We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the
TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Application of Novel Quantitative Proteomic Technologies to Identify New Serological Biomarkers in Autoimmune Diseases

Soyoung Lee, Satoshi Serada, Minoru Fujimoto and Tetsuji Naka
National Institute of Biomedical Innovation, Laboratory of Immune signal
Japan

1. Introduction

Autoimmune diseases comprise a wide variety of systemic or organ-specific inflammatory diseases, characterized by aberrant activation of immune cells that target self tissues due to misrecognizing tissue-derived proteins as foreign antigens (Hueber and Robinson, 2006; Prince, 2005). The prevalence of autoimmune diseases is approximately 2,000 ~ 3,000 per 100,000, although the prevalence varies depending on the diseases, ethnic groups and regions (Prieto and Grau, 2010). The etiology and exact pathogenesis of autoimmune diseases remain poorly understood. However, both genetic factors and environmental triggers are profoundly involved in the pathogenesis of autoimmune diseases. Notably, clinical manifestations of autoimmune disease may be different among patients, even though they have the same diagnosis, depending on the affected organs of each patient. Therefore, careful evaluation of the clinical manifestations combined with the examination of laboratory tests is required for proper diagnosis of autoimmune diseases and subsequent monitoring of the disease activity during therapy. In addition, therapeutic choices for these diseases have been limited so far and conventional therapeutics include non-steroidal anti-inflammatory drugs (NSAID), glucocorticoids, cytotoxic drugs and disease modifying anti rheumatoid drugs (DMARDs). For these reasons, autoimmune diseases have been considered to be intractable and the goal of the treatment is to control disease activity rather than to achieve remission or cure.

Recently, however, the advent of biological agents has led to the marked improvement in the treatment of rheumatoid arthritis (RA) and other inflammatory autoimmune diseases. These agents greatly contribute to improve health-related quality or daily life of patients with autoimmune diseases (Han et al., 2007; Keystone et al., 2008; Laas et al., 2009; Strand and Singh, 2007). Nevertheless, biological agents are not effective for all patients with autoimmune diseases and current biomarkers are not helpful to select an effective biological agent for individual patients. In addition, conventional inflammatory biomarkers are often inadequate to evaluate the disease activity in patients treated with biological agents. Thus, there is a growing need for the development of new biomarkers that can predict individual treatment response before starting biological therapy and evaluate the disease activity and therapeutic efficacy during therapy. In this chapter, we first outline the clinical usage and

current understanding of biological agents for the treatment with autoimmune diseases and then describe our attempt to identify new biomarkers in autoimmune diseases by taking advantage of a new proteomic approach.

2. Biological agents for the treatment of autoimmune diseases

2.1 Biological agents for autoimmune therapy in clinics

The immune response is a highly coordinated process and involves complex interactions of diverse molecules including cytokines and various cell types such as lymphocytes (Figure 1). Dysregulation in immune response such as overproduction of cytokines and aberrant activation of immune cells is implicated in autoimmune disorders. Therefore, these molecules and/or cells involved in immune response have been targeted to develop therapies in autoimmune disorders (Figure 1).
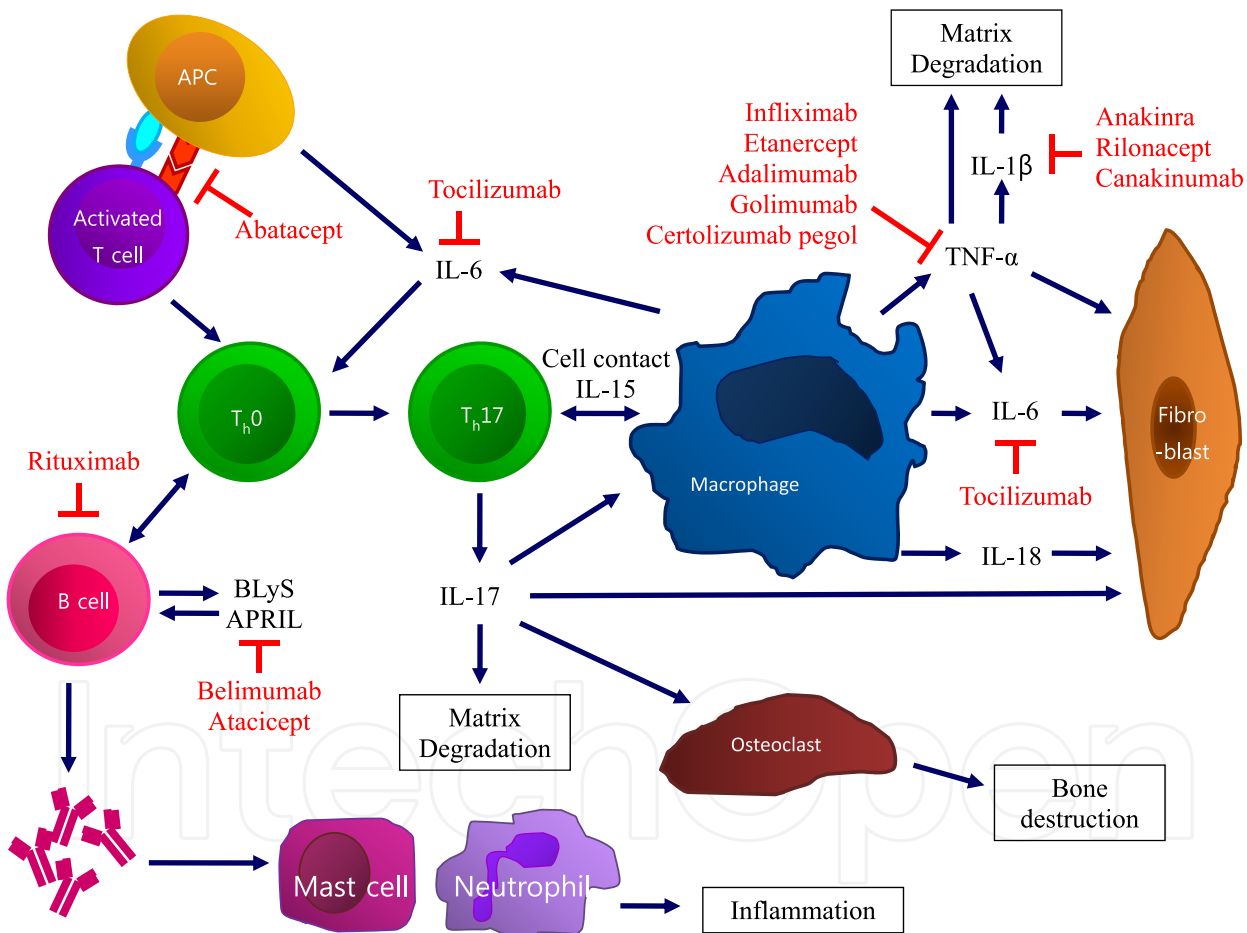
This figure summarizes the cellular interactions in the pathogenesis of RA and the interaction among antigen presenting cells (APCs), T cells, B cells, macrophages, hematopoietic cells (neutrophil, mast cell) and nonhematopoietic cells (fibroblast, connective tissue cell, and bone). These interactions are facilitated by the actions of cytokines released from the activated cells then induce the production of other pro-inflammatory and inflammatory cytokines, which contribute to the pathogenesis of RA. Also, this figure shows therapeutic biological agents proved as a RA treatment (Brennan and McInnes, 2008; McInnes and Schett, 2007).

