Investigations of Connective Tissue Diseases at the Institution for Environment and Gender Specific Medicine

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Introduction

The Institution for Environment and Gender-specific Medicine of Juntendo University was established in 2002 with the support of the Ministry of Education. At the institution, the relationship between genetics and environmental factors and/or the gender-specific mechanisms underlying the development of disorders in several fields, such as dermatology, rheumatology, gynecology, and neurology have been studied. In this report, we describe several representative studies of disorders in rheumatology, especially rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), performed at the institution, as well as our research background.

Research of RA

RA is a chronic inflammatory disorder that ultimately leads to the destruction of the joint architecture. Although the precise pathogenic mechanisms leading to the development of RA are not fully understood, proinflammatory cytokines, such as tumor necrosis factor- α (TNF- α), interleu-

kin (IL)-1 and IL-6 play pivotal roles in the induction of RA.

Recent biologic-based treatments for RA patients have shown remarkable efficacy, however, side effects of these medications, including severe infections, are sometimes observed¹⁾. As such, the development of more effective and safer therapeutic drugs are desired. To investigate the mechanisms underlying the effects of anti-TNF- α antibodies (e.g. infliximab: a representative biological preparations for RA) in RA patients, we examined the changes in the serum levels of protein biomarkers in infliximab-treated patients with RA, via a novel approach to proteomic research using a specially developed serum/plasma protein separation device (a hollow fiber membrane-based device; Toray, Tokyo, Japan)²⁾.

Among the proteins examined in the study, we identified connective tissue growth factor (CTGF) as a novel effector protein in the pathogenesis of RA.

CTGF is a bioactive cytokine in the CCN protein family consisting of four different modules³⁾. We examined the roles of CTGF in the development of RA. The serum levels of CTGF were higher in RA

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Figure-1 Inhibitory effects of infliximab on the production of CTGF induced by TNF- α from human synovial fibroblast cell line (A; MH7A), and human chondrocytes cell line (B; OUMS-27)

patients than in normal controls, and the concentration was decreased after treatments for RA patients. Immunofluorescence staining revealed that CTGF is produced by synovial cells in RA patients (data not shown).

As shown in Figure-1, TNF- α can induce the production of CTGF by a human synovial fibroblast cell line (MH7A), and infliximab can inhibit this CTGF productions (Figure-1A). These phenomena are not observed in a human chondrocytes cell line (OUMS-27) (Figure-1B). Furthermore, CTGF can increase not only the number but also the morphological size of differentiated osteoclasts in combination with macrophage colony stimulating factor (M-CSF)/receptor activator of NF- κ B ligand (RANKL) (Figure-2A,B). Bone resorption mediated by osteoclasts is also enhanced in the presence of CTGF (Figure-2C). Based on these results, we concluded that infliximab can inhibit the production of CTGF by synovial cells activated by TNF- α and suppress subsequent CTGF-mediated osteoclasts differentiation and bone resorption (Figure-3).

We next examined the effects of anti-CTGF antibodies (binding to module 1 on CTGF) on the development of RA in a mouse model of collagen-induced arthritis (CIA). As shown in Figure-4,

this antibody was able to inhibit the exacerbation of arthralgia strongly in RA model mice, and improvements in the pathological findings were also observed⁴⁾. Our antibody has very strong neutralizing activities against CTGF⁵⁾, suggesting that this CTGF antibody may be extremely effective against human RA without inducing side effects, such as severe infections.

Investigation of SLE

SLE is a representative autoimmune disease in humans and a chronic inflammatory disease that affects multiple organs. It is characterized by the activation of dendritic cells, macrophage and lymphocytes, which results in the production of several high-affinity autoantibodies and formation of immune complexes.

To investigate the gene regulations associated with the disease activity of SLE, we examined the fluctuation in the gene expressions in peripheral lymphocytes before and after treatment, using microarray analyses⁶⁾. Figure-5A shows the results of canonical pathway analyses of genes displaying significant differences in expression between the active and inactive phases of SLE. The interferon

Figure-2 Roles of CTGF in the osteoclasts differentiation (A, B) and bone resorption (C)

Figure-3 Possible effects of infliximab on the development of RA

Figure-4 Effects of anti-CTGF antibodies on the development of RA in model mice (CIA)

(IFN) signaling pathway was most frequently detected, and its map is shown in Figure-5B.

Genes displaying significantly increased expression in the active phase of SLE are shown in red, with increasing intensity corresponding to increasing differences in the gene expression in the active/inactive phases. Genes showing a significantly increased expression in the active phase of SLE or that were suggested to play an important role in pathway regulation in Figure-5B were analyzed by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) (Figure-5C). These genes included the IFN regulating factor (IRF)-related genes IFI35 and, IFIMT1, the Janus kinase (JAK) genes (JAK1 and JAK2), and signal transducer and activator of transcription (STAT) signal genes (STAT1 and STAT2).

