Review

Interleukin 6 in autoimmune and inflammatory diseases: a personal memoir

By Toshio Hirano[*1,*2,†]

(Communicated by Tasuku Honjo, m.j.a.)

Abstract: In this review, the author discusses the research that led to the identification and characterization of interleukin 6 (IL-6), including his own experience isolating IL-6, and the roles this cytokine has on autoimmune and inflammatory diseases. The cDNAs encoding B-cell stimulatory factor 2 (BSF-2), interferon (IFN)-β2 and a 26-kDa protein were independently cloned in 1986, which in turn led to the identification of each. To resolve the confusing nomenclature, these identical molecules were named IL-6. Characterization of IL-6 revealed a multifunctional cytokine that is involved in not only immune responses but also hematopoiesis, inflammation, and bone metabolism. Moreover, IL-6 makes significant contributions to such autoimmune and inflammatory diseases as rheumatoid arthritis (RA).

IL-6 activates both the STAT3 and SHP2/Gab/MAPK signaling pathways *via* the gp130 signal transducer. F759 mice, which contain a single amino-acid substitution in gp130 (Y759F) and show enhanced STAT3 activation, spontaneously develop a RA-like arthritis as they age. F759 arthritis is dependent on CD4$^+$ T cells, IL-6, and IL-17A, and is enhanced by the pX gene product from human T cell leukemia virus 1 (HTLV-1). Arthritis development in these mice requires that the F759 mutation is present in nonhematopoietic cells, but not in immune cells, highlighting the important role of the interaction between nonimmune tissues and the immune system in this disease. Furthermore, this interaction is mediated by the IL-6 amplifier through STAT3 and NF-κB. Ultimately, this model may represent a general etiologic process underlying other autoimmune and inflammatory diseases. More importantly, the understanding of IL-6 has paved the way for new therapeutic approaches for RA and other autoimmune and inflammatory diseases.

Keywords: cytokine, Interleukin 6, immune response, inflammation, autoimmune disease, rheumatoid arthritis

Introduction

The immune system is essential for human survival, as it recognizes and eliminates viruses, bacteria, and other infectious pathogens. At times, however, immune responses can be deleterious to human beings. For example, autoimmune diseases— RA, systemic lupus erythematosus, type 1 diabetes mellitus, Graves' disease, and multiple sclerosis, among others—develop when the immune system attacks the host's own tissues, resulting in a self-destructive process. Therefore, the immune system can be a double-edged sword.

Autoimmune diseases comprise a heterogeneous group of poorly understood disorders, the pathogeneses of which involve both genetic and environmental factors.[1)–4)] These diseases are generally classified into two major types: tissue-specific and systemic. Some autoimmune diseases are mediated by pathogenic and tissue-specific autoantibodies, resulting in localized effects in specifically targeted organs. In

[*1] Laboratory of Developmental Immunology, JST-CREST, Graduate School of Frontier Biosciences, Graduate School of Medicine, and WPI Immunology Frontier Research Center, Osaka University, Osaka, Japan.

[*2] Laboratory of Cytokine Signaling, RIKEN Research Center for Allergy and Immunology, Yokohama, Japan.

[†] Correspondence should be addressed: T. Hirano, Laboratory of Developmental Immunology, Graduate School of Medicine, Osaka University, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan (e-mail: hirano@molonc.med.osaka-u.ac.jp).

contrast, systemic autoimmune diseases damage multiple organ systems. In both cases, disease initiation is thought to result from a breakdown in self-tolerance, particularly when a well-defined pathogenic autoantigen has been identified and the disease specifically manifests in tissues that express the autoantigen. Interestingly, however, pathogenic antigens have not been identified for many autoimmune diseases, including RA and other tissue-specific disorders.[5),6)] This raises the possibility that a breakdown in tolerance to a tissue-specific antigen is not always required for localized autoimmune diseases. Rather, in certain diseases, the etiologic trigger may be a characteristic of the target tissue itself. In other words, aberrant activation of the immune system may be a consequence of events that are initiated by an inflammatory niche in a nonimmune target tissue in response to genetic and/or environmental factors.[1),7)–10)]

Major immune cell populations include T lymphocytes, B lymphocytes, macrophages, and antigen-presenting dendritic cells. These cells interact with each other to initiate immune responses against pathogens and, at times, the human body itself. Cytokines, such as interleukins and IFNs, are soluble factors produced by a variety of immune cells. These factors play crucial roles in immune responses, as abnormal cytokine production and activity are involved in several autoimmune and inflammatory diseases.[3),7),11)–14)] Importantly, clinical research has revealed that inhibitors of certain cytokines are beneficial to certain patients suffering from autoimmune or inflammatory diseases like RA.[15)–17)]

IL-6 is a pleiotropic cytokine that regulates multiple biological processes, including the development of the nervous and hematopoietic systems, acute-phase responses, inflammation, and immune responses.[7)] Patients with RA show high synovial concentrations of IL-6.[11)] Recent reports have highlighted the important roles of proinflammatory cytokines, such as TNF-α, IL-1, and IL-6, in the pathogenesis of RA.[12)–14)] IL-6 also contributes to the model of spontaneously occurring RA observed in F759 mice and SKG mice, and in antigen-induced RA models, such as collagen-induced arthritis and adjuvant-induced arthritis.[18)–22)] Furthermore, some patients with RA respond to treatment with anti–IL-6 receptor antibodies.[16),17)] Nine IL-6 family cytokines have been identified, including IL-6, oncostatin M, LIF, CNTF, CT-1, IL-11, and IL-27.[23),24)] All of these signaling molecules share gp130 as a receptor subunit and signal transducer. It has been established that gp130 is involved in two major signaling pathways: the JAK–signal transducer and activator of transcription 3 (STAT3) pathway, which is mediated by the YxxQ motif of gp130, and the SHP2–Gab-Ras-Erk–MAPK pathway, which is regulated *via* the cytoplasmic Y759 residue of gp130.[24)–26)] Importantly, F759 mice, which show enhanced STAT3 activation *via* gp130, a signal transducer of IL-6 family cytokines, spontaneously develop RA-like arthritis.[18),24)]

Recently, the *in vivo* roles of IL-6 in T cell functioning have been reconsidered, because this molecule is involved in interactions between two critical CD4$^+$ T cell populations: regulatory T (Treg) cells and T helper 17 (Th17) cells. Of note, Th17 cells secrete several proinflammatory cytokines, such as IL-17, and contribute to the induction of various chronic inflammatory conditions, including autoimmune diseases. Interestingly, T cell receptor (TCR)-induced development of Foxp3$^+$CD4$^+$ T cells requires TGF-β, whereas IL-6–mediated signaling *via* STAT3 suppresses the development of this population while increasing the number of Th17 cells by enhancing RORγt expression.[27)–33)] On the other hand, maturation of natural thymus-derived Foxp3$^+$CD4$^+$ T cells *in vivo* is not affected by IL-6–mediated signaling under steady state.[34)] Further, follicular helper T cells, which have recently emerged as a separate CD4$^+$ T helper lineage that specifically supports B cell activities, differentiate from naïve CD4$^+$ T cells in the presence of IL-6 *via* Bcl6 expression.[35)] Thus, IL-6 is involved in fate controlling of cytokine secreting CD4$^+$ T cells, which play a role in the development of autoimmune diseases.

Cytokines in the 1970s

When the author graduated from Osaka University Medical School in 1972, it was known that interactions among immune cells, such as T lymphocytes, B lymphocytes, and macrophages, are required for immune responses. Studies had shown that antigenic stimulation causes immune cells to produce several soluble factors, which we now call cytokines, and that these soluble factors are required for a range of immune responses. For example, antigens in the presence of T lymphocytes induce the differentiation of B lymphocytes into immunoglobulin-producing plasma cells. In 1971 and 1972, Richard W. Dutton, Anneliese Schimpl, and Eberhard Wecker reported the presence of soluble factors that induced immunoglobulin production in B cells.[36),37)] The molecular characteristics of these soluble factors, however, were

completely unknown. Many immunologists called each factor by a different name based on the biological activity the investigator was examining; thus, at times, it appeared as if each factor had as many aliases as there were immunologists. At that time, I was interested in characterizing the molecular nature of soluble factors that acted on B cells. In 1973, I applied for a visiting fellowship at the United States National Institutes of Health, and joined Dr. Albert A. Nordin's immunology laboratory at The Gerontology Research Center (now called The National Institute on Aging). There in Baltimore, I first met Dr. Tadamitsu Kishimoto and Dr. Kiyoshi Takatsu, both of whom were members of Dr. Kimishige Ishizaka's laboratory at John Hopkins University and have since taught me a great deal. After spending three years in Dr. Nordin's laboratory researching cytotoxic T cell differentiation and the soluble factors involved, I returned to Osaka University Medical School in 1976 and two years later moved to Osaka Prefectural Habikino Hospital, now the Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, where I saw a number of patients with tuberculous pleurisy.