Fig. 1. An overview of the pathogenesis of rheumatoid arthritis (RA) and the cytokine targets.

Every biological agent used in clinics today has its own specific targets and can be grouped as follows according to its aims: 1) tolerance induction, 2) inhibition of MHC, antigen, and T cell receptor interaction, 3) Inhibition of cellular function and cell-cell interaction, 4) Interference with cytokines, 5) apoptosis (Table 1). Among them, the anticytokine biological

Strategy	Biologic Agent	Molecule	Target	Indication
Tolerance induction	Abetimus	ds-DNA	Anti-ds-DNA	SLE
Inhibition of MHC, antigen, and T cell receptor interaction			Under studying	
Inhibition of cellular unction and cell-cell interaction	Abatacept	CTLA4-Ig	CD80/86	RA, JIA
	Rituximab	mAb to CD20	B cells	RA, JIA
	Belimumab	mAb to BLyS	BLyS	SLE
Interference with cytokines	Etanercept	TNFRII/FcIgG1	TNF-α, TNF-β	RA, PsA, Ps, AS, JIA
	Infliximab	mAb to TNF-α	TNF-α	RA, PsA, Ps, AS, JIA, IBD, uveitis
	Golimumab	mAb to TNF-α	TNF-α	RA, PsA, AS
	Adalimumab	mAb to TNF-α	TNF-α	RA, PsA, Ps, AS, JIA, IBD, uveitis
	Certolizumab pegol	Pegylated mAb to TNF-α	TNF-α	CD, RA
	Anakinra	IL-1 Ra	IL-1	RA, sJIA
	Rilonacept	IL-1R/IL-1AcP/IgG1	IL-1	CAPS, sJIA
	Canakinumab	mAb to IL-1	IL-1	CAPS, sJIA
	Tocilizumab	mAb to IL-6R	IL-6	JIA
Apoptosis		mAb to Fas	Fas	

AS: Ankylosing spondylitis, CAPS: Cryoprin-associated periodic syndrome, CD: Crohn's disease, ds-DNA: double strand-DNA, IBD: Inflammatory bowel disease, JIA: Juvenile idiopathic arthritis, mAb: Monoclonal antibody, Ps: Psoriasis, PsA: Psoriatic arthritis, RA: Rheumatoid arthritis, sJIA: Systemic-typed JIA, SLE: Systemic lupus erythematosus. (Textbook of Pediatric Rheumatology, 6th ed. 2011, Saunders)

Table 1. Summary of biological agents

agents that suppress the action of proinflammatory cytokines such as TNF, IL-1 and IL-6 are well-known and widely used in clinics. These agents were developed as therapies in RA and recommended for the treatment of patients whose disease does not respond to conventional therapies (Gomez-Reino and Carmona, 2006). RA patients treated with the anticytokine biological agents show dramatic improvement of their clinical symptoms and the levels of inflammatory biomarkers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Subsequently, these agents have been applied to the treatment of other inflammatory autoimmune diseases and have had a significant impact on patients' prognosis and survival (Andreakos et al., 2002; Efthimiou and Markenson, 2005; Maini et al., 2006; Nishimoto et al., 2009; Yokota et al., 2008).

However, it has been reported that substantial numbers of patients with autoimmune diseases still do not respond to one or more anticytokine biological agents. Among biological agents, TNF inhibitors have been extensively investigated with regard to the frequency of inadequate responders (Launois et al., 2011; Lovell et al., 2008; Maini et al., 2006; Yokota et al., 2008), because anti-TNF antibodies were the first agents approved as the therapy of RA. For example, 20~40 % of patients treated with a TNF inhibitor failed to achieve an improvement of 20 % in American College of Rheumatology criteria (Emery et al., 2008; Rubbert-Roth and Finckh, 2009). More patients lose efficacy during therapy, as shown by a report that 21 % of RA patients initially treated with etanercept no longer receive this therapy after 24 months (Feltelius et al., 2005).

Recently, patients who had an inadequate response or adverse events with one anticytokine agent are often treated with another biologic agent (Gomez-Reino and Carmona, 2006; Hyrich et al., 2007; Karlsson et al., 2008; Rubbert-Roth and Finckh, 2009). In the case of the treatment failure with the first TNF inhibitor, one survey reported that over 94 % of practicing rheumatologists in the United States of America have switched from one TNF inhibitor to another (Yazici et al., 2009). Interestingly, while some surveys reported that the efficiency of second TNF inhibitors is less than that of the first TNF inhibitor (Gomez-Reino and Carmona, 2006; Hyrich et al., 2007; Karlsson et al., 2008; Rubbert-Roth and Finckh, 2009), a large cohort study from the UK revealed that patients who switched their therapy from an initial TNF inhibitor continued to receive the second TNF inhibitor for mean length of 6 months and only 16 % of patients stopped it again due to poor response (Hyrich et al., 2007). This observation indicates that biological agents that share the common target do not always show the same effect on patients. One reason for the inefficacy of the first TNF inhibitor but not of the second one is the development of neutralizing antibody against the first agent, which may not interfere with the action of the second TNF inhibitor. Nevertheless, this observation also raises the possibility that these agents may have their own mode of action. Supporting the latter notion, there are differences in the efficacy between TNF inhibitors depending on diseases (Ackermann and Kavanaugh, 2007; Nash and Florin, 2005; Ramos-Casals et al., 2008; Sfikakis et al., 2007; Triolo et al., 2002; Veres et al., 2007). For example, while anti-TNF antibodies are effective for both RA and Crohn's disease, TNF receptor-Fc fusion protein (TNFR-Fc) is effective for RA but not for Crohn's disease.

