The Crafoord Prize Lectures 2009

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THE INTERLEUKIN-1, THE FIRST INTERLEUKIN

Charles A. Dinarello

HOW EXTENSIVE THE VIEW FROM A MOUNTAIN TOP IS!
– A STORY OF INTERLEUKIN 6

Toshio Hirano

INTERLEUKIN 6: FROM BENCH TO BEDSIDE
– 40 YEARS IN IMMUNOLOGY

Tadamitsu Kishimoto

The Crafoord Prize Lecture held by Laureate in polyarthritis 2009, Charles A. Dinarello:

Interleukin-1, the First Interleukin

Your Majesties, President of the Academy, members of the Crafoord family, members of the Royal Swedish Academy of Science, ladies and gentlemen: It is indeed a great event in the career of a physician or a scientist to be awarded the Crafoord Prize. I am truly honored and grateful that the Royal Swedish Academy of Sciences selected our work on Interleukin-1 for the Crafoord Prize in polyarthritis.

Fever, the perennial indicator of disease in history

The molecule, interleukin-1 (IL-1), is a relatively new name for a small protein that mankind has sought to understand for several thousand years. One can imagine how early *Homo sapiens,* suffering from repeated infections or injuries, paid attention to feelings of cold and chills followed by hot skin and sweating. The response would re-occur a few hours later and repeatedly until the infection cleared or more often, the person died. This was the fever response and it was such a reliable event that the Sumerians had a pictograph of a flaming brazier, which was placed over the head of an individual to indicate fever. As human cultures developed, specific persons were charged with caring for the sick and these early 'physicians' would recognize fever as a diagnostic as well as a prognostic marker of illness. We have no trouble finding written documentation on the phases of fever by these physicians.

Seeking a mechanism for fever

With the sophistication of the Greek and Persians, we have a great deal of clinical descriptions for the various forms of fever that occur in malaria, in typhoid infections, wounds and other infections. Although I am not a scholar of Chinese or Indian medical history, it would not surprise me that these civilizations had their share of astute clinical descriptions of the association of fever with disease. One of the more relevant observations was made by Roman physicians caring for injured soldiers. The Roman physicians wrote that upon the release or drainage of pus from wounds, fevers would rapidly cease and initiate the return to health. Pus is the collection of large numbers of white blood cells gathering at the sites of infection or injured tissue. We will return to this issue of fever and pus later. During the middle ages, it is somewhat unfortunate that the observations of the Romans were replaced by theories that fever was the result of demonic possession. With the discovery of the circulation of blood came the concept that heat of fever was generated by "friction" in the blood vessels. Certainly, this explanation had its basis on the rapid heart rate that occurs during fever. Then with the discovery of bacteria came the concept that the heat of fever was the result of bacterial "fermentation" in the intestines. That explanation gained considerable support since a large number of infections were due to intestinal bacterial infections.

Laboratory investigations

During the Renaissance, we see the beginning of clinical thermometry with precise measurements of elevated body temperatures during fever and the return to baseline temperature with sweating. In the latter half of the 19th century, clinical thermometry became increasingly accurate and investigations into the mechanism of fever began in earnest. The association of pus and fever found new champions and in the early 1940's laboratory studies on

the mechanism for fever began in earnest. Two investigators deserve particular recognition: Eli Menkin and Paul Beeson. Menkin was a Russian-trained physician who immigrated to the United States and Beeson was of the generation of American academic clinical investigators that split their time between caring for patients and performing laboratory studies. What did these men do that I rank them so highly? They were convinced that the body produced its own endogenous fever-inducing substance in response to infectious agents as well as other disease processes. Today, such a concept seems obvious as sort of a 'unifying theory' for such a common physiological response but these investigators had the first real evidence.

Both Menkin and Beeson forced the formation of sterile pus in the peritoneal cavity of rabbits using a sterile irritant and then took out the pus cells, incubated them for a few hours and then injected the fluid released from the pus cells into the ear vein of trained rabbits and induced fever. Menkin called the fever-producing substance released from the pus cells 'pyrexin' and Beeson called it 'granulocyte pyrogen'. Beeson went one important step further: he showed that 'granulocyte pyrogen' was a protein substance and not contaminated by any substances from bacteria. Nearly all bacterial products, such as 'endotoxin' also produce fever in trained rabbits. Bacterial products contaminate water, laboratory equipment and are usually resistant to heat inactivation. In fact, there is little difference between the fever from endotoxins and 'granulocyte pyrogen'. This issue of fever from endotoxins and other bacterial products would plague fever research for the next 40 years and was not fully understood until a few years ago by the functional description of Toll-like receptors by Bruce Beutler.

