining predominated in psoriasis, but was only occasionally present in atopic dermatitis. In the cases with intermediate features ("psoriasiform dermatitis") there was significant molecular heterogeneity; some cases were psoriasis-like (predominantly *IL17A* positive), others eczema-like (predominantly *IL13* positive), and yet others had either an overlapping (both *IL17A* and *IL13* positive).

Conclusions:

RNA in situ hybridization for cytokines appears to be highly specific given the reproducible and expected patterns observed in cases of atopic dermatitis and psoriasis. The approach appears to work much more robustly for cytokine detection than immunohistochemistry, which typically has high background and/or non-specific staining. The approach has the disadvantage of detecting RNA as opposed to protein. We believe there are many potential applications of this approach for diagnosis, treatment selection, and clinical trial design in eczema. In particular, personalized treatment selection is conceivably possible with this approach. Further study to evaluate this possibility is underway.