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CHRONIC KIDNEY DISEASE. PATHOPHYSIOLOGY, PROGRESSION & RISK FACTORS - 1

FP304 ABERRANT APRIL EXPRESSION IN TONSILLAR GERMINAL CENTER B CELLS IN IGA NEPHROPATHY PATIENTS

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Introduction and Aims: A proliferation-inducing ligand (APRIL) is a critical mediator for antibody-producing plasma cell survival. Although recent clinical and basic studies suggest that APRIL may be involved in the pathogenesis of IgA nephropathy (IgAN), its role in IgAN remains unclear. Since immunological disorders in mucosal immunity are recently discussed in the pathogenesis, we examined the clinical impact of mucosal APRIL expression in IgAN patients and assessed underlying mechanisms.
Methods: In addition to clinical background before and after tonsillectomy, the expressions of APRIL and its receptors (TACI; transmembrane activator and calcium modulator cyclophilin ligand interactor, BCMA; B-cell maturation antigen) in the tonsils from IgAN patients (n=56) and control patients (chronic tonsillitis without renal diseases, n=12) were evaluated by real-time PCR, immunohistochemistry (IHC) and flow cytometric analysis (FCM). For IHC and FCM with tonsillar samples of IgAN and control patients, we used two anti-APRIL antibodies specifically recognizing APRIL-producing cells and secreted form of APRIL (Stalk-1 and Aprily-2, respectively) and evaluated correlation between these expression pattern and clinical phenotypes. We examined the presence and patterns of APRIL genetic variants mRNAs in tonsillar B cells from IgAN patients and controls. The expression levels of APRIL in some B cell lines under TLR9 stimulation with CpG-ODN were also evaluated by real-time PCR.
Results: Tonsillar transcriptional levels of APRIL and its receptors in IgAN patients were significantly higher than those in control patients ($P<0.05$). IHC revealed that Stalk-1+ cells in IgAN patients were detected not only in the epithelial areas but also germinal centers (GC) much more than those in controls. Percentage of Stalk-1+ GC (27.4±21.3%) in IgAN patients was significantly higher than that in controls (7.2 ±6.81%, $P=0.0005$), and correlated with amount of proteinuria ($P=0.0017$) and treatment responses, such as decrease of proteinuria ($P=0.0003$). Furthermore, percentage of Stalk-1+ GC was correlated with the serum levels of IgG-IgA immune complex (IC) in IgAN patients ($P=0.0304$), but not the serum levels of galactose-deficient IgA1 (Gd-IgA1). Furthermore, IHC revealed that majority of Stalk-1+ cells in the tonsillar GC expressed CD19. FCM showed that the percentage of Stalk-1+ GC B cells in total tonsillar GC B cells was significantly higher in IgAN patients than controls ($P=0.0314$). Interestingly, some IgAN patients showed co-localization of Stalk-1+ and Aprily-2+ B cells in GC. Genetic analysis with tonsillar B cells indicates the increase of genetic variants lacking APRIL cleavage sites in IgAN patients. Furthermore, in vitro experiments further showed that TLR9 stimulation increased APRIL expression in B cell lines.
Conclusions: APRIL+ GC B cells in tonsils may determine the disease activity of IgAN patients, presumably via the production of anti-glycan or poly-reactive antibody prior to IC formation with Gd-IgA1. Genetic variation of APRIL and aberrant TLR9 activation in tonsillar B cells may be involved in underlying mechanisms of the tonsillar APRIL overexpression in IgAN patients.