

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

To the Editor:

Ankylosing spondylitis (AS) is a chronic inflammatory disorder characterized by progressive and destructive arthritis of the spine and pelvis¹. B cells are involved in the pathogenesis of autoimmune diseases through antibody production, cytokine release, and antibody presentation to auto-reactive T cells. In AS, the role of B cells in the pathogenesis is still incompletely understood². 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 genes³. 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 erythematosus⁴, myasthenia gravis⁵, rheumatoid arthritis (RA)⁶, Sjögren syndrome⁷, and AS^{8,9} compared to healthy controls. A previous study of VH gene usage in patients with AS reported overexpression and rearrangement of the VH2 gene⁸. 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, who had visited the Eulji University Hospital and the Hospital for Rheumatic Disease, Hanyang University, in Korea. Patients with RA satisfied the American College of Rheumatology 1987 classification criteria for the diagnosis of RA. Patients with AS who fulfilled the modified New York classification criteria were selected for our study. Patients with RA or AS were enrolled consecutively for the study. The study protocol was approved by the institutional review board at Hanyang

University Hospital and Eulji University Hospital. All participants provided written informed consent prior to enrollment and data collection.

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 μ l of cDNA was performed using SYBR Green Realtime PCR Master Mix (TOYOBO). Quantification of relative gene expression was calculated by the comparative Ct method ($2^{-\Delta\Delta Ct}$), 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 study⁸.

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 VH usage 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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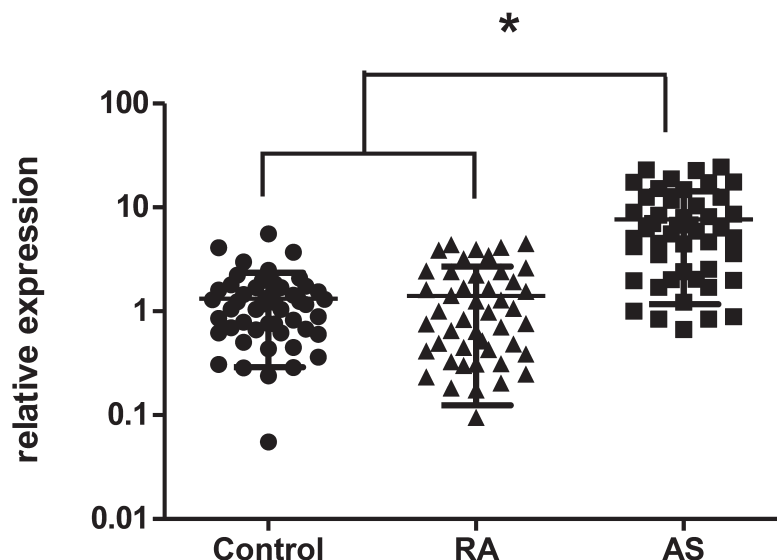


Figure 1. Variable heavy chain (VH)2 transcript levels in healthy control, rheumatoid arthritis (RA), and ankylosing spondylitis (AS) groups. Quantitative real-time PCR was performed with the VH2 primer set. Patients with AS exhibited significantly higher expression of VH2 compared to healthy subjects ($n = 50$) and patients with RA ($n = 46$).

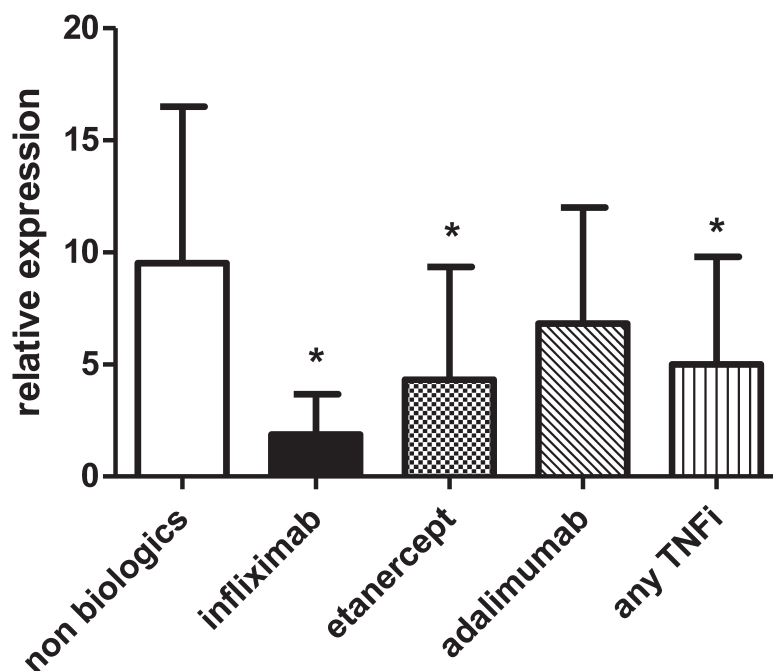


Figure 2. Association of variable heavy chain (VH)2 expression with tumor necrosis factor (TNF) inhibitors. Statistical significance test was done by Mann-Whitney U test. Infliximab, etanercept, and anti-TNF-treated groups demonstrated significantly lower expression of VH2 compared to the nonbiologic group.

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REFERENCES

- Pang WS, Davis JC. Clinical aspects of ankylosing spondylitis. In: Weisman MH, Reveille JD, Van der Heijde D, eds. Ankylosing spondylitis and the spondyloarthropathies, 1st ed. Philadelphia: Mosby; 2006:65-74.
- Baeten D, Kruithof E, Breban M, Tak PP. Spondylarthritis in the absence of B lymphocytes. 2008;58:730-3.
- Foreman AL, Van de Water J, Gougeon ML, Gershwin ME. B cells in autoimmune diseases: insights from analyses of immunoglobulin variable (IgV) gene usage. Autoimmun Rev 2007;6:387-401.
- Isenberg D, Spellerberg M, Williams W, Griffiths M, Stevenson F. Identification of 9G4 idiotope in systemic lupus erythematosus. Br J Rheumatol 1993;32:876-82.
- Sims GP, Shiono H, Willcox N, Stott DI. Somatic hypermutation and selection of B cells in thymic germinal centers responding to acetylcholine receptor in myasthenia gravis. J Immunol 2001;167:1935-44.
- Robbins DL, Kenny TP, Coloma MJ, Gavilondo-Cowley JV, Soto-Gil RW, Ghen PP, et al. Serologic and molecular characterization of human monoclonal rheumatoid factor derived from rheumatoid synovial cells. Arthritis Rheum 1990;33:1185-95.
- Dorner T, Hansen A, Jacobi A, Lipsky PE. Immunoglobulin repertoire analysis provides new insights into the immunopathogenesis of Sjögren's syndrome. Autoimmun Rev 2002;1:119-24.
- Kim YJ, Kim NY, Lee MK, Choi HJ, Baek HJ, Nam CH. Overexpression and unique rearrangement of VH2 transcripts in immunoglobulin variable heavy chain genes in ankylosing spondylitis patients. Exp Mol Med 2010;42:319-26.
- Voswinkel J, Weisgerber K, Pfreundschuh M, Gause A. B lymphocyte involvement in ankylosing spondylitis: the heavy chain variable segment gene repertoire of B lymphocytes from germinal center-like foci in the synovial membrane indicates antigen selection. Arthritis Res 2001;3:189-95.

J Rheumatol 2014;41:3; doi:10.3899/jrheum.131195