Isolating and purifying human leukocytic pyrogen

As a medical student at Yale University, I chose for my thesis advisors Elisha Atkins and Phyllis Bodel and to work on 'granulocyte pyrogen'. By this time, the fever-producing substance was also called 'endogenous pyrogen' or 'leukocytic pyrogen'. I continued to work on the pyrogen at the NIH with my mentor Sheldon Wolff. We had a very challenging project: the purification of 'leukocytic pyrogen' from human cells to homogeneity and the determination of its N-terminal amino acids. We began the project in 1971. There were two hurdles to overcome: how to purify the protein in the absence of endotoxins or other bacterial products and how to recover enough to perform an amino acid sequence. Cell lines did not produce enough leukocytic pyrogen and so we used human blood cells. We made all buffers and custom made glass columns for fear of bacterial product contamination. All chromatography materials had to be free of endotoxins. We made a glass gel-filtration column 5.1 x 182 cm. During iso-electric focusing, we observed that there were two 'leukocytic pyrogens' and these later became know as IL-1β and IL-1α. At the final stages of purification, we could not see 'leukocytic pyrogen' by standard methods on gels and so we radiolabeled the proteins and proceeded with the purification steps until there was only one radioactive band. Upon intravenous injection into rabbits, this single band induced fever at an estimated 25 ng making 'leukocytic pyrogen' one of the most potent biologically active proteins. In 1977 we published the purification of 'leukocytic pyrogen'.

Leukocytic pyrogen does more than cause fever

By 1977, interest in fever and 'leukocytic pyrogen' had expanded to include liver and metabolic upheavals that take place during infections. Ralph Kampschmidt studied 'leukocytic endogenous mediator' as a product of pus cells to explain these changes. Jean-Michel Dayer

and Steven Krane studied 'mononuclear cell factor' for prostaglandin production. Jeremy Saklatvala studied 'catabolin' for its ability to destroy cartilage. At the other end of the universe, immunologists became interested in small proteins released from macrophages that stimulated lymphocytes. Byron Waksman, Igal Gery and Fritz Bach called the substance 'lymphocyte activating factor'. Having a method to purify 'leukocytic pyrogen', Lanny Rosenwasser and I published in 1979 that 'leukocytic pyrogen' and 'lymphocyte activating factor' were, in fact, the same molecule. The concept that a single molecule could possess so many varied biological activities was met with considerable skepticism in the scientific community and some continued to claim that our preparations were not pure, or worse, contaminated with bacterial products. Nevertheless, the field had become crowded with names for these different activities and the nomenclature IL-1 and IL-2 was born out of necessity. IL-1 was the name for the macrophage product and IL-2 for the lymphocyte product. At the time, there was no amino acid sequence for either IL-1 or IL-2, they were just names with biological activities. This was the status of 'cytokines' in 1979.

