Letters to the Editor

Citrullinated filaggrin is decreased in oral keratinocytes in rheumatoid arthritis

Sirs,

Anti-citrullinated peptide autoantibodies (ACPA) are specific for rheumatoid arthritis (RA). There is local ACPA production in the synovial tissue and citrullinated peptides show peculiar interaction with the HLA-DR B1 shared epitope (1, 2). In RA synovium, peptidylarginine deiminases (PADI) 2 and 4 mediate conversion of arginine into citrulline residues in condensed fibrin and intracellular vimentin, respectively (3, 4). Citrullinated fibrin is also found in other synovial inflammatory conditions (5). Oral keratinocytes (OK) also present citrullination of the protein filaggrin in perinuclear granules. Although filaggrin is not present in the synovium, citrullinated filaggrin might act as a booster in enhancing ACPA expansion in patients already primed by synovium citrullinated proteins. In fact, 48-91% of RA patients have autoantibodies reactive to OK citrullinated filaggrin as documented by the anti-perinuclear factor test (APF) (6). The presence of citrullinated epitopes in OK is documented in 10-23% of healthy subjects (7, 8). We studied the content of citrullinated epitopes in OK in patients with RA (APF-positive and APF-negative) and in patients with other autoimmune diseases (OAD) as well as its possible associations with RA clinical parameters.

Serum and OKs were obtained from 78 RA patients, 62 non-RA patients, and 51 non-autoimmune individuals (NAI). RA patients had the modified Sharp's index (9) determined. OKs were washed twice in 0.15M phosphate buffered saline pH 7.4 (PBS), briefly exposed to 0.5% Triton X-100, and re-suspended in PBS to a final concentration of 10^5cells/mm^3. OKs were deposited onto glass slides, dried at room temperature and kept frozen at –70ºC for up to 2 weeks. Filaggrin immunodetection in OKs was determined by standard indirect immunofluorescence using a pool of APF-reactive human sera diluted 1/10 and appropriate fluorescein conjugate. Immunodetection was scored as the frequency of OKs with citrulline-positive perinuclear granules (citrulline-positive OKs) among 200 consecutively examined OKs. APF activity in the serum was determined by the standard technique at 1/10 screening dilution (10). Reading was performed at X400 magnification by two independent blinded observers.

The frequency of citrulline-positive OKs was not correlated with age, ethnicity and gender in RA, non-RA, NAI, and in all individuals altogether (data not shown). The frequency of citrulline-positive OKs was significantly reduced in RA as compared to NAI (Fig. 1). The frequency of circulating APF did not differ in RA patients with (54%) and those without citrulline-positive OKs (64%). APF titer did not correlate with the frequency of citrulline-positive OKs in RA patients (r = 0.032). The frequency of citrulline-positive OKs in RA patients showed no association with the presence of extra-articular manifestations (p = 0.142), disease duration (p = 0.924), age of disease onset (p = 0.952), number of swollen joints (p = 0.148), functional class (p = 0,294), presence of joint deformities (p = 0.71), morning stiffness (p = 0.496), and Sharp's index (r = 0.091). Filaggrin immunodetection in OKs was not associated with the use of prednisone (p = 0.622), methotrexate (p = 0.852), chloroquine (p = 0.988), and sulfasalazine (p = 0.165).

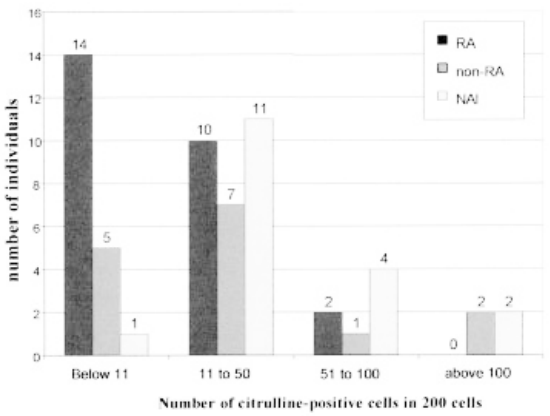

Fig. 1. Distribution of patients with rheumatoid arthritis (RA), patients with other autoimmune rheumatic diseases (non-RA) and non-autoimmune individuals (NAI), according to the semi-quantitative immunodetection of citrullinated epitopes in oral keratinocytes. The frequency of cells carrying citrulline-positive perinuclear granules (citrulline-positive OKs) was scored among 200 sequentially examined oral keratinocytes.

Foot note: Anova = 4.775; p = 0.012. Tukey: RA x NAI (p = 0.009); non-RA x NAI (p = 0.273); RA x non-RA (p = 0.432).

The under-expression of filaggrin in OKs in RA is an original finding and indicates a reduced citrullinating activity in the oral epithelium of these patients. The absence of association with circulating APF or with several clinical parameters of the disease suggests that the citrullinating activity in OKs does not contribute to the humoral response to citrullinated epitopes or to the pathophysiology of RA. It also harmonizes with previous reports that antigens other than filaggrin are the primary auto-immunogens involved in eliciting and maintaining the ACPA. In fact, it was recently observed that the presence of synovial intracellular citrullinated proteins was associated with the titer of circulating ACPA in RA patients (11). The preliminary finding hereby presented of no association between the under-expression of filaggrin in OKs in RA and the ongoing therapy shall be formally evaluated by prospective studies.

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