Restoration of Overexpressed Variable Heavy Chain 2 Transcripts with Tumor Necrosis Factor Inhibitors in Ankylosing Spondylitis

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To the Editor:

Ankylosing spondylitis (AS) is a chronic inflammatory disorder characterized by progressive and destructive arthritis of the spine and pelvis. B cells are involved in the pathogenesis of autoimmune diseases through antibody production, cytokine release, and antibody presentation to autoreactive T cells. In AS, the role of B cells in the pathogenesis is still incompletely understood. It has been hypothesized that the production of high affinity monoreactive autoantibodies in autoimmune disease could arise from intrinsic abnormalities in the generation of immunoglobulin genes. Immunoglobulin gene usage can be regarded as an important factor of pathogenesis of autoimmune diseases. Investigation of variable heavy chain (VH) gene usage is important for determining whether usage of particular gene families is distorted. Several studies have investigated the VH gene usage in various autoimmune diseases, including systemic lupus erythematosus, myasthenia gravis, rheumatoid arthritis (RA), Sjögren syndrome, and AS compared to healthy controls. A previous study of VH gene usage in patients with AS reported overexpression and rearrangement of the VH2 gene. However, it has not been clear whether this phenomenon was specific for AS or is a general feature in other rheumatic diseases. To find out whether VH2 overexpression was a phenomenon specific for AS, we investigated VH2 gene usage in larger scale samples of AS, with patients with RA as disease controls. Further, we analyzed the correlation of VH2 expression with clinical characteristics of AS.

To analyze the Ig VH gene usage, peripheral blood mononuclear cells (PBMC) were collected from 50 healthy controls, 46 patients with RA, and 47 patients with AS. Amplification of 0.5 μl of cDNA was performed using SYBR Green Realtime PCR Master Mix (TOYOBO). Quantification of relative gene expression was calculated by the comparative Ct method (2−ΔΔCt), as described by the manufacturer. Data were normalized to human acidic ribosomal protein (HuPo) mRNA levels.

The Q-PCR results showed that VH2 gene expression levels were exclusively higher in patients with AS, when compared to patients with RA or healthy controls. Significant difference was shown in the expression level of VH2 gene to HuPo (1.31 ± 1.03, 1.41 ± 1.29, and 7.70 ± 6.53, respectively; p < 0.0001; Figure 1). These results were consistent with those of the previous study.

In a subgroup analysis of patients with AS, peripheral arthritis and uveitis was not associated with VH2 expression (p = 0.328; p = 0.540). However, treatments with tumor necrosis factor-α (TNF-α) inhibitors were associated with downregulation of VH2 expression. VH2 expression level was significantly lower in the TNF-α-treated group than in the nonbiologics group (5.00 ± 4.79, 9.53 ± 6.98, respectively; p = 0.012). Infliximab reduced VH2 expression level the most significantly (1.88 ± 1.79, p = 0.002), followed by etanercept (4.32 ± 5.03, p = 0.014; Figure 2).

The influence of TNF-α inhibitor on VH2 expression had not been reported previously, to our knowledge. Further investigations are required to confirm that TNF-α inhibitor treatments affect VH2 expression level, to verify the mechanism by which TNF-α inhibitor restores VH2 expression, and to investigate whether abnormal VH2 activations play a role in the pathogenesis of AS.

Our study revealed that VH2 gene is overexpressed in patients with AS and decreased with TNF-α inhibitor treatment.

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Total RNA was isolated from PBMC by RNeasy mini kit (Qiagen), and cDNA was synthesized by Maxime RT PreMix [Oligo-(dT)-15 primer] kit (Intron Biotechnology), following manufacturers’ instruction. Q-PCR was performed on 50 controls, 46 patients with RA, and 47 patients with AS. Amplification of 0.5 μl of cDNA was performed using SYBR Green Realtime PCR Master Mix (TOYOBO). Quantification of relative gene expression was calculated by the comparative Ct method (2−ΔΔCt), as described by the manufacturer. Data were normalized to human acidic ribosomal protein (HuPo) mRNA levels.

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Figure 1. Variable heavy chain (VH2) transcript levels in healthy control, rheumatoid arthritis (RA), and ankylosing spondylitis (AS) groups. Quantitative real-time PCR was performed with the VH2 primer set. Patients with AS exhibited significantly higher expression of VH2 compared to healthy subjects (n = 50) and patients with RA (n = 46).
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