Cloning the cDNA for human IL-1β

How to resolve this issue of multiple and varied biological activities attributed to a single small protein? In the early 1980's, cDNA cloning was in its infancy but an effort to isolate the cDNA coding for IL-1 would resolve several problems as recombinant IL-1 could be tested for the various activities and there would be plenty of the molecule to study. Together with Phil Auron, Drew Webb and Alex Rich, we began isolating large amounts polyA RNA from human blood monocytes in February, 1982. We likely used 60 liters of human blood or possible more during that year. By 1984, we had succeeded and true to our previous experiences on IL-1, our paper was rejected by *Nature*. On the eve of the publication of the cDNA for IL-1 in *Proceedings of the National Academy of Sciences*, we proposed that the acute phase response was due to a single molecule, known by various names, but capable of transforming nearly all cells and organs from normal function to disease-associated functions (Figure 1). Indeed, the concept was proven to
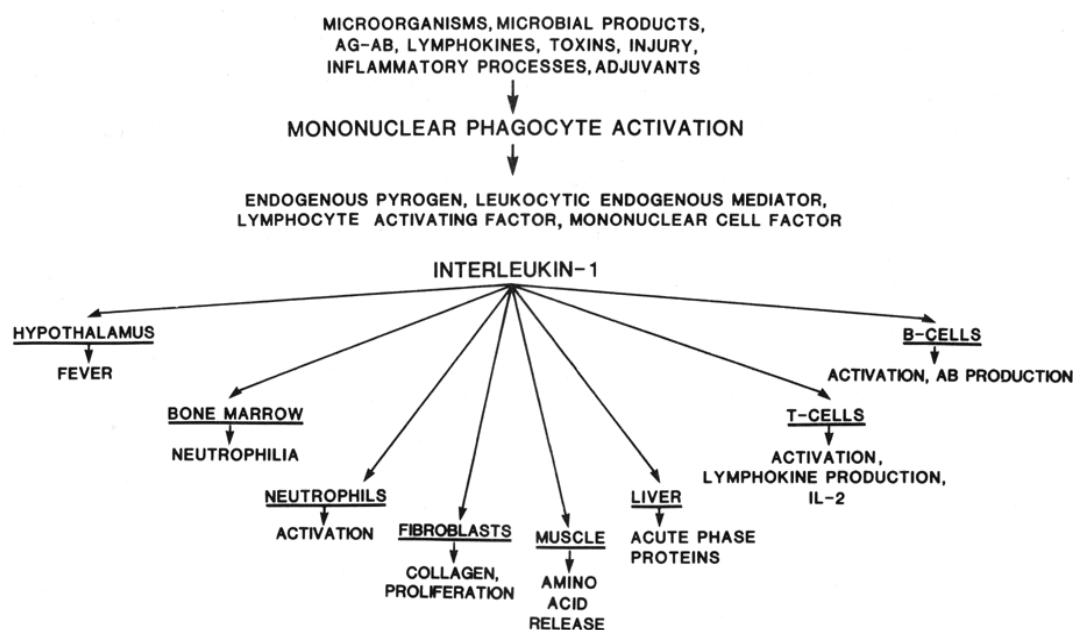
Figure 1. The IL-1 hyothesis in 1984 on the eve of the cloning of IL-1β. From Dinarello CA. Interleukin-1 and the pathogenesis of the acute-phase response. *N Engl J Med* 311:1413-1418, 1984.

be correct as the various biological activities of purified 'leukocytic pyrogen' (IL-1) were confirmed by recombinant IL-1 (Figure 2). In many ways, this was the birth of cytokine biology as the mystery was lifted and investigations for disease mechanisms as well as therapeutic targets could begin.

Expanding the new world of cytokine biology

Also in 1984, the mouse form of IL-1 was cloned by LoMedico and Mizel. However, the mouse cDNA of IL-1 was different from ours. There are two IL-1's: IL-1α and IL-1β. Both can bind to the same receptor and elicit the same responses. Today there are 11 members of the IL-1 family including IL-18 and IL- 33. Although IL-1 was the first cytokine to demonstrate how nature orchestrates disease, other cytokines such as tumor necrosis factor (TNFα) and IL-6 were to follow. Today, it is clear that nature intended to have cytokines elicit not only multiple biological activities but also overlapping activities. For fever, IL-1α and IL-1β are clearly the most potent in producing fever in humans at 10 ng/kg. TNFα produces fever at 150 ng/kg and IL-6 at 1-10 μg/kg. The human genome codes for a mere 39,000 genes, most of which are expressed only during embryonic development. Thus multiple activities of a single gene product is an efficient use of the limited number of genes that we use to function.
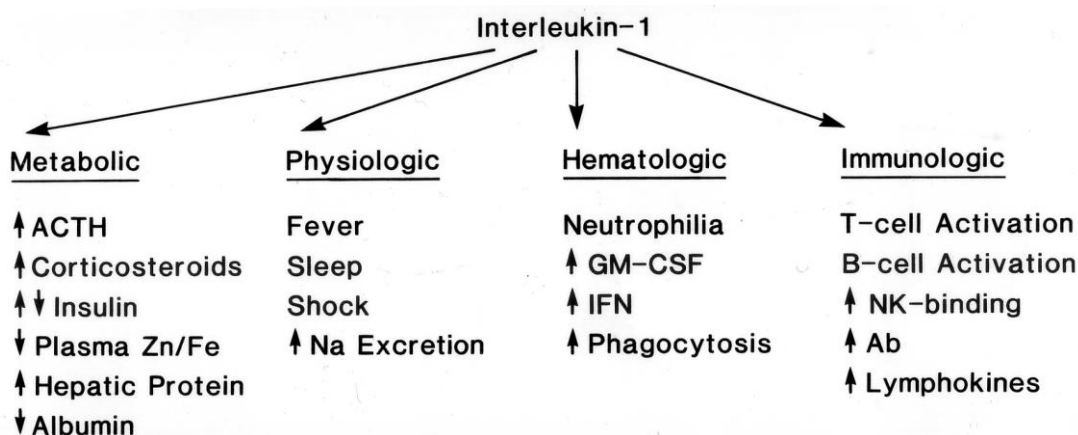