A steep climb leading to the discovery of IL-6

The research I was conducting there demonstrated that purified protein derivative (PPD)-stimulated pleural effusion cells from patients with pulmonary tuberculosis produced soluble factors capable of inducing immunoglobulin production in B cells (Fig. 1).[38] Because this activity appeared to be very robust and a large number of lymphocytes could be obtained from each patient (up to 1×10^9 cells/patient), I decided to isolate the active factor and began to devise a purification protocol in 1978. In early 1980, Muraguchi, Kishimoto, and their colleagues,[39] as well as Teranishi, myself, and our colleagues[40] independently showed that culture supernatant fractions from stimulated human peripheral blood mononuclear cells and tonsillar mononuclear cells, respectively, induce immunoglobulin production in Epstein-Barr virus–transformed B lymphoblastoid cell lines. Kishimoto's group called this factor "TRF" or "BCDF",[39),41] whereas our group called it "TRF-like factor" or "BCDFII".[40),42),43] We partially purified the TRF-like factor and showed that it was present in gel filtration fractions corresponding to molecular weights of 22 kDa and 36 kDa, and that its isoelectric point was between 5 and 6 (Fig. 1).[40] The biological activity and physicochemical properties of the soluble factor were the same as those of a cytokine, now known as IL-6.[7] I moved to Kumamoto University Medical School in 1980 as an Associate Professor in Dr. Onoue's laboratory where I continued my efforts to purify and characterize this factor. In early 1984, I began working at Osaka University as an Associate Professor in Dr. Kishimoto's laboratory and by the end of the year, finally succeeded in purifying the factor and determining the sequence of its 14 N-terminal amino acids.[44),45] This success allowed me a brief reprieve from my work just before the new year. The next steps, however, were much harder than I expected. Several attempts to clone cDNA encoding the active protein completely failed. This raised the possibility that the identified sequence may have been incorrect or may have represented other proteins that had been co-purified with the active molecule. These worries gave me serious ill including a severe arrhythmia and kept me up at night through to the end of 1985. Thankfully, a medical checkup showed that my arrhythmia was psychogenic. I then attempted to purify the protein using 100 liters of newly obtained culture supernatants. We were lucky enough to obtain several protein fragments and their partial amino-acid sequences in March, 1986. Then, to clone the cDNA, we used three probes corresponding to three purified protein fragments. I speculated that this approach was more likely to succeed than using only one probe that corresponded to the N-terminal portion of the protein.

Standing atop the mountain: Isolation of IL-6

After 8 years of effort, my goal suddenly came into view. At 11 am on Sunday morning, May 25th, 1986, I found a clone that was bound by all three probes. It was no longer a dream; it was a reality. I was very confident that we had finally cloned a cDNA encoding the active protein, which we called "BSF-2" at that time and had previously called BCDF, BCDFII, or TRF-like factor. The sequence of the cDNA showed that BSF-2 is synthesized as a precursor consisting of 212 amino acids and is processed into a mature form consisting of 184 amino acids. From the molecular weight, isoelectric point and its induction of immunoglobulin production in an Epstein-Barr virus transformed B cell line, it was likely that BSF-2 was in fact the same molecule as the TRF-like factor that we had been analyzing. We published our results in a November 1986 issue of *Nature*.[46] To my surprise, the sequence we reported was identical to that of an IL-1–induced 26-kDa protein reported by Robert Hageman and Walter
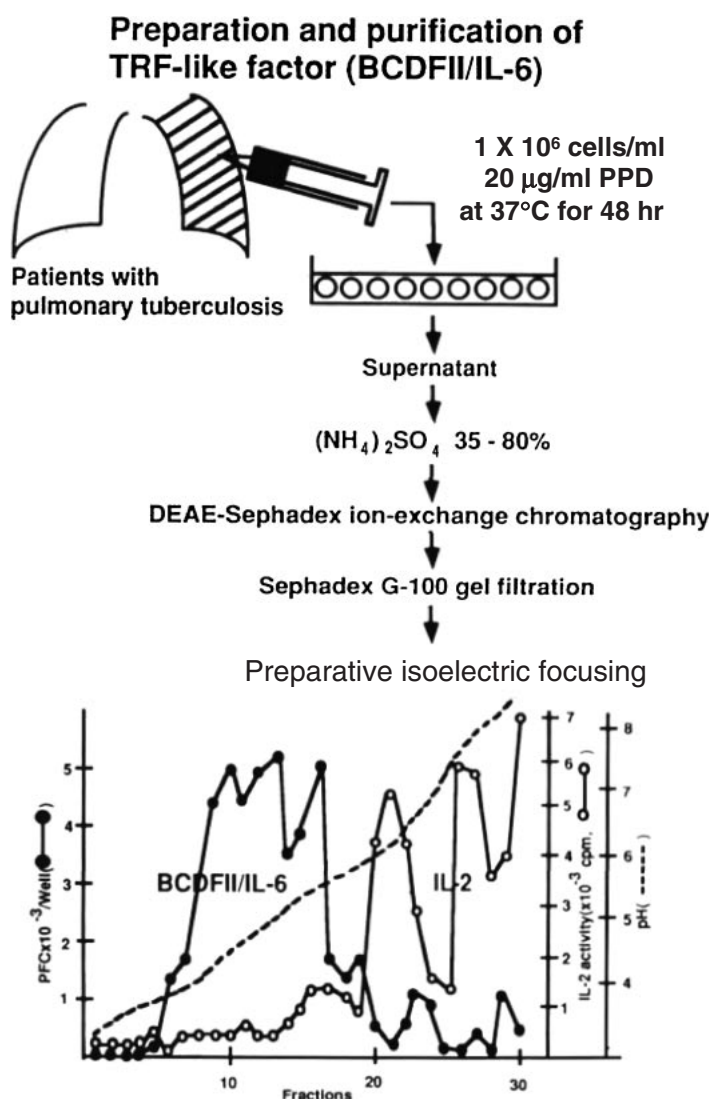
Preparation and purification of
TRF-like factor (BCDFII/IL-6)

1 X 10⁶ cells/ml
20 µg/ml PPD
at 37°C for 48 hr

Patients with
pulmonary tuberculosis

Supernatant

$(NH_4)_2SO_4$ 35 - 80%

DEAE-Sephadex ion-exchange chromatography

Sephadex G-100 gel filtration

Preparative isoelectric focusing

Fig. 1.   PPD-stimulated pleural effusion cells produce soluble factors capable of inducing immunoglobulin production in B cells. The culture supernatant from the pleural effusion cells or tonsillar mononuclear cells contained an active factor that had the same biologic and physicochemical properties of the cytokine now known as IL-6.

Fiers in the September issue of *The European Journal of Biochemistry*[47] and that of IFN-$\beta 2$ reported by Asher Zilberstein and Michel Revel in the October issue of *The EMBO Journal*.[48] A January 1988 issue of *Science* reported that an orphan interferon had found a new home.[49] Uncertainties about the roles of IFN-$\beta 2$ were being resolved as researchers found that it has numerous activities in the body's defenses.[50] Furthermore, plasmacytoma/hybridoma/myeloma growth factor and hepatocyte-stimulating factor, which regulates the biosynthesis of a variety of acute-phase proteins, were also found to be identical to this factor.[51]–[55] To resolve these nomenclature

issues, Dr. William E. Paul chaired a nomenclature meeting in New York City on December 14, 1988, at which the name "interleukin-6" was proposed (Fig. 2).[56] It is worth noting that two important molecules acting on B cells, IL-4 and IL-5 were also cloned in 1986.[57],[58]