The other treatment option after the failure with TNF inhibitors is to switch from TNF inhibitors to other biological agents with different targets. The Abatacept Trial in Treatment of Anti-TNF INadequate responders (ATTAIN) study investigated the effect of abatacept (CTLA4-Ig), an inhibitor of T cell co-stimulatory signal, on patients with active RA and an inadequate response to previous anti-TNF therapy. At 6 months, the ACR20 response rate was

50.4% in the abatacept group versus 19.5% in the placebo group and sustained improvements in ACR responses were achieved after 2 years of threatment with abatacept (Genovese et al., 2005; Rubbert-Roth and Finckh, 2009). In Randomized Evaluation of Long-Term Efficacy of Rituximab in RA (REFLEX) trial, B cell-depleting anti-CD20 antibody, rituximab, was administered to active RA patients with an inadequate response to TNF inhibitor. Among 208 patients treated with rituximab, 51 % of patients achieved an ACR20 response compared to 18 % of patients treated with placebo (Cohen et al., 2006). In addition, the patients treated with rituximab, by themselves, reported clinically meaningful and statistically improvements of pain, functional disability, and health-related quality of life (Keystone et al., 2008).

Recently, the Research on Actemra Determining efficacy after Anti-TNF failures (RADIATE) study examined the efficacy and safety of anti-IL-6 receptor (IL-6R) antibody, tocilizumab, in patients with active RA who had failed TNF inhibitor. Especially, 50.0 % of patients treated with tocilizumab at the 8 mg/kg of dose achieved ACR20 as well as rapid and sustained improvement of RA symptoms compared to 10.1 % of patients treated with placebo achieved ACR20 (Emery et al., 2008) (Figure 2). These findings are in accordance with the supposition that biological agents targeting different molecules have distinctive mechanism of action and show different effects on patients.
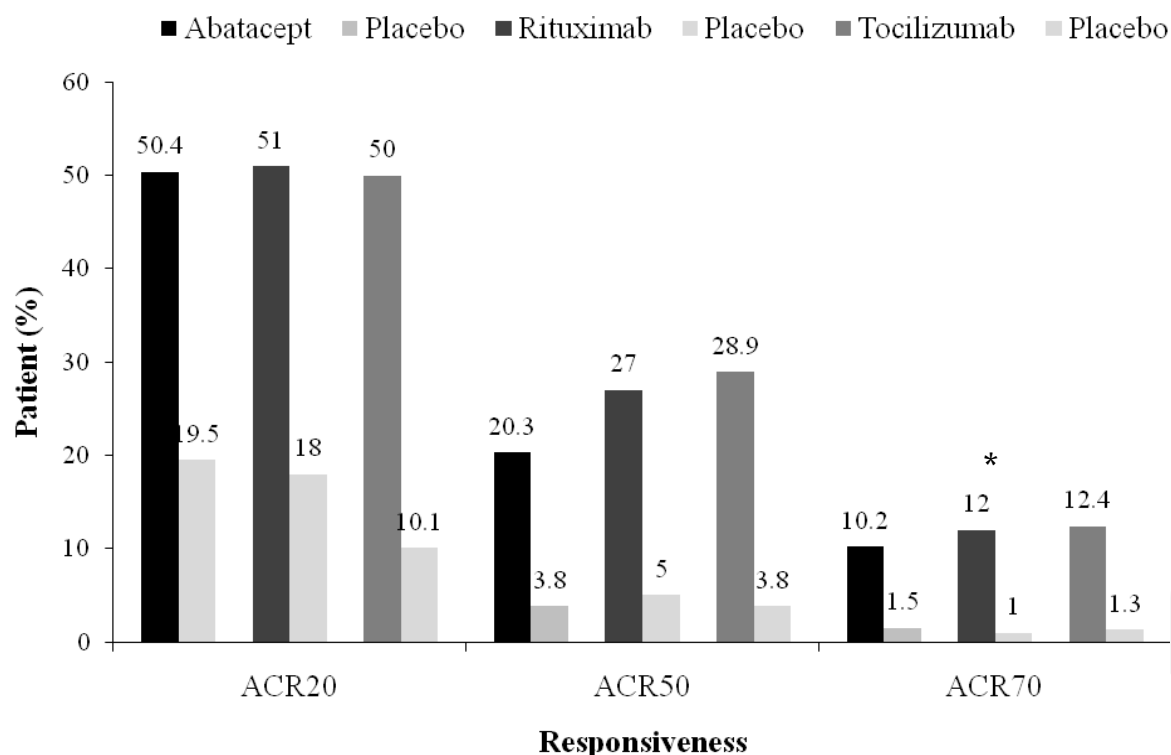
The second biological agents with other mechanisms with TNF inhibitors such as abatacept, rituximab and tocilizumab were used for patients who failed to initial TNF inhibitors. Bars show percentages of patients achieving a response according to the American College of Rheumatology 20% improvement criteria (ACR20), 50% improvement criteria (ACR50), and 70% improvement criteria (ACR70). The ACR20, ACR50, ACR70 responses in patients treated with abatacept, rituximab and tocilizumab were significantly higher than patients treated with placebo ($p<.001$, $*p=.003$).

Fig. 2. Responsiveness of treatment with the second biological agents in the patients with RA refractory to initial TNF inhibitors.

2.2 Biological therapeutic agents tested in animal models of autoimmune disorders

The analyses on murine disease models have contributed greatly to gain insight into pathogenesis and therapeutic strategy of autoimmune disorders. These models are also useful to clarify the detailed mechanisms of action of biological agents. We have recently investigated several disease models and reveled that anticytokine biological agents have different mechanism of action and show different effects on clinical manifestations of disease models (Fujimoto et al., 2008; Terabe et al., 2011).