Figure 2. Confirmation of the multiple biological activities of recombinant IL-1β.

Blocking IL-1 in rheumatoid arthritis

Validation of the role of a cytokine, such as IL-1, in a disease process or a biological function is best accomplished with specific blockade or neutralization of the endogenous cytokine rather than exogenous application of the cytokine. We have learned a great deal from blocking IL-1 in humans with specific diseases. An important advance was the isolation of the IL-1 receptor antagonist by Hannum, Eisenberg and Arend. The IL-1 receptor antagonist is a naturally occurring molecule and a member of the IL-1 family. Dayer and Seckinger showed that the antagonist specifically binds to the IL-1 receptor and prevents binding of IL-1 to the cell. We first recognized the existence of the IL-1 receptor antagonist in 1981 in human serum during experimental fever. Today, a pharmacological form of the IL-1 receptor antagonist, known as anakinra or Kineret, is used to treat the signs and symptoms of rheumatoid arthritis. Anti-IL-1β

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neutralizing antibodies are also effective in treating rheumatoid arthritis. Thus, IL-1β has been validated in rheumatoid arthritis. IL-1β also plays a role in osteoarthritis.

IL-1β and the 'auto-inflammatory diseases'

As shown in Figure 3, there are several agents that can be used to treat rheumatoid arthritis. Blocking TNFα is particularly effective. However, there are some diseases that are primarily 'IL-1 diseases' in that blocking TNFα has little or no effect. These diseases are called "auto-inflammatory" diseases and are distinguished from 'auto-immune' diseases. In auto-immune diseases, the T-cell is the prominent cell and TNFα and IFNγ play prominent roles. In auto-inflammatory diseases, the macrophage is the prominent cell and IL-1β and IL-6 are dominant. This novel concept of 'auto-inflammatory diseases' should be credited to two physicians: Alan Wanderer and Hal Hoffman.
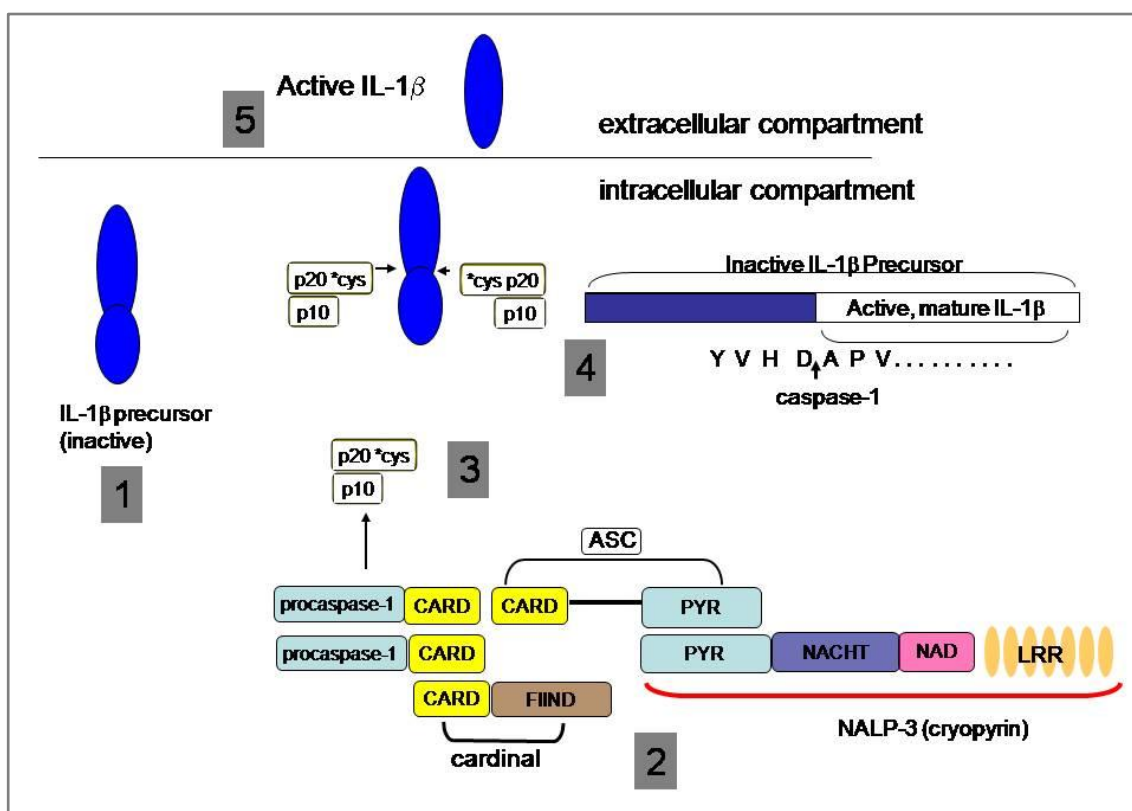