I had wished to isolate the factor that stimulates B cells to produce immunoglobulin. Once we cloned the factor, however, it was clear that IL-6 acts on a variety of cells and tissues in addition to B cells. For instance, IL-6 induces hepatocytes to express various acute-phase proteins; activates osteoclasts to destroy bone tissue; functions as a growth factor for myeloma
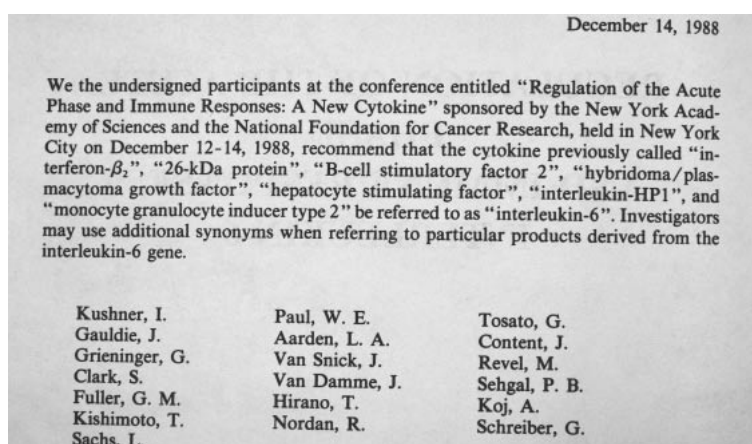
December 14, 1988

We the undersigned participants at the conference entitled "Regulation of the Acute Phase and Immune Responses: A New Cytokine" sponsored by the New York Academy of Sciences and the National Foundation for Cancer Research, held in New York City on December 12-14, 1988, recommend that the cytokine previously called "interferon-β_2", "26-kDa protein", "B-cell stimulatory factor 2", "hybridoma/plasmacytoma growth factor", "hepatocyte stimulating factor", "interleukin-HP1", and "monocyte granulocyte inducer type 2" be referred to as "interleukin-6". Investigators may use additional synonyms when referring to particular products derived from the interleukin-6 gene.

Kushner, I.	Paul, W. E.	Tosato, G.
Gauldie, J.	Aarden, L. A.	Content, J.
Grieninger, G.	Van Snick, J.	Revel, M.
Clark, S.	Van Damme, J.	Sehgal, P. B.
Fuller, G. M.	Hirano, T.	Koj, A.
Kishimoto, T.	Nordan, R.	Schreiber, G.
Sachs, L.		

Fig. 2.   At a nomenclature meeting chaired by Dr. W. E. Paul, the name "interleukin-6" was proposed for these synonymous molecules (Dec. 14, 1988; New York).[56]
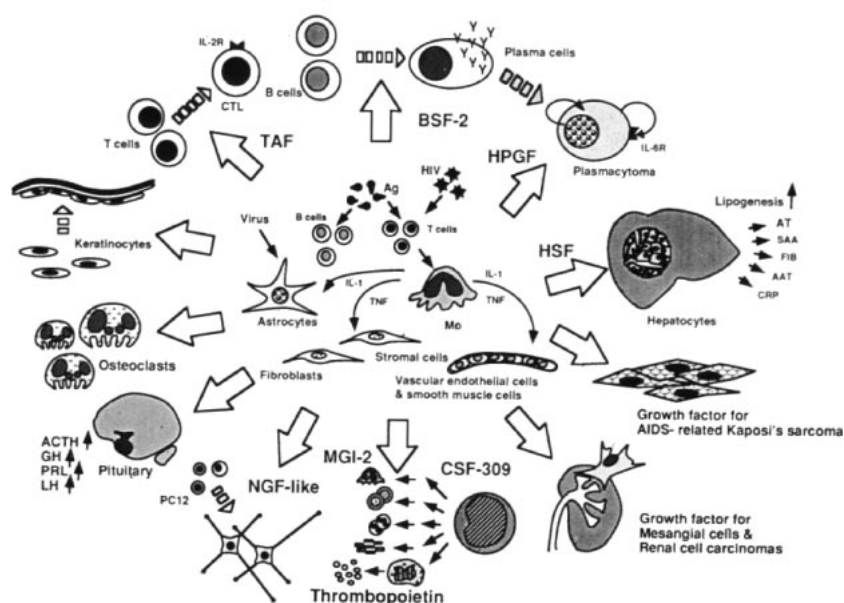
Fig. 3.   Multifunctional cytokine IL-6 is produced by a variety of cell types, including T cells, B cells, macrophages, astrocytes, stromal cells, vascular endothelial cells, smooth muscle cells, and fibroblasts. IL-6 induces antibody expression (B cell stimulatory factor 2) and cytotoxic T-lymphocyte activity (T cell activation factor). IL-6 was also referred to as a hybridoma/plasmacytoma growth factor and a hepatocyte stimulatory factor. IL-6 acts as a growth factor for AIDS-related Kaposi's sarcoma, mesangial cells, and renal cell carcinomas, and as a thrombopoietin, a nerve growth factor, a hormone-inducing factor in the pituitary gland, an osteoclast-inducing factor, and a keratinocyte growth factor.

and plasmacytoma cells; increases platelet production; and precipitates fever and cachexia. IL-6 is therefore a multifunctional cytokine that plays roles in immune responses, inflammation, hematopoiesis, and the endocrine and nervous systems (Fig. 3).[7],[59]–[61]

After IL-6 was cloned, we isolated its receptor in the late 1980s. In fact, we cloned a cDNA encoding the IL-6 receptor using expression cloning, which had just been introduced by Brian Seed and his colleagues.[62] Then, we cloned the signal-transducing receptor subunit gp130,[63],[64] demonstrating that the IL-6 receptor comprises an IL-6–specific alpha chain subunit and the gp130 signal transducer (Fig. 4). Interestingly, gp130 functions not only as a receptor subunit for IL-6, but also as
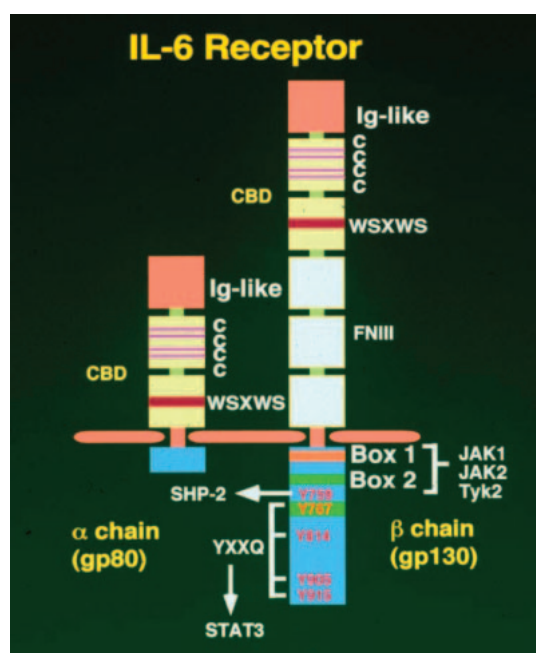
Fig. 4. IL-6 receptor is composed of two subunits: an IL-6–specific alpha chain subunit and the signal transducer gp130.

a signal transducer for other cytokines, including IL-11, OSM, LIF, CT-1, CNTF, and IL-27 (Fig. 5).[23),24)] Although the results had already exceeded my expectations, this was not the end of the story.

The view from the mountaintop: IL-6 is critically involved in autoimmune diseases, including RA

Patients with cardiac myxoma show a variety of autoimmune symptoms, such as hypergammaglobu-

linemia, the presence of autoantibodies, and increased acute-phase protein levels. Each of these symptoms is ameliorated when the tumor cells are resected, suggesting that cardiac myxoma cells induce autoimmunity. We found that cardiac myxoma cells produce IL-6,[45)] providing the first evidence of a role for IL-6 in an autoimmune disease. We then found that patients with RA have high synovial levels of IL-6 (Fig. 6),[11)] suggesting, for the first time, that IL-6 is involved in RA, a chronic autoimmune polyarthritis. Although both genetic and environmental factors contribute to this disease, the specific etiology is unknown. Patients with RA show a variety of symptoms, including polyclonal plasmacytosis accompanied by the production of rheumatoid factor, increased levels of acute-phase proteins, enhanced bone resorption, and increased platelet numbers, all of which do not at first glance appear closely related. The pleiotropic nature of IL-6, however, may shed some light on this puzzle. I speculated that dysregulation of IL-6 gene expression or activity is intimately related to the development of RA.[7),65)]