These data indicated that JAK inhibitors were able to inhibit IFN-mediated gene expression, thereby potentially improving the SLE disease activity. To confirm this point, we orally administered a JAK inhibitor (Tofacitinib; TOFA; Pfizer, New York City, NY, USA) to a mouse models of SLE (NZB/NZWf1; B/Wf1) and evaluated its effects. As shown in Figure-6A, TOFA was able to suppress proteinuria (a) and the anti-DNA antibody titer (b), and reduce the weight of the spleen (c). The pathological findings of SLE-related nephropathy were improved by TOFA administration (Figure-6B). These effects were further enhanced when TOFA was administered in combi-

nation with a steroid (dexamethasone) (Figure-6). Thus, TOFA may be useful as a new therapeutic agent for SLE⁷⁾.

Our hypothesis regarding the developments of SLE is outlined in Figure-7 based on several of our SLE investigations^{8) 9)}. The etiology of SLE seems to comprise both genetic and environmental factors. Certain environmental factors, such as infection, stress, and physicochemical elements, may influence the epigenetics, such as DNA-methylation, via DNA-methyltransferase 1 (DNMT-1; a DNA methylation-regulating enzyme). These events can promote gene transcription/translation, thereby inducing the hyper production of IFN and B cell-activating factor belonging to the tumor necrosis factor family (BAFF), which are major cytokines associated with the SLE disease activity. Some genetic elements may also be related to the promotion of gene transcription/translations.

The hyper production of such cytokines may contribute to the production of autoantibodies to self (internal)-antigens, which are released from cells by NETosis (a type of cell death in neutrophils). We therefore believe that the hyper-responsiveness of immune responses mediated by epigenetics may play an important role in the development of SLE. In fact, we previously reported that the DNMT-1 levels were significantly decreased in patients with SLE compared with normal controls¹⁰⁾.

Figure-5 Fluctuations of gene expressions in peripheral CD3 positive lymphocytes of SLE patients before and after treatments A: Canonical pathway analyses, B: IFN signaling pathway, C: qRT-PCR analyses (closed column; active phase, open column; inactive phase of SLE).

Other studies on connective tissue diseases

In addition to the studies mentioned above, we are performing our own studies on connective tissue diseases as follows: 1) A study on the fluctuation of serum microRNA levels by treatment for diseases such as polymyositis, RA, and SLE; 2) A study on the effects of nutrition on the levels of lipids (such as omega 3 and 6) and their association with RA onset; and 3) A study on the effects of

anti-CTGF antibodies on the development of scleroderma.

These studies are aimed at determining the etiology of the above-mentioned disorders, leading to the development of new therapeutic drugs.

Background of our research

The study of philosophy was developed in ancient Greece. Science, which was originally a field of

Figure-6 Effects of JAK inhibitor (TOFA) on the development of SLE in model mice (B/WF1)
A: Proteinuria, anti-DNA antibody titer, weight of spleen. B: Pathology of nephritis.

Figure-7 Hypothesis regarding the developments of SLE

philosophy (natural philosophy), was separated from philosophy and came to be called “nature science” by the UK Science Promotion Society in 1834 (Figure-8). From the 19th century to the 20th century, the discovery of pathogenic microorganisms as external factors increased the importance of etiology research in medical science (translational

research). In 1953, the discovery of the DNA double helix structure further increased the importance of the genetic components as internal factors causing disease. The causes of many diseases, including collagen diseases, seems to be based on the integration of such external (infection, nutrition, stress, physicochemical factors, etc.) and internal

Figure-8 Historical relationship among philosophy, science and technology

(genetic) factors. The importance of the regulatory system of gene expressions has recently been recognized, termed epigenetics. However, epigenetics are susceptible to environmental factors, such as infection and stress, and part of this gene control system is passed on to offspring.

Given the findings from medical studies throughout history, with the aim of establishing new diagnostic methods and treatments for connective tissue diseases, we have drafted our study protocols with close attention to the following points;

- 1) Perform research to determine disease etiology, including environment and genetic elements and epigenetics aspects.
- 2) Etiology is complex. Therefore, to clarify disease etiologies, it is important to search for regulators of disease activity. For that reason, we must comprehensively grasp fluctuations in genes and proteins before and after treatment and identify those elements suggested to be associated with the etiology.
- 3) Incorporate the latest science and technology into research, such as bioinformatics.

Technology developed rapidly during the Industrial Revolution. While science has always developed in close concert with technology, the role of philosophy in science has shown a relative decline over the years (Figure-8). William Nelson Joy, a renowned computer scientist, said that powerful technologies of the 21st century, such as robotics engineering, genetic engineering, and nanotechnol-

ogy, would be used for arms race expansion competitions and come to threaten the existence of human beings; he therefore insisted that such technologies be abandoned in the essay, "Why the future does not need us." However, it is clear that recent developments in science and technology have made great contributions to the well-being of mankind. For instance, RA, which was once an incurable disease, can now be completely cured, and new essential treatments rooted in the pathogenesis of SLE have also been developed. In the past, philosophy was at the center of liberal arts education (academics seeking human freedom). In modern times, new approaches to philosophy and liberal arts may be needed in order to avoid invoking a humanitarian crisis through our scientific efforts, as William Nelson Joy feared.

Many young doctors are expected to contribute to patients' future well-being through their research as well as clinical efforts.

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