Figure 3. Auto-immune and auto-inflammatory diseases.

Of the auto-inflammatory diseases, a group called the periodic fever syndromes have attracted a great deal of attention because these disease have known genetic defects that result in increased release of IL-1β. Hence the periodic fever syndromes are uniquely IL-1β-driven diseases and therefore successfully treated with IL-1β blockade. Although they are rare diseases, their clinical, hematological and metabolic manifestations are common to most acute as well as inflammatory conditions. For example, fever, elevated white blood cell counts, increased levels of hepatic acute phase proteins as well as general fatigue, myalgia and serosal inflammation are common to most inflammatory diseases. The arch-typical member of the periodic fever

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syndromes is familial Mediterranean fever (FMF). Patients with this autosomal recessive disorder have recurrent attacks.

The genetic defect in FMF results in a change in a protein called 'pyrin' as described by Daniel Kastner. A sub-group of auto-inflammatory diseases, due to a single amino acid change, are called cryopyrin associated periodic syndromes (CAPS). In CAPS, the affected protein is called 'cryopyrin' because patients with CAPS exposed to cold (cryo) develop fever (pyrin) within a few hours. Another periodic fever disease is Hyper IgD syndrome described by Jos van der Meer. Due to a mutation in mevalonic acid synthase, patients with Hyper IgD syndrome produce elevated IL-1β. In each of the periodic fever syndromes, the basic defect is loss of tight control of the release of IL-1β. How is the release of IL-1β controlled?

As shown in Figure 4, IL-1β is first synthesized and an inactive precursor requiring cleavage to an active cytokine by the enzyme caspase-1. Caspase-1 is an intracellular enzyme, a cysteine protease, which itself requires cleavage from the inactive pro-caspase-1 to the active enzyme. The activation of caspase-1 requires the aggregation of group of intracellular proteins called the 'caspase-1 inflammasome' (Figure 3). One of the proteins that make up the 'inflammasome' is the protein cyropyrin. Therefore, mutations in cryopyrin result in an 'overactive' inflammasome with greater activation of caspase-1 and more release of IL-1β.

Chronic Inflammatory Diseases

Auto-immune Diseases
T-cell dominant
TNFα > IL-1β
co-cytokine = Interferon-γ

- rheumatoid arthritis
- juvenile rheumatoid arthritis
- ankylosing spondylitis
- psoriasis
- Crohn's bowel disease

Responsive to:

Anti-TNF-α
TNF soluble receptors
CTLA-4-Ig
Anti-IL-23
Anti-CD20
Anti-IL-6R
IL-1Ra
Anti-IL-1β

Auto-inflammatory Diseases
Monocyte/macrophage dominant
IL-1β > TNFα
co-cytokine = IL-6

Uniquely responsive to IL-1β blockade

- adult and juvenile Still's disease; pericarditis
- cryopyrin mutations
- Familial Mediterranean Fever
- Hyper IgD Syndrome
- Behçet's Syndrome
- Macrophage Activation Syndrome
- Normocomplementemic urticarial vasculitis
- Anti-synthetase syndrome (myositis)
- PAPA Syndrome; Blau's Syndrome
- Urate Crystal Disease (Gouty Arthritis)
- Type 2 Diabetes
- Smoldering Multiple Myeloma

Figure 4. The release of IL-1β following activation of the caspase-1 inflammasome.