In the early 1990s, after being appointed Professor at the Osaka University Medical School, I proposed a working hypothesis for the mechanisms that underlie chronic inflammatory proliferative disease (CIPD) and certain autoimmune diseases in which IL-6 is thought to play a role.[7),65)] In the model, constitutive activation of a set of transcription factors—for example, NF-κB—critically governs the onset and progression of these diseases (Fig. 7). During the initial phase, inflammation and transcription factor activation can be induced by a variety of stimuli, including infection, foreign materi-
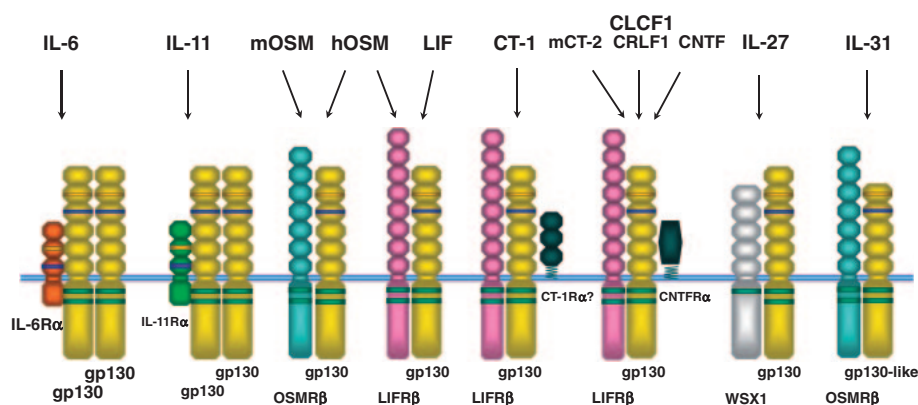

Fig. 5. gp130 is not only a receptor subunit for IL-6, but also functions as a signal transducer for other cytokines like IL-11, OSM, LIF, CT-1, CNTF, and IL-27.
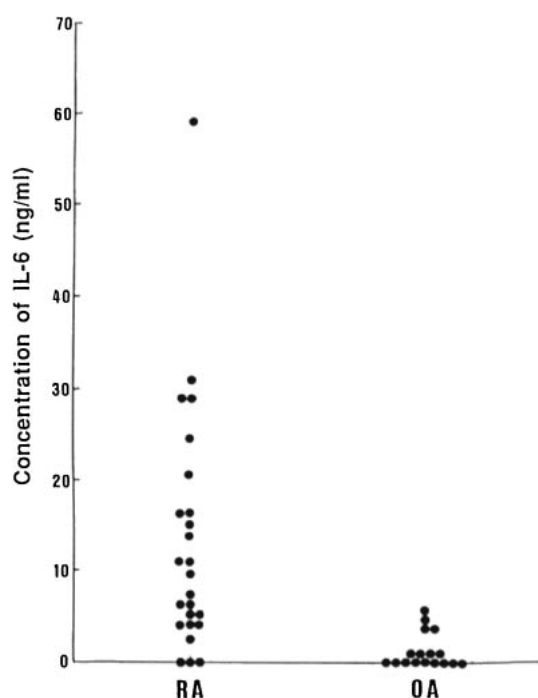
Fig. 6.   High levels of IL-6 are present in the synovial fluids of RA patients.[11] OA, osteoarthritis.

als, and/or injury that lead to the expression of IL-6 and other cytokines, MHC molecules, adhesion molecules, autoantigens, and transcription factors. In the context of MHC molecules, autoantigens may

then be recognized by autoreactive T cells, activating the T cells to propagate the inflammatory response. Because the first phase could occur in nonimmune cells or tissues, this model implies that interactions between nonimmune and immune systems may play critical roles in autoimmune diseases and CIPD.

To understand the molecular networks regulated by IL-6, we investigated IL-6 receptor–related signal transduction pathways. We showed that IL-6 induces two major signal transduction pathways: STAT3 and SHP2/Gab/MAPK signaling, which involve the gp130 YxxQ and Y759 motives, respectively (Fig. 8).[24),25)] We then attempted to clarify the *in vivo* roles of each of these gp130-associated signaling cascades. We generated a series of knock-in mouse lines in which gp130-mediated SHP2 or STAT3 signaling was selectively disrupted.[26)] To create SHP2 signal–deficient mice (F759 mice), we mutated Y759 of gp130 to a phenylalanine residue. F759 mice show enhanced STAT3 activation through gp130 because Y759 is required for SOCS3-mediated negative feedback mechanisms. The most intriguing finding was that F759 mice spontaneously develop a RA-like joint disease (F759 arthritis) (Fig. 9).[18)] This was the first definitive evidence that showed a critical role for IL-6 in a spontaneous autoimmune disease. Interestingly, like RA, F759 arthritis is a late onset disease, characterized by a symmetrical and progressive presentation. F759 mice show a variety of
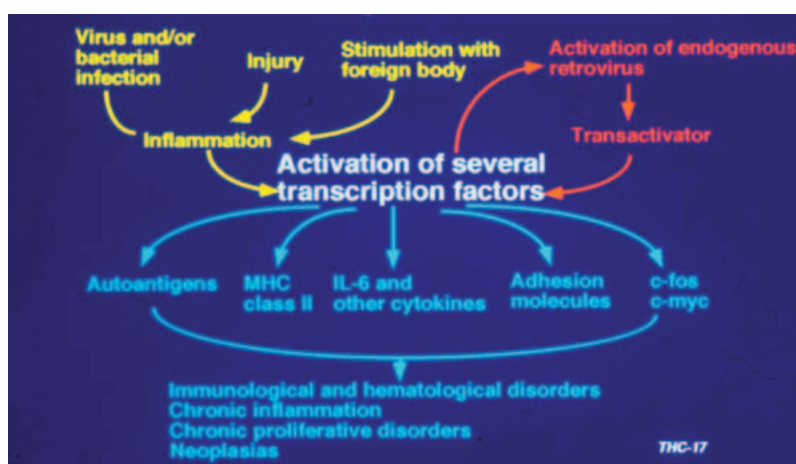

Fig. 7.   A working hypothesis for the mechanisms involved in certain autoimmune diseases and CIPD. During the initial phase, inflammation and transcription factor activation can be induced by a variety of stimuli, including infection, foreign materials, and/or injury, leading to the expression of IL-6 and other cytokines, MHC molecules, adhesion molecules, autoantigens, and transcription factors. Autoantigens in the context of MHC molecules may be recognized by autoreactive T cells, activating the T cells to propagate the inflammatory response. Because the first phase can occur in nonimmune cells or tissues, this model implies that interactions between nonimmune and the immune systems may play critical roles in autoimmune diseases and CIPD.
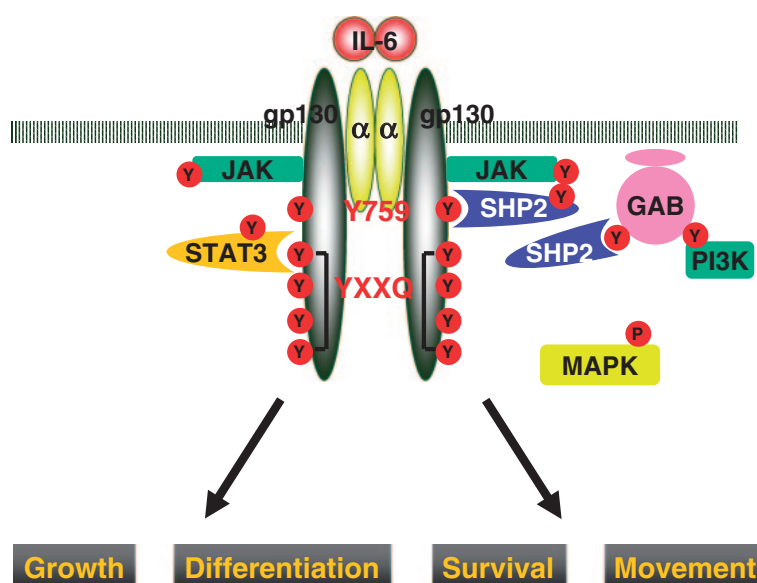
Fig. 8.   IL-6 induces two major signal transduction pathways, STAT3 signaling and SHP2/Gab/MAPK signaling. Activation of these pathways depends on the gp130 YxxQ motives and Y759, respectively.
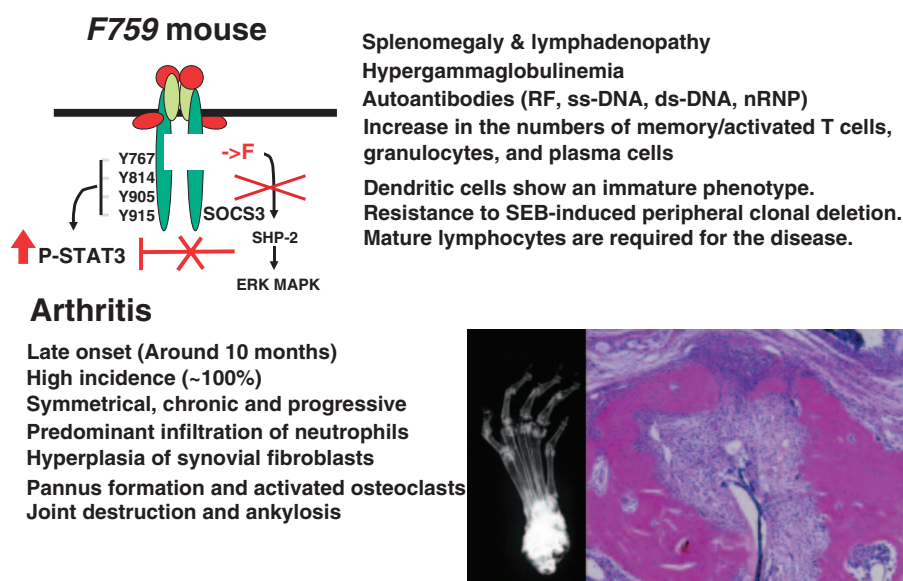