We analyzed the effect of two anticytokine agents, anti-IL-6R monoclonal antibody (mAb) and TNFR-Fc, on collagen-induced arthritis (CIA), a murine model of human RA (Fujimoto et al., 2008). In accordance with the pivotal proinflammatory role of IL-6 and TNF in this arthritis model, both agents could inhibit the development of arthritis. However, while anti-IL-6R mAb potently inhibited the differentiation of Th17 cells, a highly inflammatory subset of T helper cells, TNFR-Fc exhibited no effect on Th17 cells. This observation suggests that these two agents have different action points: IL-6 blockade acts on initial phase of adoptive immune response and regulates T helper cell differentiation, whereas TNF inhibitors act much later, presumably at inflamed sites. Our study also suggests that IL-6 inhibitors may be applicable to other Th17-related autoimmune diseases. Indeed, anti-IL-6R mAb suppressed disease in a murine model of multiple sclerosis via the inhibition of Th17 cell differentiation (Serada et al., 2008). The different modes of action in anti-IL-6R mAb and TNF inhibitors may explain the difference in their efficiency in a murine model of uveoretinitis. Anti-IL-6R mAb treatment had a significant protective effect in experimental autoimmune uveoretinitis (EAU) mice, but either TNFR-Fc or anti-TNF mAb treatment did not (Hohki et al., 2010). Interestingly, in the EAU model, anti-IL-6R mAb not only suppressed Th17 cell differentiation but also suppressed autoantigen-specific Th1 cells via the generation of induced regulatory T cells, supporting the notion that IL-6 inhibitors act on initial phase of adoptive immune response (Haruta et al., 2011).

Confusingly, biological agents may act differently on different autoimmune diseases. Indeed, anti-IL-6R mAb and anti-TNF mAb, but not TNFR-Fc exerted similar effect on a murine inflammatory bowel disease (IBD) model (Terabe et al., 2011). This model is a T cell dependent colitis and is induced by the transfer of purified naïve CD4 T cells into lymphopenic mice. Both anti-IL-6R mAb and anti-TNF mAb successfully inhibited colitis, whereas TNFR-Fc did not show any protective effect on colitis. In addition, anti-IL-6R mAb and anti-TNF mAb could comparably inhibit the expansion of colitogenic T cells in this model, although like in other models, anti-IL-6R mAb additionally could modulate the profile of T helper cell differentiation (Terabe et al., 2011). Thus, anti-IL-6R mAb and anti-TNF mAb may share a similar mode of action in the inhibition of IBD. It is also notable that TNFR-Fc failed to inhibit inflammation in this colitis model (Terabe et al., 2011). Similar discrepancy in the effect of anti-TNF mAb and TNFR-Fc has been observed in human IBD. Many mechanisms have been proposed so far to explain the difference of action between these two agents. For example, anti-TNF mAb binds not only to soluble TNF-α, but also to membrane-bound TNF-α, leading to the induction of antibody-dependent and complement dependent cytotoxicity (Maini, 2004). The anti-TNF mAb may also have more capacity than TNFR-Fc to induce apoptosis via reverse signaling with cross-linking by binding firmly to transmembrane TNF(Terabe et al., 2011). Nevertheless, these hypotheses are still controversial and it remains to be explained why anti-TNF mAb and TNFR-Fc have differential effectiveness in some autoimmune diseases such as Crohn's disease. We believe that further study on this murine IBD model is useful for elucidation of this issue.

3. Biomarkers

3.1 A need for new biomarkers in the era of biological agents

Given the difference in mechanism and therapeutic effect of each biologic agent, it is desirable to select an effective biological agent on each patient before initiating therapy or after failure of the initial therapy. However, no reliable guidance is available at present for the selection of biological therapies. There is a growing need for the development of biomarkers that predict individual treatment response before therapy.

In addition, in patients treated with biological agents in whom immune response is substantially suppressed, conventional laboratory biomarkers such as CRP and ESR do not always reflect disease activity. In particular, since serum CRP is primarily dependent on liver by circulating IL-6, CRP is unable to reflect disease activity in patients treated with IL-6 inhibitors. Moreover, conventional markers may also be inadequate for the detection of inflammation unrelated to original diseases. In RA patients after joint surgery, anti-IL-6R mAb tocilizumab completely suppressed the increase in CRP and partially suppressed the rise in body temperature (Hirao et al., 2009). More importantly, biological agents may mask typical symptoms of bacterial infection and inhibit the elevation of serum biomarkers. Indeed, RA patients treated with tocilizumab did not present characteristic clinical symptoms and typical elevation of serum CRP after bacterial pneumonia and septic shock (Fujiwara et al., 2009). Even without biologic treatment, current inflammatory biomarkers are not useful to distinguish infection from flares of autoimmune diseases. This is an important issue in clinical settings, because therapeutic strategies for infection and disease flares are completely opposite. Infection must be treated primarily with antibiotics and discontinuation of biological agents should be considered. In contrast, disease flares should be treated intensively with the same or alternative biological agents. Thus, new biomarkers are needed for the detection and discrimination of inflammation by either infection or disease flares.
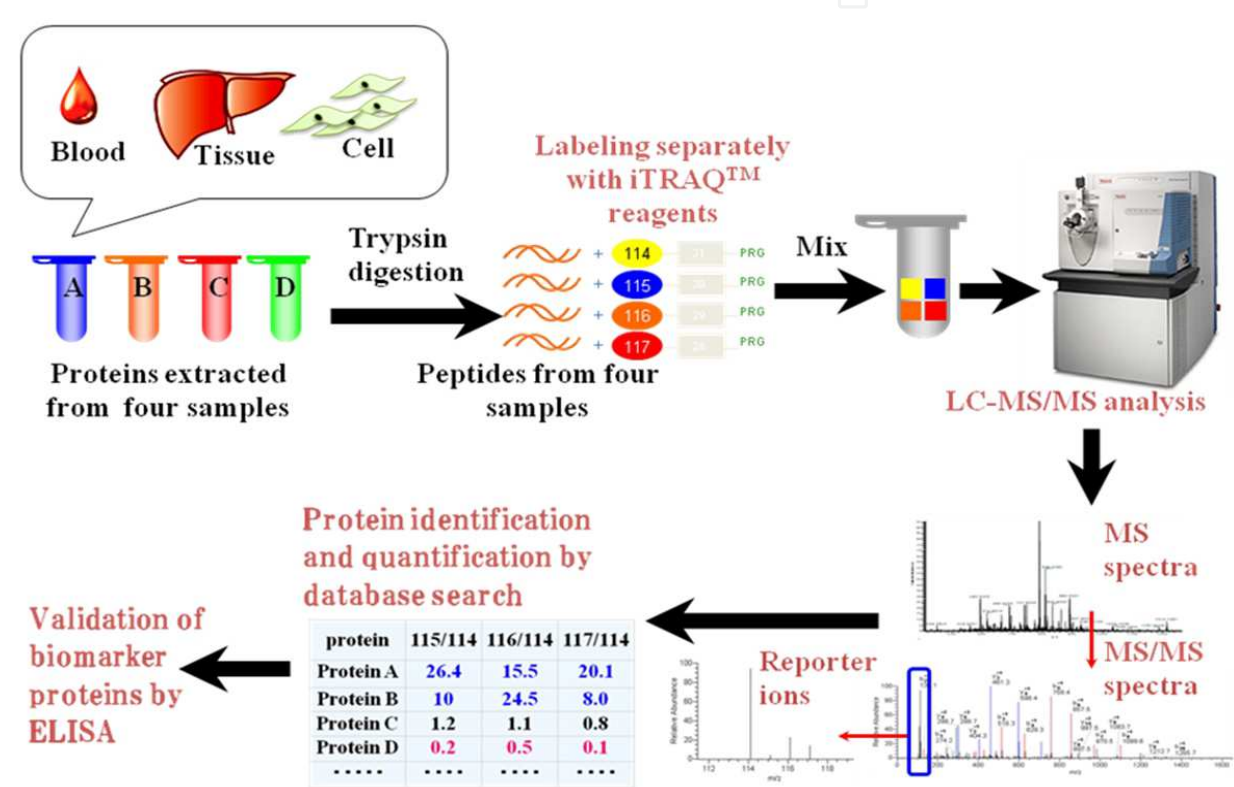
Even after the successful repression of disease with biologic therapies, it remains unknown yet whether biological agents can be terminated safely without disease recurrence. Therefore, a biomarker that indicates clinical remission or cure of autoimmune diseases is helpful to determine the timing to stop biological agents.