What is particularly unique about patients with mutations in cryopyrin is the severity of their disease and a relatively small increase in the levels of circulating IL-1β. Monocytes from the blood of these patients spontaneously release more IL-1β compared to cells from healthy control

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subjects. When cultured in vitro with bacterial stimulants, the release of IL-1β is approximately 4-5 fold greater than that from control subjects. Thus, the difference is relatively small when compared to the difference in clinical manifestations. The 4-5 fold difference was also observed in the levels of IL-1β circulating in patients with CAPS compared to healthy controls. Using a neutralizing anti-IL-1β monoclonal antibody that forms complexes with IL-1β in the serum, investigators observed that in healthy subjects 6 ng of IL-1β are produced each day whereas in patients with CAPS, 31 ng of IL-1β are produced each day. Patients with CAPS treated with anti-IL-1β antibody experienced a near complete clinical response which was long-lasting and also associated with a reduction in the production of IL-1β. This latter observation was anticipated, since IL-1β induces its own production. In fact, the term "auto-inflammatory" connotes the self induction of inflammation and IL-1β-induced IL-1β appears to be part of the disease process.

Role of IL-1β in the loss of insulin production in diabetes

During the early days of IL-1 studies, we sustained high losses of IL-1 activity during purification. But fortunately, due to the potency of IL-1, it was possible to carry-out experiments using nanogram amounts of the cytokine. In 1985, we began a collaboration with Thomas Mandrup-Poulsen, Klaus Bendtzen and Jørn Nerup. These Danish physician-scientists were searching for molecules that killed the insulin-producing beta-cells in Type 1 diabetes. They reasoned that inflammatory cells might secrete mediators with direct cytotoxic action on beta-cells. In fact, cell-free supernatants from activated human immune cells did kill beta-cells. They next screened various cytokines including preparations of purified IL-1 from our laboratory (Figure 5). Unexpectedly, IL-1 was very potent in inducing death in cultured beta-cells. Also, depletion of IL-1 from the immune cell supernatants using anti-IL-1 antibody removed all activity. Indeed, the final proof came when they reported that recombinant IL-1β induced beta-cell death and that IL-1 receptor antagonist completely prevented the toxic effect of IL-1 on these vital cells.
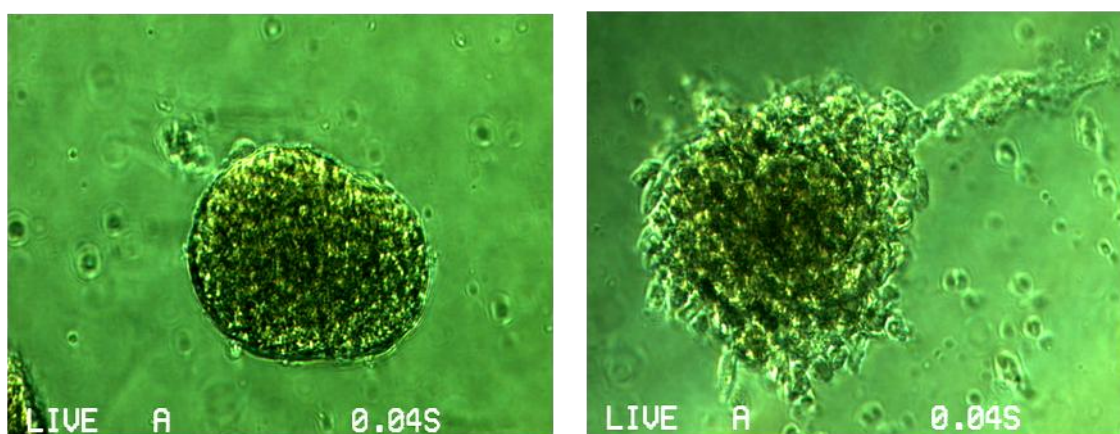

Figure 5. Destructive effect of IL-1 on the pancreatic islet. Courtesy of Morten Tonnesen and Thomas Mandrup-Poulsen, Hagedorn Research Institute.