Fig. 9.   F759 mice show enhanced STAT3 activation in response to IL-6 owing to the Y759F mutation in gp130, which inhibits SOCS3-mediated negative feedback. These mutant mice spontaneously develop a RA-like joint disease (F759 arthritis). Characteristics of the disease are included in the figure: late onset; nearly 100% incidence; symmetrical, chronic, and progressive symptoms; infiltration of neutrophils into the joints; hyperplasia of synovial fibroblasts; pannus formation and the presence of activated osteoclasts; joint destruction and ankylosis; splenomegaly and lymphadenopathy; hypergammaglobulinemia; autoantibody productions; increased numbers of memory/activated T cells, granulocytes, plasma cells, and immature dendritic cells; resistance to super antigen-induced T cell death; and a requirement for lymphocytes during disease development.

immunological abnormalities, including hypergammaglobulinemia, autoantibody production, and an increased number of memory activated T cells. These effects result from a point mutation in gp130, leading to enhanced STAT3 activation by IL-6. We then asked whether HTLV-1 infection serves as an environmental trigger for F759 arthritis. Iwakura and his colleagues had shown that HTLV-1 pX transgenic
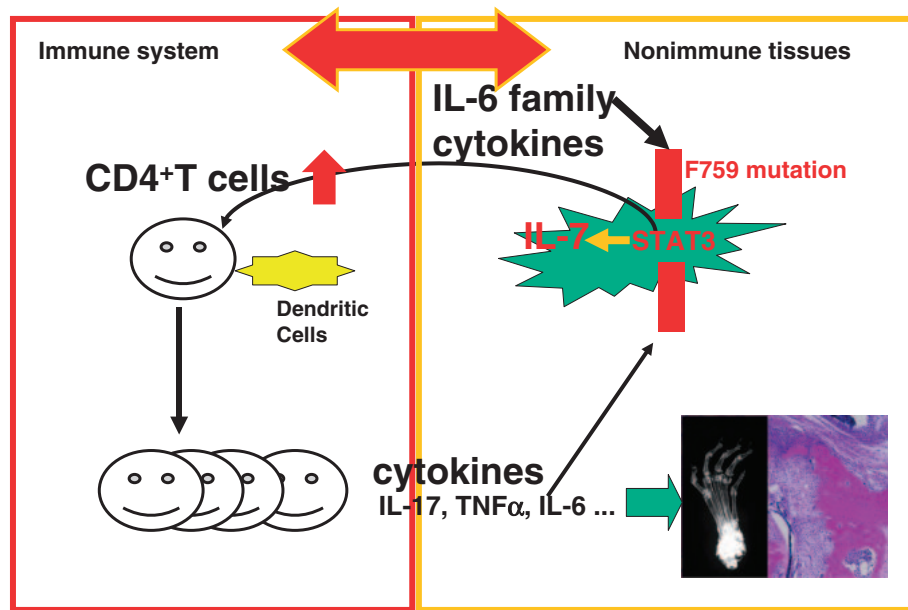
Fig. 10. Interactions between nonimmune tissues and the immune system play critical etiologic roles in autoimmune diseases. The F759 mutation in nonimmune cells, particularly in type 1 collagen^{+} fibroblasts, induces excessive IL-7 production, followed by activation of CD4^{+} T cells in the presence of dendritic cells. Activated CD4^{+} T cells express cytokines, including IL-17A, TNF-α, and IL-6, which leads to the development of arthritis.

mice, a model of HTLV-1 infection, developed arthritis in certain genetic backgrounds.[66)] Similarly, we found that HTLV-1 pX enhanced F759 arthritis in a C57BL/6 background.[67)] Thus, the F759 mutation enhanced STAT3 activation *via* IL-6, whereas HTLV-1 pX activated NF-κB, suggesting that both STAT3 and NF-κB are involved in F759 arthritis.

Using bone-marrow transplantation studies and various knock-out mouse strains, we demonstrated that F759 arthritis is CD4^{+} T cell–dependent, and that the gp130 F759 mutation must be present in nonhematopoietic cells, but not in immune cells. In response to IL-6 stimulation, these nonhematopoietic cells from F759 mice show enhanced production of the T-cell survival factor IL-7, leading to the activation of CD4^{+} T cells *via* homeostatic proliferation, an important step in the development of F759 arthritis (Fig. 10).[19)] Thus, our results demonstrate that interactions between nonimmune tissues and the immune system make critical contributions to autoimmune F759 arthritis and suggest that nonimmune systems may play a more general etiologic role in autoimmune diseases.

Classification of CD4^{+} T cells into T helper 1 (Th1) and Th2 cells has provided a framework for understanding the contributions of various CD4^{+} T cell subtypes to autoimmune diseases.[68)–70)] Further-
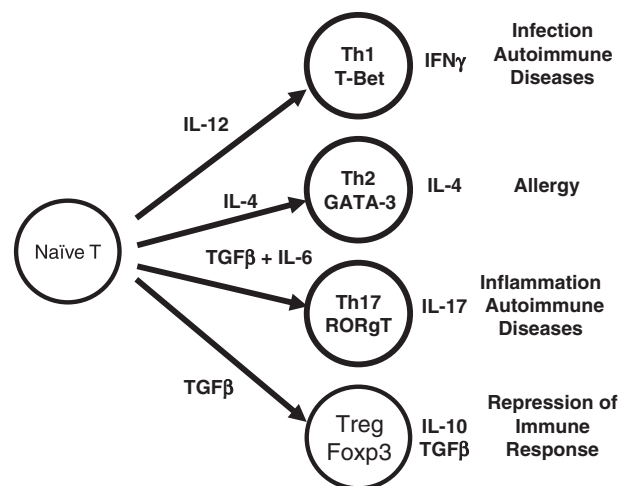

Fig. 11. IL-6 together with TGFβ induces Th17 cell development, a pivotal step in the development of autoimmune diseases and inflammation.

more, recent studies have identified Th17 cells as a previously unknown arm of the CD4^{+} T cell effector response (Fig. 11). These cells secrete several proinflammatory cytokines, including IL-17A, an important cytokine in such autoimmune diseases as collagen-induced arthritis, experimental autoimmune encephalomyelitis (EAE), and the arthritis disorders

that develop in SKG and IL-1 receptor antagonist–deficient mice.[71)–74)] F759 arthritis is also dependent on IL-17A.[75)] Another T cell subset, Treg cells, has been shown to regulate immune responses, while abnormalities in these cells cause autoimmune diseases.[76)] Additionally, IL-6 together with TGF-β induces Th17 cell development, whereas TGF-β induces Treg cell development. Thus, IL-6 is a key cytokine involved in T cell differentiation (Fig. 11).[30),31)] We have shown that gp130 and STAT3 in T cells are essential for Th17 development.[34)] Taken together, these results indicate that IL-6 makes significant contributions to both normal immune responses and autoimmune diseases.