Collectively, the development of a number of novel biomarkers, such as those that can help to select biological agents before therapy, can precisely evaluate disease activity and therapeutic effect during the therapy or can instruct the timing of therapy completion after achievement of remission, are warranted for the appropriate clinical management of patients receiving biological therapies.

3.2 Serum proteome analysis using the new technology iTRAQ

The pathogenesis of autoimmune diseases involves alterations in the expression of genes that control pathways regulating self tolerance. However, gene transcripts may not faithfully reflect their protein levels. In addition, post-translational modifications are not amenable to the study of transcriptional profiling (Hueber and Robinson, 2006). Recently, there has been the remarkable improvement of the proteomic approaches as represented by the development of sophisticated methods of protein sample preparation and the improvement of the sensitivity, accuracy and resolution in mass spectrometer. Therefore, direct proteomic measurement may provide greater utility for the discovery of new biomarkers monitoring autoimmune diseases in the post genomic era. Current efforts to

identify autoimmune disease biomarkers have focused on three groups of proteins reflective of the autoimmune disease process. These groups include 1) degradation products arising from destruction of the affected tissues, 2) enzymes that play a role in tissue degradation, and 3) cytokines and other proteins associated with immune system activation and the inflammatory response (Prince, 2005). Recent proteomics technologies have enabled us to screen these markers from proteins extracted from tissues and sera from patients (Hueber and Robinson, 2006). Accordingly, there are many proteomic studies that analyzed protein profiles and searched new biomarkers in autoimmune diseases (Dwivedi et al., 2009; Ferraccioli et al., 2010; Ling et al., 2010; Serada et al., 2010; Takeuchi et al., 2007). The quantitative proteome analysis by mass spectrometry (MS) usually involves differential isotope labeling of proteins and peptides metabolically, enzymatically or chemically using

protein	115/114	116/114	117/114
Protein A	26.4	15.5	20.1
Protein B	10	24.5	8.0
Protein C	1.2	1.1	0.8
Protein D	0.2	0.5	0.1
.....	....	....	....

In a single experiment of iTRAQ analysis, 4 to 8 samples differentially labeled with iTRAQ reagents can be quantitatively analyzed by mass spectrometry (this figure shows a four-plex reagent experiment). First, proteins extracted from cells, tissue and/or body fluid such as blood are reduced, alkylated and digested with trypsin. Second, obtained peptides in each sample are labeled with each iTRAQTM reagent at N-terminal amino group or epsilon amino group from lysine. The iTRAQ tags are isobaric and the labeling with iTRAQ reagents results in the uniform increase in molecular weight of peptides in every sample. After labeling, samples are mixed into one tube and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Mass spectrometry is performed by full scan MS, followed by MS/MS spectra of peptides. In MS/MS spectra, iTRAQ tag-specific reporter ions (114.1, 115.1, 116.1, 117.1 in the figure) are detected in low m/z region, and these reporter ion intensities represent the abundance of the peptide from each sample. Peptide sequence information is obtained from high m/z region of MS/MS spectra and the protein is identified by database search after comparing obtained MS/MS spectra with theoretical MS/MS spectra in the database. Usually, candidate biomarker proteins obtained by iTRAQ analysis are further verified by other methods such as ELISA analysis.

Fig. 3. Flowchart of iTRAQ analysis

external reagent tags. These methods address some of the limitations faced in traditional gel-based proteomic approaches. However, these approaches still suffer from some limitations such as inability to multiplex and to quantify zero protein expression level. In contrast, a novel quantitative proteomic technology, isobaric tagging of peptides enable simultaneous identification and quantification of peptides by tandem MS and permit parallel proteome analysis of more than two samples (Aggarwal et al., 2006).

The isobaric tags for relative and absolute quantitation (iTRAQ), which is one such method commercialized, uses four amine specific isobaric reagents to label the primary amines of peptides from four to eight different biological samples. The labeled peptides from each sample are mixed, separated using two-dimensional liquid chromatography and analyzed using MS and tandem mass spectrometry (Figure 3). The isobaric tagging strategy provides multiple independent measures of the relative abundance of a protein. The capability of iTRAQ for protein quantitation has been verified by analyzing standard mixtures of proteins of known proportions (Aggarwal et al., 2006). The iTRAQ approach has now been successfully used to identify and quantify the proteins in variety of prokaryotic and eukaryotic samples (Aggarwal et al., 2006; Cong et al., 2006; DeSouza et al., 2005; Dwivedi et al., 2009; Hardt et al., 2005; Zhang et al., 2005).

3.3 Leucin-rich-α-2 glycoprotein as a novel biomarker

We reported for the first time that the iTRAQ technology is applicable to identify novel biomarkers in sera from patients with autoimmune diseases (Serada et al., 2010). Before we publish our results, a study was published and reported the serum proteome of RA patients treated with anti-TNF mAb therapy. They provided evidence that iTRAQ strategy can be used to obtain quantitative data that reflect changes in the serum proteome after targeted therapeutic interventions (Dwivedi et al., 2009).