The spin-off of these studies helped create an entire area of investigation in diabetes, that is, the effect of cytokines on the insulin-producing islets. Marc Donath brought the field of cytokines to the problem of type 2 diabetes by demonstrating that glucose-induced beta-cell destruction could be blocked by the IL-1 receptor antagonist. Thus, IL-1β itself was produced by the beta-

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cell, particularly when induced by high glucose. To test his hypothesis for a causative role of IL-1β in type 2 diabetes, Donath and Mandrup-Poulsen carried out a randomized, placebo-controlled study of anakinra in patients with type 2 diabetes. Glycemia was significantly reduced in the anakinra group compared to the placebo group. In a follow-up study, patients responding to anakinra used 66% less insulin during the remainder of the year. The therapeutic benefit of blocking IL-1 in type 2 diabetes has been confirmed using a monoclonal antibody to IL-1β.

IL-1β in the progress on multiple myeloma

The incidence of multiple myeloma in the population continues to increase and few survive. Multiple myeloma is caused by large numbers of malignant cells in the bone marrow, called myeloma cells. Although IL-6 is the dominant growth factor for myeloma cells, it is IL-1β that drives IL-6 production. A pre-condition of multiple myeloma is called indolent or smoldering multiple myeloma in which the myeloma cells increase in number by the growth factor IL-6. It was hypothesized by Drs. Lust and Donovan that if one could prevent the IL-1β-driven production of IL-6 in indolent or smoldering multiple myeloma before the rapid expansion of the malignant cells, one could prevent progression to full blown multiple myeloma. Indeed, they administered anakinra daily to patients at high risk to progress to full blown multiple myeloma and instead of the expected progression to full-blown disease with one year, a large majority of the patients did not progress. Many patients remain disease free after 4 years. This remarkable study by Mayo Clinic physicians is being expanded using antibodies to IL-1β.

Conclusions

Although science advances in parallel with technology, human inquisitiveness marches to its own drummer. During these thousands of years of human intelligence, people knew that there was more going on than just an elevation of body temperature. Today we see the therapeutic benefit of reducing local and systemic inflammation by blocking the 'fever molecule', IL-1. But the therapeutic success came only after the persistent efforts of countless investigators to fully understand the physiologic upheaval in the febrile patient. This upheaval was so important in evolution that nature did not risk imparting the response to a single cytokine or a single receptor. Indeed, it is no accident of nature that the cytoplasmic signaling domain of the IL-1 receptor is nearly identical to those of over ten Toll-like receptors. Today we view cytokines such as IL-1, tumor necrosis factor and IL-6 as legitimate targets to reduce inflammatory disease. But these cytokines and Toll-like receptors served evolution well by providing mechanisms for the host to defend itself against infection as well as provide for repair. We still depend on them today.

Charles A. Dinarello
Lund 13 May 2009

The Crafoord Prize Lecture held by Laureate in polyarthritis 2009, Toshio Hirano

How extensive the view from a mountain top is!
– A story of interleukin 6

Your Majesties, President of the Academy, members of the Crafoord Family, members of the Academy, ladies and gentlemen, it is my great honor to be awarded the 2009 Crafoord Prize in Polyarthritis jointly with Professor Charles Dinarello and Professor Tadamitsu Kishimoto by the Royal Swedish Academy of Sciences. At this special occasion, I would like to express my sincere thanks to Prof. Bo Sundqvist, President of the Academy, Prof. Gunnar Öquist, permanent secretary of the Academy, Dr. Catharina Svanborg, chairman of the prize committee. I also express my sincere thanks to the Academy and the Crafoord Foundation and members of the Crafoord Family, in particular Ms. Ebba Fischer, chairman of the Crafoord Foundation. I thank all my colleagues, without whose collaboration and help it would not have been possible for us to isolate interleukin 6 and clarify its properties and roles in inflammatory diseases. And I would not be standing here today without the support of my wife, Chiyoko Hirano. I thank my dear daughters, Yuko and Yoko. While my father is no longer with us, I know he is watching me with great pleasure. Although, my 92 year old mother is not here today, I know this award pleased her very much.

We do not know what the view from a mountain top will be. It is easy to find out if we check a guide before climbing. But in real life, as we know, there are no guides for all the mountains we have to climb. Life creates many challenges and it is not until we have reached one summit that we can see what lies before us. It may be a breathtaking view of the ocean. It may be a straight road showing us the correct direction to go in. We may even see a delightful view of a city, like Granada in Spain as shown in this picture, which my uncle in law painted to celebrate my prize award. Or we may see many more mountains, new challenges to be climbed. Only the one who stands on the top of the mountain can know what this view is, how extensive it is, how beautiful it is and what it will lead to.