Our research has also demonstrated that IL-6 not only induces IL-17A expression, but is also a target gene for IL-17A in nonimmune cells like fibroblasts. Importantly, IL-6 is a critical downstream target gene for IL-17A in F759 arthritis. We showed that IL-17A–induced NF-κB activation and subsequent IL-6 gene expression are augmented in the presence of IL-6. This synergistic induction of IL-6 expression is mediated by an interaction between NF-κB and STAT3. IL-6 then induces Th17 cells to produce IL-17A, completing a positive feedback loop in nonimmune cells. We have named this positive loop the "IL-6 amplifier" (Fig. 12).[75)] Of note, we showed that the development of F759 autoimmune arthritis requires the IL-6 amplifier in type 1 collagen$^+$ nonimmune tissues. The IL-6 amplifier plays a central role in the interactions between the immune system and nonimmune tissues through the activation of both NF-κB and STAT3. This signaling amplifier is activated in F759 mice, for example, in which IL-6–mediated STAT3 activation is enhanced by the lack of SOCS3-mediated negative feedback. Also consistent with this idea, HTLV1-pX activates NF-κB and enhances F759 arthritis. We also showed that MOG-specific, Th17 cell–induced EAE is dependent on STAT3 in type 1 collagen$^+$ nonimmune tissues.[75)] Collectively, these data led us to hypothesize that a number of different events, including antigen-specific T cell development, viral infection, injury, and/or physical stimulation, are capable of activating the IL-6 amplifier through STAT3 and/or NF-κB in nonimmune tissues, and thereby may critically contribute to autoimmune diseases (Fig. 13). In this scenario, tissue-specific autoimmune disease could be triggered by not only tissue-specific immune response but also any event that activates STAT3 and/or NF-κB in the target tissue (*e.g.*, activation of the IL-6 amplifier).
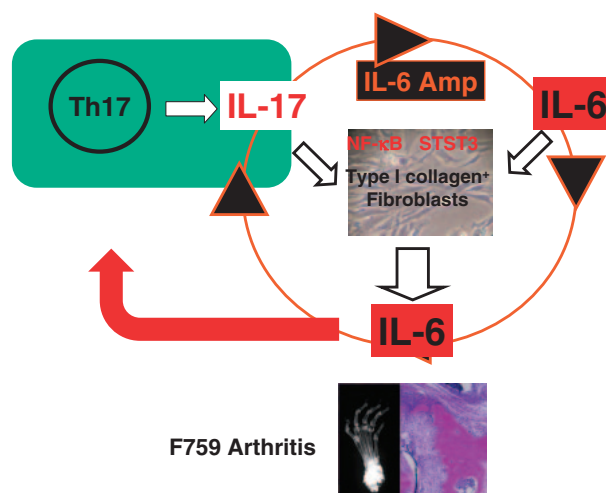

Fig. 12. The IL-6 amplifier plays a critical role in F759 arthritis.

Conclusions

Thirty-eight years ago, when I graduated from the Osaka University Medical School, I could not have imagined where this research would take me. We discovered IL-6 is an essential multifunctional cytokine involved not only in immune responses, but also embryonic development, inflammation, and the hematopoietic and nervous systems. We have now begun to understand how autoimmune diseases such as RA develop and the relationship with IL-6. For example, dysregulation of IL-6 signaling induces autoimmune arthritis in F759 mice; and we have identified an IL-6 amplifier, which critically contributes to F759 arthritis. Thus, it is possible that any factor capable of chronically activating NF-κB and/or STAT3 in nonimmune tissues will precipitate RA, a model that may be generally applicable to other autoimmune diseases (Fig. 13). Therapeutically targeting IL-6 receptors has been shown beneficial for many RA patients.[16),17)] Thus, our scientific research during the last 30 years has helped elucidate the immunological underpinnings of and laid the foundation for a new biologic drug for RA and potentially other autoimmune and inflammatory diseases.
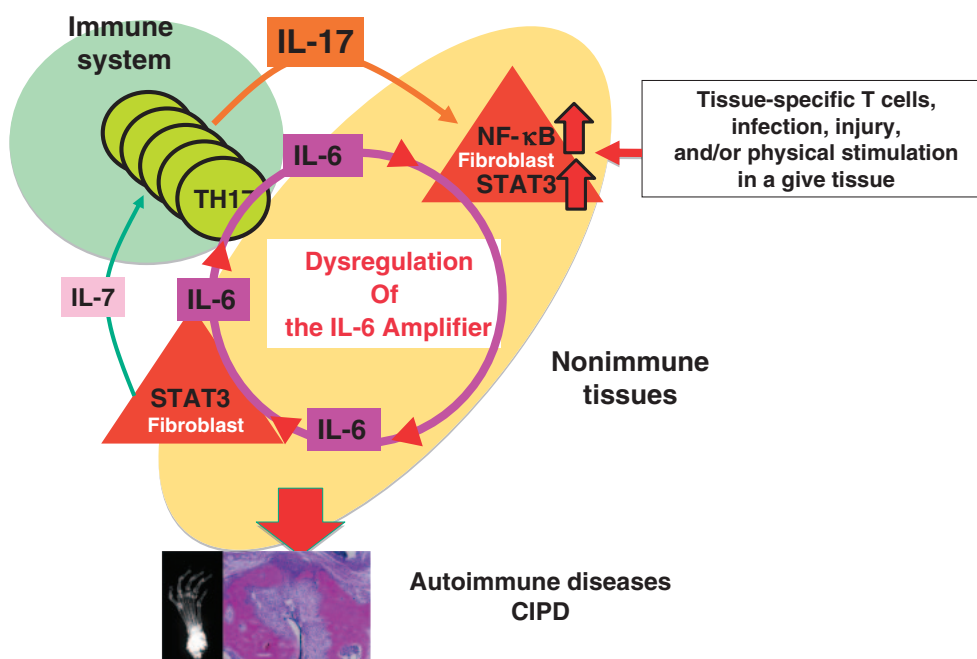
Acknowledgements

Fig. 13.   Any factor that activates STAT3 and/or NF-κB in a nonimmune tissue outside of the immune system may trigger autoimmune diseases or CIPD through dysregulation of the IL-6 amplifier.

Taniguchi, Takeshi Watanabe, and Shigekazu Nagata for their valuable advice and encouragement. I also wish to thank many friends and colleagues, including Drs. Tetsuya Taga, Kiyoshi Yasukawa, Katsuhiko Yamasaki, Tsuyoshi Teranishi, Shizuo Akira, Atsushi Muraguchi, Hitoshi Kikutani, Kazuyuki Yoshizaki, and Yoichiro Iwakura. Finally, I would like to acknowledge a number of people who have worked in my laboratory, including those who have already left to start their own laboratories: Drs. Koichi Nakajima, Tadashi Matsuda, Katsuhiko Ishihara, Masahiko Hibi, Tsuneyasu Kaisho, Yojiro Yamanaka, Toshiyuki Fukada, Keigo Nishida, Motoyuki Ito, Takuya Ohtani, Toru Atsumi, Satoru Yamasaki, Shinichiro Sawa, Daisuke Kamimura, Hideki Ogura, Yukihisa Sawa, Masaaki Murakami, and many other colleagues. This review was written using the transcript from the 2009 Crafoord Prize Lecture, which was given at Lund University in Sweden. We also thank Dr. Karagiannis (Osaka University, Japan) for carefully reading this manuscript.

References

1) Marrack, P., Kappler, J. and Kotzin, B. (2001) Autoimmune disease: why and where it occurs. Nat. Med. **7**, 899–905.

2) Ermann, J. and Fathman, C.G. (2001) Autoimmune diseases: genes, bugs and failed regulation. Nat. Immunol. **2**, 759–761.

3) O'Shea, J.J., Ma, A. and Lipsky, P. (2002) Cytokines and autoimmunity. Nat. Rev. Immunol. **2**, 37–45.

4) Mathis, D. and Benoist, C. (2004) Back to central tolerance. Immunity **20**, 509–516.

5) Mocci, S., Lafferty, K. and Howard, M. (2000) The role of autoantigens in autoimmune disease. Curr. Opin. Immunol. **12**, 725–730.

6) Skapenko, A., Leipe, J., Lipsky, P.E. and Schulze-Koops, H. (2005) The role of the T cell in autoimmune inflammation. Arthritis Res. Ther. **7**, S4–S14.

7) Hirano, T. (1998) Interleukin 6 and its receptor: ten years later. Int. Rev. Immunol. **16**, 249–284.

8) Matsumoto, I., Staub, A., Benoist, C. and Mathis, D. (1999) Arthritis Provoked by Linked T and B Cell Recognition of a Glycolytic Enzyme. Science **286**, 1732–1735.

9) Hirano, T. (2002) Cytokines in autoimmune disease and chronic inflammatory proliferative disease. Cytokine Growth Factor Rev. **13**, 297–298.

10) Hirano, T. (2002) Revival of the autoantibody model in rheumatoid arthritis. Nat. Immunol. **3**, 342–344.