We used iTRAQ technology to obtain profiles of serum proteome in RA patients before and after TNF inhibitor treatment. We then listed serum proteins that declined remarkably after treatment. Our strategy was verified by the detection of familiar biomarkers including CRP and serum amyloid A (SAA) as reduced serum proteins after treatment. Among the candidate proteins that declined after therapy, we focused on an uncharacterized protein called leucine-rich-α-2 glycoprotein (LRG) and examined further on this protein using other methods such as Western blot and ELISA. Indeed, taking advantage of ELISA analysis of many serum samples from RA patients, we found that serum levels of LRG significantly declined after therapy with TNF inhibitors and correlate well with disease activity of RA patients. In addition, LRG levels were significantly high in patients with other autoimmune diseases such as Crohn's disease and Behcet disease. As expected, the LRG correlated well with a conventional biomarker CRP in patients with these autoimmune inflammatory diseases. Interestingly, however, while CRP correlated with serum IL-6 levels, LRG did not. In accordance with this, in some Crohn's disease patients with active disease, CRP levels remained low but serum LRG concentrations were significantly elevated. Thus, LRG exhibits similarity with CRP but also has a unique property. Moreover, because serum LRG concentrations of Crohn's disease patients before starting therapy were higher in the non-responders to anti-TNF therapy than in the responders, LRG may predict therapeutic responses to TNF inhibitors in Crohn's disease patients (Serada et al., 2010).

Until now, LRG has been reported to be expressed by liver cells and neutrophils, and regulated by multiple factors and produced at local inflammatory sites. According to the

previous reports, it seems that LRG is not a unique biomarker in autoimmune disease but rather is a generalized inflammatory biomarker, because serum LRG levels are reported to be increased in patients with bacterial infection and several types of cancers. Nevertheless, serum LRG satisfies the condition of an inflammatory biomarker in the point that its concentration is high at diagnosis, correlated well with disease activity and is a possible predictor of the responsiveness to biological agents. For these reasons, serum LRG is a novel inflammatory biomarker potentially surrogate for CRP. Further studies are in progress in our laboratory to determine the pathophysiological function of LRG and the clinical benefit of LRG measurement.

4. Conclusion

Autoimmune diseases including RA are not only rare but also difficult to treat. In the clinical field, biological agents have emerged as attractive therapeutic options for these diseases, because of their rapid and/or dramatic effectiveness to intractable diseases. However, biological agents are expensive and their usage is occasionally accompanied with severe adverse effects such as immunosuppression and fatal infection. To maximize the therapeutic potential and to minimize the adverse effects of biological agents, novel biomarkers are required for the selection of agents, monitoring of the disease activity and therapeutic efficacy or differential diagnosis of infection. In this respect, LRG we identified from iTRAQ analysis is a candidate of novel biomarkers useful for clinical practice of biological agents since it correlates with disease activity and therapeutic effectiveness of biological agents. In addition, the application of iTRAQ analysis, the novel quantitative proteomic approach, is useful for the identification of new serological biomarkers in patients with autoimmune diseases. Further studies using this approach may lead to the development of additional new biomarkers and may help to clarify the pathogenesis and identify therapeutic targets in autoimmune diseases.

5. References

Ackermann, C., and Kavanaugh, A. (2007). Tumor necrosis factor as a therapeutic target of rheumatologic disease. Expert Opin Ther Targets *11*, 1369-1384.

Aggarwal, K., Choe, L.H., and Lee, K.H. (2006). Shotgun proteomics using the iTRAQ isobaric tags. Brief Funct Genomic Proteomic *5*, 112-120.

Andreakos, E.T., Foxwell, B.M., Brennan, F.M., Maini, R.N., and Feldmann, M. (2002). Cytokines and anti-cytokine biologicals in autoimmunity: present and future. Cytokine Growth Factor Rev *13*, 299-313.

Brennan, F.M., and McInnes, I.B. (2008). Evidence that cytokines play a role in rheumatoid arthritis. J Clin Invest *118*, 3537-3545.

Cohen, S.B., Emery, P., Greenwald, M.W., Dougados, M., Furie, R.A., Genovese, M.C., Keystone, E.C., Loveless, J.E., Burmester, G.R., Cravets, M.W., *et al.* (2006). Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. Arthritis Rheum *54*, 2793-2806.

Cong, Y.S., Fan, E., and Wang, E. (2006). Simultaneous proteomic profiling of four different growth states of human fibroblasts, using amine-reactive isobaric tagging reagents and tandem mass spectrometry. Mech Ageing Dev *127*, 332-343.

DeSouza, L., Diehl, G., Rodrigues, M.J., Guo, J., Romaschin, A.D., Colgan, T.J., and Siu, K.W. (2005). Search for cancer markers from endometrial tissues using differentially labeled tags iTRAQ and cICAT with multidimensional liquid chromatography and tandem mass spectrometry. J Proteome Res *4*, 377-386.

Dwivedi, R.C., Dhindsa, N., Krokhin, O.V., Cortens, J., Wilkins, J.A., and El-Gabalawy, H.S. (2009). The effects of infliximab therapy on the serum proteome of rheumatoid arthritis patients. Arthritis Res Ther *11*, R32.

Efthimiou, P., and Markenson, J.A. (2005). Role of biological agents in immune-mediated inflammatory diseases. South Med J *98*, 192-204.

Emery, P., Keystone, E., Tony, H.P., Cantagrel, A., van Vollenhoven, R., Sanchez, A., Alecock, E., Lee, J., and Kremer, J. (2008). IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomised placebo-controlled trial. Ann Rheum Dis *67*, 1516-1523.

Feltelius, N., Fored, C.M., Blomqvist, P., Bertilsson, L., Geborek, P., Jacobsson, L.T., Lindblad, S., Lysholm, J., Rantapaa-Dahlqvist, S., Saxne, T., and Klareskog, L. (2005). Results from a nationwide postmarketing cohort study of patients in Sweden treated with etanercept. Ann Rheum Dis *64*, 246-252.

Ferraccioli, G., De Santis, M., Peluso, G., Inzitari, R., Fanali, C., Bosello, S.L., Iavarone, F., and Castagnola, M. (2010). Proteomic approaches to Sjogren's syndrome: a clue to interpret the pathophysiology and organ involvement of the disease. Autoimmun Rev *9*, 622-626.