11) Hirano, T., Matsuda, T., Turne, M., Miyasaka, N., Buchan, G., Tang, B. *et al.* (1988) Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis. Eur. J. Immunol. **18**, 1797–1801.

12) Ishihara, K. and Hirano, T. (2002) IL-6 in autoimmune disease and chronic inflammatory proliferative disease. Cytokine Growth Factor Rev. **13**,

357–368.

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

14) Feldmann, M., Brennan, F. and Maini, R. (1996) Role of cytokines in rheumatoid arthritis. Annu. Rev. Immunol. **14**, 397–440.

15) Feldmann, M. and Maini, R.N. (2001) Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned? Annu. Rev. Immunol. **19**, 163–196.

16) Nishimoto, N., Kishimoto, T. and Yoshizaki, K. (2000) Anti-interleukin 6 receptor antibody treatment in rheumatic disease. Ann. Rheum. Dis. **59**, i21–i27.

17) Nishimoto, N. and Kishimoto, T. (2004) Inhibition of IL-6 for the treatment of inflammatory diseases. Curr. Opin. Pharmacol. **4**, 386–391.

18) Atsumi, T., Ishihara, K., Kamimura, D., Ikushima, H., Ohtani, T., Hirota, S. *et al.* (2002) A point mutation of Tyr-759 in interleukin 6 family cytokine receptor subunit gp130 causes autoimmune arthritis. J. Exp. Med. **196**, 979–990.

19) Sawa, S., Kamimura, D., Jin, G.H., Morikawa, H., Kamon, H., Nishihara, M. *et al.* (2006) Autoimmune arthritis associated with mutated interleukin (IL)-6 receptor gp130 is driven by STAT3/IL-7-dependent homeostatic proliferation of CD4+ T cells. J. Exp. Med. **12**, 1459–1470.

20) Hata, H., Sakaguchi, N., Yoshitomi, H., Iwakura, Y., Sekikawa, K., Azuma, Y. *et al.* (2004) Distinct contribution of IL-6, TNF-alpha, IL-1, and IL-10 to T cell-mediated spontaneous autoimmune arthritis in mice. J. Clin. Invest. **114**, 582–588.

21) Alonzi, T., Fattori, E., Lazzaro, D., Costa, P., Probert, L., Kollias, G. *et al.* (1998) Interleukin 6 is required for the development of collagen-induced arthritis. J. Exp. Med. **187**, 461–468.

22) Ohshima, S., Saeki, Y., Mima, T., Sasai, M., Nishioka, K., Nomura, S. *et al.* (1998) Interleukin 6 plays a key role in the development of antigen-induced arthritis. Proc. Natl. Acad. Sci. USA **95**, 8222–8226.

23) Murakami, M., Kamimura, D. and Hirano, T. (2004) New IL-6 (gp130) family cytokine members, CLC/NNT1/BSF3 and IL-27. Growth Factors **2004**, 2.

24) Kamimura, D., Ishihara, K. and Hirano, T. (2003) IL-6 signal transduction and its physiological roles: the signal orchestration model. Rev. Physiol. Biochem. Pharmacol. **149**, 1–38.

25) Fukada, T., Hibi, M., Yamanaka, Y., Takahashi-Tezuka, M., Fujitani, Y., Yamaguchi, T. *et al.* (1996) Two signals are necessary for cell proliferation induced by a cytokine receptor gp130: involvement of STAT3 in anti-apoptosis. Immunity **5**, 449–460.

26) Ohtani, T., Ishihara, K., Atsumi, T., Nishida, K., Kaneko, Y., Miyata, T. *et al.* (2000) Dissection of signaling cascades through gp130 *in vivo*: Reciprocal roles for STAT3- and SHP2-mediated signals in immune responses. Immunity **12**, 95–105.

27) Cua, D.J., Sherlock, J., Chen, Y., Murphy, C.A., Joyce, B., Seymour, B. *et al.* (2003) Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature **421**, 744–748.

28) Harrington, L.E., Hatton, R.D., Mangan, P.R., Turner, H., Murphy, T.L., Murphy, K.M. *et al.* (2005) Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat. Immunol. **6**, 1123–1132.

29) Park, H., Li, Z., Yang, X.O., Chang, S.H., Nurieva, R., Wang, Y.H. *et al.* (2005) A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat. Immunol. **6**, 1133–1141.

30) Veldhoen, M., Hocking, R., Atkins, C., Locksley, R. and Stockinger, B. (2006) TGF in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity **24**, 179–189.

31) Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T., Oukka, M. *et al.* (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature **441**, 235–238.

32) Mangan, P.R., Harrington, L.E., O'Quinn, D.B., Helms, W.S., Bullard, D.C., Elson, C.O. *et al.* (2006) Transforming growth factor-beta induces development of the T(H)17 lineage. Nature **441**, 231–234.

33) Ivanov, I.I., McKenzie, B.S., Zhou, L., Tadokoro, C.E., Lepelley, A., Lafaille, J.J. *et al.* (2006) The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell **126**, 1121–1133.

34) Nishihara, M., Ogura, H., Ueda, N., Tsuruoka, M., Kitabayashi, C., Tsuji, F. *et al.* (2007) IL-6-gp130-STAT3 in T cells directs the development of IL-17+ Th with a minimum effect on that of Treg in the steady state. Int. Immunol. **19**, 695–702.

35) Linterman, M.A. and Vinuesa, C.G. (2010) Signals that influence T follicular helper cell differentiation and function. Semin. Immunopathol. (in press).

36) Dutton, R.W., Falkoff, R., Hirst, J.A., Hoffman, M., Kappler, J.W., Kattman, J.F. *et al.* (1971) Is there evidence for a nonantigen specific diffusable chemical mediator from the thymus-derived cell in the initiation of the immune response? Pro. Immunol. **1**, 355.

37) Schimpl, A. and Wecker, E. (1972) Replacement of T cell function by a T cell product. Nat. New Biol. **235**, 15–17.

38) Hirano, T., Teranishi, T., Toba, T., Sakaguchi, N., Fukukawa, T. and Tsuyuguchi, I. (1981) Human helper T cell factor(s) (ThF). I. Partial purification and characterization. J. Immunol. **126**, 517–522.

39) Muraguchi, A., Kishimoto, T., Miki, Y., Kuritani, T., Kaieda, T., Yoshizaki, K. *et al.* (1981) T cell-replacing factor(TRF)-induced IgG secretion in human B blastoid cell line and demonstration of acceptors for TRF. J. Immunol. **127**, 412–416.

40) Teranishi, T., Hirano, T., Arima, N. and Onoue, K.

(1982) Human helper T cell factor(s) (ThF). II. Induction of IgG production in B lymphoblastoid cell lines and identification of T cell replacing factor (TRF)-like factor(s). J. Immunol. **128**, 1903–1908.

41) Okada, M., Sakaguchi, N., Yoshimura, N., Hara, H., Shimizu, K., Yoshida, N. *et al.* (1983) B cell growth factor and B cell differentiation factor from human T hybridomas: Two distinct kinds of BCGFs and their synergism in B cell proliferation. J. Exp. Med. **157**, 583–590.

42) Hirano, T., Teranishi, T. and Onoue, K. (1984) Human helper T cell factor(s). III. Characterization of B cell differentiation factor I (BCDF I). J. Immunol. **132**, 229–234.

43) Hirano, T., Teranishi, T., Lin, B. and Onoue, K. (1984) Human helper T cell factor(s). IV. Demonstration of a human late-acting B cell differentiation factor acting on Staphylococcus aureus Cowan I-stimulated B cells. J. Immunol. **133**, 798–802.

44) Hirano, T., Taga, T., Nakano, N., Yasukawa, K., Kashiwamura, S., Shimizu, K. *et al.* (1985) Purification to homogeneity and characterization of human B-cell differentiation factor (BCDF or BSFp-2). Proc. Natl. Acad. Sci. USA **82**, 5490–5494.

45) Hirano, T., Taga, T., Yasukawa, K., Nakajima, K., Nakano, N., Takatsuki, F. *et al.* (1987) Human B cell differentiation factor defined by an anti-peptide antibody and its possible role in autoantibody production. Proc. Natl. Acad. Sci. USA **84**, 228–231.

46) Hirano, T., Yasukawa, K., Harada, H., Taga, T., Watanabe, Y., Matsuda, T. *et al.* (1986) Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature **324**, 73–76.