Fujimoto, M., Serada, S., Mihara, M., Uchiyama, Y., Yoshida, H., Koike, N., Ohsugi, Y., Nishikawa, T., Ripley, B., Kimura, A., *et al.* (2008). Interleukin-6 blockade suppresses autoimmune arthritis in mice by the inhibition of inflammatory Th17 responses. Arthritis Rheum *58*, 3710-3719.

Fujiwara, H., Nishimoto, N., Hamano, Y., Asanuma, N., Miki, S., Kasayama, S., and Suemura, M. (2009). Masked early symptoms of pneumonia in patients with rheumatoid arthritis during tocilizumab treatment: a report of two cases. Mod Rheumatol *19*, 64-68.

Genovese, M.C., Becker, J.C., Schiff, M., Luggen, M., Sherrer, Y., Kremer, J., Birbara, C., Box, J., Natarajan, K., Nuamah, I., *et al.* (2005). Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. N Engl J Med *353*, 1114-1123.

Gomez-Reino, J.J., and Carmona, L. (2006). Switching TNF antagonists in patients with chronic arthritis: an observational study of 488 patients over a four-year period. Arthritis Res Ther *8*, R29.

Han, C., Smolen, J.S., Kavanaugh, A., van der Heijde, D., Braun, J., Westhovens, R., Zhao, N., Rahman, M.U., Baker, D., and Bala, M. (2007). The impact of infliximab treatment on quality of life in patients with inflammatory rheumatic diseases. Arthritis Res Ther *9*, R103.

Hardt, M., Witkowska, H.E., Webb, S., Thomas, L.R., Dixon, S.E., Hall, S.C., and Fisher, S.J. (2005). Assessing the effects of diurnal variation on the composition of human

parotid saliva: quantitative analysis of native peptides using iTRAQ reagents. Anal Chem *77*, 4947-4954.

Haruta, H., Ohguro, N., Fujimoto, M., Hohki, S., Terabe, F., Serada, S., Nomura, S., Nishida, K., Kishimoto, T., and Naka, T. (2011). Blockade of interleukin-6 signaling suppresses not only th17 but also interphotoreceptor retinoid binding protein-specific Th1 by promoting regulatory T cells in experimental autoimmune uveoretinitis. Invest Ophthalmol Vis Sci 52, 3264-3271.

Hirao, M., Hashimoto, J., Tsuboi, H., Nampei, A., Nakahara, H., Yoshio, N., Mima, T., Yoshikawa, H., and Nishimoto, N. (2009). Laboratory and febrile features after joint surgery in patients with rheumatoid arthritis treated with tocilizumab. Ann Rheum Dis *68*, 654-657.

Hohki, S., Ohguro, N., Haruta, H., Nakai, K., Terabe, F., Serada, S., Fujimoto, M., Nomura, S., Kawahata, H., Kishimoto, T., and Naka, T. (2010). Blockade of interleukin-6 signaling suppresses experimental autoimmune uveoretinitis by the inhibition of inflammatory Th17 responses. Exp Eye Res *91*, 162-170.

Hueber, W., and Robinson, W.H. (2006). Proteomic biomarkers for autoimmune disease. Proteomics *6*, 4100-4105.

Hyrich, K.L., Lunt, M., Watson, K.D., Symmons, D.P., and Silman, A.J. (2007). Outcomes after switching from one anti-tumor necrosis factor alpha agent to a second anti-tumor necrosis factor alpha agent in patients with rheumatoid arthritis: results from a large UK national cohort study. Arthritis Rheum *56*, 13-20.

Karlsson, J.A., Kristensen, L.E., Kapetanovic, M.C., Gulfe, A., Saxne, T., and Geborek, P. (2008). Treatment response to a second or third TNF-inhibitor in RA: results from the South Swedish Arthritis Treatment Group Register. Rheumatology (Oxford) *47*, 507-513.

Keystone, E., Burmester, G.R., Furie, R., Loveless, J.E., Emery, P., Kremer, J., Tak, P.P., Broder, M.S., Yu, E., Cravets, M., *et al.* (2008). Improvement in patient-reported outcomes in a rituximab trial in patients with severe rheumatoid arthritis refractory to anti-tumor necrosis factor therapy. Arthritis Rheum *59*, 785-793.

Laas, K., Peltomaa, R., Puolakka, K., Kautiainen, H., and Leirisalo-Repo, M. (2009). Early improvement of health-related quality of life during treatment with etanercept and adalimumab in patients with rheumatoid arthritis in routine practice. Clin Exp Rheumatol *27*, 315-320.

Launois, R., Avouac, B., Berenbaum, F., Blin, O., Bru, I., Fautrel, B., Joubert, J.M., Sibilia, J., and Combe, B. (2011). Comparison of Certolizumab Pegol with Other Anticytokine Agents for Treatment of Rheumatoid Arthritis: A Multiple-treatment Bayesian Metaanalysis. J Rheumatol.

Ling, X.B., Park, J.L., Carroll, T., Nguyen, K.D., Lau, K., Macaubas, C., Chen, E., Lee, T., Sandborg, C., Milojevic, D., *et al.* (2010). Plasma profiles in active systemic juvenile idiopathic arthritis: Biomarkers and biological implications. Proteomics *10*, 4415-4430.

Lovell, D.J., Reiff, A., Ilowite, N.T., Wallace, C.A., Chon, Y., Lin, S.L., Baumgartner, S.W., Giannini, E.H., and Pediatric Rheumatology, C. (2008). Safety and efficacy of up to eight years of continuous etanercept therapy in patients with juvenile rheumatoid arthritis. Arthritis and Rheumatism *58*, 1496-1504.

Maini, R.N., Taylor, P.C., Szechinski, J., Pavelka, K., Broll, J., Balint, G., Emery, P., Raemen, F., Petersen, J., Smolen, J., *et al.* (2006). Double-blind randomized controlled clinical trial of the interleukin-6 receptor antagonist, tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate. Arthritis Rheum *54*, 2817-2829.

Maini, S.R. (2004). Infliximab treatment of rheumatoid arthritis. Rheum Dis Clin North Am *30*, 329-347, vii.

McInnes, I.B., and Schett, G. (2007). Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol *7*, 429-442.

Nash, P.T., and Florin, T.H. (2005). Tumour necrosis factor inhibitors. Med J Aust *183*, 205-208.