47) Haegeman, G., Content, J., Volckaert, G., Derynck, R., Tavernier, J. and Fiers, W. (1986) Structural analysis of the sequence encoding for an inducible 26-kDa protein in human fibroblasts. Eur. J. Biochem. **159**, 625–632.

48) Zilberstein, A., Ruggieri, R., Korn, J.H. and Revel, M. (1986) Structure and expression of cDNA and genes for human interferon-β-2, a distinct species inducible by growth-stimulatory cytokines. EMBO J. **5**, 2529–2537.

49) Marx, J.L. (1988) Orphan interferon finds a new home. Science **239**, 25–26.

50) Sehgal, P.B., May, T.T., Tamm, I. and Vilcek, J. (1987) Human β2 interferon and B-cell differentiation factor BSF-2 are identical. Science **235**, 731–732.

51) Andus, T., Geiger, T., Hirano, T., Northoff, H., Ganter, U., Bauer, J. *et al.* (1987) Recombinant human B cell stimulatory factor 2 (BSF-2/IFN-beta 2) regulates β-fibrinogen and albumin mRNA levels in Fao-9 cells. FEBS Lett. **221**, 18–22.

52) Gauldie, J., Richards, C., Harnish, D., Lansdorp, P. and Baumann, H. (1987) Interferon β2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. Proc. Natl. Acad. Sci. USA **84**, 7251–7255.

53) Van Damme, J., Opdenakker, G., Simpson, R.J., Rubira, M.R., Cayphas, S., Vink, A. *et al.* (1987) Dentification of the human 26-kDa protein, interferon β2 (IFNβ2), as a B cell hybridoma/plasmacytoma growth factor induced by interleukin 1 and tumor necrosis factor. J. Exp. Med. **165**, 914–919.

54) Kawano, M., Hirano, T., Matsuda, T., Taga, T., Horii, Y., Iwato, K. *et al.* (1988) Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. Nature **332**, 83–85.

55) Van Snick, J., Cayphas, S., Szikora, J.P., Renauld, J.C., Van Roost, E., Boon, T. *et al.* (1988) cDNA cloning of murine interleukin-HP1:homology with human interleukin 6. Eur. J. Immunol. **18**, 193–197.

56) Sehgal, P.B., Grienger, G. and Tosato, G. (eds.) (1989) Regulation of the acute phase and immune responses: Interleukin-6. Ann. New York Acad. Sci. **557**, 1–583.

57) Noma, Y., Sideras, P., Naito, T., Bergstedt-Lindquist, S., Azuma, C., Severinson, E. *et al.* (1986) Cloning of cDNA encoding the murine IgG1 induction factor by a novel strategy using SP6 promoter. Nature **319**, 640–646.

58) Kinashi, T., Harada, N., Severinson, E., Tanabe, T., Sideras, P., Konishi, M. *et al.* (1986) Cloning of complementary DNA encoding T-cell replacing factor and identity with B-cell growth factor II. Nature **324**, 70–73.

59) Hirano, T. and Kishimoto, T. (1990) Interleukin-6. Handbook of Experimental Pharmacology **95/I**, 633–665.

60) Van Snick, J. (1990) Interleukin-6: an overview. Annu. Rev. Immunol. **8**, 253–278.

61) Heinrich, P.C., Castell, J.V. and Andus, T. (1990) Interleukin-6 and the acute phase response. Biochem. J. **265**, 621–636.

62) Yamasaki, K., Taga, T., Hirata, Y., Yawata, H., Kawanishi, Y., Seed, B. *et al.* (1988) Cloning and expression of the human interleukin-6 (BSF-2/IFN beta 2) receptor. Science **241**, 825–828.

63) Hibi, M., Murakami, M., Saito, M., Hirano, T., Taga, T. and Kishimoto, T. (1990) Molecular cloning and expression of an IL-6 signal transducer, gp130. Cell **63**, 1149–1157.

64) Taga, T., Hibi, M., Hirata, Y., Yamasaki, K., Yasukawa, K., Matsuda, T. *et al.* (1989) Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. Cell **58**, 573–581.

65) Hirano, T. (1992) Interleukin-6 and its relation to inflammation and disease. Clin. Immunol. Immunopathol. **62**, S60–S65.

66) Iwakura, Y., Tosu, M., Yoshida, E., Takiguchi, M., Sato, K., Kitajima, I. *et al.* (1991) Induction of inflammatory arthropathy resembling rheumatoid arthritis in mice transgenic for HTLV-I. Science **253**, 1026–1028.

67) Ishihara, K., Sawa, S., Ikushima, H., Hirota, S., Atsumi, T., Kamimura, D. *et al.* (2004) The point mutation of tyrosine 759 of the IL-6 family cytokine receptor gp130 synergizes with HTLV-1 pX in promoting rheumatoid arthritis-like arthritis. Int. Immunol. **16**, 455–465.

68) Zhu, J., Yamane, H., Cote-Sierra, J., Guo, L. and Paul, W.E. (2006) GATA-3 promotes Th2 responses through three different mechanisms: induction of Th2 cytokine production, selective growth of Th2 cells and inhibition of Th1 cell-specific factors. Cell Res. **16**, 3–10.

69) Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu. Rev. Immunol. **7**, 145–173.

70) Glimcher, L.H. and Murphy, K.M. (2000) Lineage commitment in the immune system: the T helper lymphocyte grows up. Genes Dev. **14**, 1693–1711.

71) Iwakura, Y. and Ishigame, H. (2006) The IL-23/IL-17 axis in inflammation. J. Clin. Invest. **116**, 1218–1222.

72) Hirota, K., Hashimoto, M., Yoshitomi, H., Tanaka, S., Nomura, T., Yamaguchi, T. *et al.* (2007) T cell self-reactivity forms a cytokine milieu for spontaneous development of IL-17$^+$ Th cells that cause autoimmune arthritis. J. Exp. Med. **204**, 41–47.

73) Bettelli, E., Oukka, M. and Kuchroo, V.K. (2007) T$_H$-17 cells in the circle of immunity and autoimmunity. Nat. Immunol. **8**, 345–350.

74) Afzali, B., Lombardi, G., Lechler, R.I. and Lord, G.M. (2007) The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease. Clin. Exp. Immunol. **148**, 32–46.

75) Ogura, H., Murakami, M., Okuyama, Y., Tsuruoka, M., Kitabayashi, C., Kanamoto, M. *et al.* (2008) Interleukin-17 Promotes Autoimmunity by Triggering a Positive-Feedback Loop via Interleukin-6 Induction. Immunity **29**, 628–636.

76) Sakaguchi, S. (2000) Regulatory T cells: key controllers of immunologic self-tolerance. Cell **101**, 455–458.

Profile

Toshio Hirano was born in 1947. He graduated from the Faculty of Medicine at Osaka University in 1972 and started his research carrier in 1973 with studies on the regulation of the development of cytotoxic T lymphocytes at the Gerontology Research Center (now called the National Institute on Aging), the National Institutes of Health, the USA. He returned to the Faculty of Medicine at Osaka University in 1976 to study B lymphocyte differentiation. In 1978, he moved to Osaka Prefectural Habikino Hospital, now the Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, where he started his pioneering work on the characterization and isolation of soluble factors inducing B lymphocyte differentiation into antibody forming plasma cells. This study led to the discovery of interleukin 6 in 1986. Subsequent extensive studies demonstrated that IL-6 is a multifunctional cytokine involved in not only immune response but also acute phase reaction, inflammation, hematopoiesis, and so on and that IL-6 plays critical roles in autoimmune and inflammatory diseases such as rheumatoid arthritis. These basic studies have paved the way for drug development for these diseases. He was promoted to Professor at Osaka University in 1989 (to present time). He acted as Director at Biomedical Research Center of Faculty of Medicine (1997–1999) and Dean of Graduate School of Frontier Biosciences (2004–2006). He is currently Dean of Graduate School of Medicine (2008 to present time). He is also Group Director at Research Center for Allergy and Immunology, RIKEN (2001 to present time). Between 2005 and 2006, he was President of the Japanese Society for Immunology. He was awarded Erwin von Balz Prize in 1986, CIBA-GEIGY Rheumatism Prize in 1990, The Sandoz Prize for Immunology in 1992, Osaka Science Prize in 1997, Mochida Memorial Prize in 1998, ISI Citation Laureate Award, 1981–98 in 2000, The Fujihara Prize in 2004, Medical Award of The Japan Medical Association in 2005, The Emperor's Purple Ribbon Medal in 2006 and The Crafoord Prize from the Royal Swedish Academy of Science in 2009.