Nishimoto, N., Miyasaka, N., Yamamoto, K., Kawai, S., Takeuchi, T., and Azuma, J. (2009). Long-term safety and efficacy of tocilizumab, an anti-IL-6 receptor monoclonal antibody, in monotherapy, in patients with rheumatoid arthritis (the STREAM study): evidence of safety and efficacy in a 5-year extension study. Ann Rheum Dis *68*, 1580-1584.

Prieto, S., and Grau, J.M. (2010). The geoepidemiology of autoimmune muscle disease. Autoimmun Rev *9*, A330-334.

Prince, H.E. (2005). Biomarkers for diagnosing and monitoring autoimmune diseases. Biomarkers *10 Suppl 1*, S44-49.

Ramos-Casals, M., Brito-Zeron, P., Soto, M.J., Cuadrado, M.J., and Khamashta, M.A. (2008). Autoimmune diseases induced by TNF-targeted therapies. Best Pract Res Clin Rheumatol *22*, 847-861.

Rubbert-Roth, A., and Finckh, A. (2009). Treatment options in patients with rheumatoid arthritis failing initial TNF inhibitor therapy: a critical review. Arthritis Res Ther *11 Suppl 1*, S1.

Serada, S., Fujimoto, M., Ogata, A., Terabe, F., Hirano, T., Iijima, H., Shinzaki, S., Nishikawa, T., Ohkawara, T., Iwahori, K., *et al.* (2010). iTRAQ-based proteomic identification of leucine-rich alpha-2 glycoprotein as a novel inflammatory biomarker in autoimmune diseases. Ann Rheum Dis *69*, 770-774.

Sfikakis, P.P., Markomichelakis, N., Alpsoy, E., Assaad-Khalil, S., Bodaghi, B., Gul, A., Ohno, S., Pipitone, N., Schirmer, M., Stanford, M., *et al.* (2007). Anti-TNF therapy in the management of Behcet's disease--review and basis for recommendations. Rheumatology (Oxford) *46*, 736-741.

Strand, V., and Singh, J.A. (2007). Improved health-related quality of life with effective disease-modifying antirheumatic drugs: evidence from randomized controlled trials. Am J Manag Care *13 Suppl 9*, S237-251.

Takeuchi, T., Nakanishi, T., Tabushi, Y., Hata, A., Shoda, T., Kotani, T., Shimizu, A., Takubo, T., Makino, S., and Hanafusa, T. (2007). Serum protein profile of rheumatoid arthritis treated with anti-TNF therapy (infliximab). J Chromatogr B Analyt Technol Biomed Life Sci *855*, 66-70.

Terabe, F., Fujimoto, M., Serada, S., Shinzaki, S., Iijima, H., Tsujii, M., Hayashi, N., Nomura, S., Kawahata, H., Jang, M.H., *et al.* (2011). Comparative analysis of the effects of anti-IL-6 receptor mAb and anti-TNF mAb treatment on CD4+ T-cell responses in murine colitis. Inflamm Bowel Dis *17*, 491-502.

Triolo, G., Vadala, M., Accardo-Palumbo, A., Ferrante, A., Ciccia, F., Giardina, E., Citarrella, P., Lodato, G., and Licata, G. (2002). Anti-tumour necrosis factor monoclonal antibody treatment for ocular Behcet's disease. Ann Rheum Dis *61*, 560-561.

Veres, G., Baldassano, R.N., and Mamula, P. (2007). Infliximab therapy for pediatric Crohn's disease. Expert Opin Biol Ther *7*, 1869-1880.

Yazici, Y., Krasnokutsky, S., Barnes, J.P., Hines, P.L., Wang, J., and Rosenblatt, L. (2009). Changing patterns of tumor necrosis factor inhibitor use in 9074 patients with rheumatoid arthritis. J Rheumatol *36*, 907-913.

Yokota, S., Imagawa, T., Mori, M., Miyamae, T., Aihara, Y., Takei, S., Iwata, N., Umebayashi, H., Murata, T., Miyoshi, M., *et al.* (2008). Efficacy and safety of tocilizumab in patients with systemic-onset juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled, withdrawal phase III trial. Lancet *371*, 998-1006.

Zhang, Y., Wolf-Yadlin, A., Ross, P.L., Pappin, D.J., Rush, J., Lauffenburger, D.A., and White, F.M. (2005). Time-resolved mass spectrometry of tyrosine phosphorylation sites in the epidermal growth factor receptor signaling network reveals dynamic modules. Mol Cell Proteomics *4*, 1240-1250.
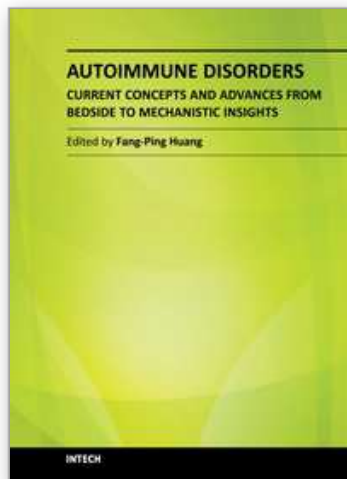
Autoimmune Disorders - Current Concepts and Advances from Bedside to Mechanistic Insights

Edited by Dr. Fang-Ping Huang

Autoimmune disorders are caused due to break down of the immune system, which consequently fails in its ability to differentiate "self" from "non-self" in the context of immunology. The diseases are intriguing, both clinically and immunologically, for their diversified clinical phenotypes and complex underlying immunological mechanisms. This book offers cutting-edge information on some of the specific autoimmune disease phenotypes, respective diagnostic and prognostic measures, classical and new therapeutic options currently available, pathogenesis and underlying mechanisms potentially involved, and beyond. In the form of Open Access, such information is made freely available to clinicians, basic scientists and many others who will be interested regarding current advances in the areas. Its potential readers will find many of the chapters containing in-depth analysis, interesting discussions and various thought-provoking novel ideas.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Soyoung Lee, Satoshi Serada, Minoru Fujimoto and Tetsuji Naka (2011). Application of Novel Quantitative Proteomic Technologies to Identify New Serological Biomarkers in Autoimmune Diseases, Autoimmune Disorders - Current Concepts and Advances from Bedside to Mechanistic Insights, Dr. Fang-Ping Huang (Ed.), ISBN: 978-953-307-653-9, InTech, Available from: http://www.intechopen.com/books/autoimmune-disorders-current-concepts-and-advances-from-bedside-to-mechanistic-insights/application-of-novel-quantitative-proteomic-technologies-to-identify-new-serological-biomarkers-in-a

INTECH
open science | open minds