Today's View

Today, we know that human beings cannot survive without an immune system. The history of humankind is the history of fighting against a variety of infectious diseases, such as the black plaque, cholera, smallpox, influenza, tuberculosis and so on. An epidemic of infectious diseases often determines which country finally wins a battle. We have won the battle against a variety of infectious diseases since Edward Jenner discovered that cowpox induced protection against human smallpox in 1796. This procedure is called "vaccination". We are, however, still fighting against a variety of infectious diseases caused by a newly generated microorganisms. A patient with severe combined immunodeficiency syndrome, which is caused by the loss of function mutation of interleukin-2 receptor gamma chain essential for lymphocyte development, suffers severe infectious diseases. Thus it is out of question that we human beings cannot survive on the earth without the immune system. However, immune responses often bring disadvantages to human beings. There are several autoimmune diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus, type 1 diabetes mellitus, Graves' disease, multiple sclerosis and so on. Both genetic and environmental factors are involved in causing autoimmune diseases. Autoimmune diseases are caused by the attack of immune system against self-antigen, resulting

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in self-destruction. T lymphocytes, B lymphocytes, macrophages and antigen presenting dendritic cells are major players in the immune system. They interact with each other to initiate immune responses against pathogens and even self-components of our body. Cytokines, such as interleukin 1 and 6, which are soluble factors produced by immune cells, play crucial roles in immune responses and abnormal production of cytokine or their abnormal functions are involved in several autoimmune diseases including RA. Now we know that there are a lot of cytokines and inhibitors of these cytokines, including IL-1, IL-6 and TNFa which are beneficial for autoimmune diseases, in particular RA.

Past view

What did I know 37 years ago when I graduated from Osaka University Medical School in 1972? We already knew that the interaction of immune cells, such as T lymphocyes, B lymphocytes and macrophages are required for immune responses. In response to antigenic stimulation such as pathogens, immune cells produce several soluble factors, now called cytokines, and these soluble factors are required for immune responses. For example, antigenic stimulation induces the differentiation of B lymphocytes into immunoglobulin-producing plasma cells under the help of T lymphocytes. In 1971 and 1972, M. Dutton and both Schimpl and Wecker, respectively reported the presence of soluble factors, which could replace the helper functions of T lymphocytes and thereby they called it T cell replacing factor (TRF). However, the molecular natures of these soluble factors were completely unknown. Immunologists called each factor by his/her own name based on the biological activity he/she examined, and thereby there were a lot of names corresponding to the numbers of immunologists.

 I wished to study Immunology and in 1973 I applied to the visiting fellowship at National Institutes of Health, in The Unites States and participated in the laboratory of Immunology being supervised by Dr. Albert A. Nordin at The Gerontology Research Center, which is now called The National Institute on Aging in Baltimore. In Baltimore, I first met Dr. Kishimoto and Dr. Takatsu, both of whom were members of Dr. Kimishige Ishizaka's laboratory at John Hopkins University and since then I have learned a lot from them. My encounter with Dr. Kishimoto has very much influenced my life as an immunologist. But at that time I did not know my future. After three years research on the regulation of cytotoxic T cell differentiation and soluble factors involved in this process at Dr. Nordin's Lab, NIH, I returned to Osaka University Medical School in 1976 and thereafter moved to Osaka Prefectural Habikino Hospital in 1978, which is now called Osaka Prefectural Medical Center for Respiratory and Allergic Diseases. I saw many patients with tuberculous pleurisy there.

A steep mountain path

I found that the purified protein derivative (PPD)-stimulated pleural effusion cells of patients with pulmonary tuberculosis produce soluble factors capable of inducing immunoglobulin production in B cells (1). Since this activity seemed to be very strong and large amounts of lymphocytes (up to 1 X 109/patient) could be obtained from one patient I thought it might be possible to purify the active factors. Then I decided to isolate this active factor and started its purification together with Dr. Tsuyoshi Teranishi in 1978. We found that the fractions corresponding to the molecular weight of 22KDa and the isoelectric point of 5 contained factors acting on B cell lines transformed by Epstein-Barr virus to induce immunoglobulin production. These biological activity and physicochemical properties of the soluble factors, at that time we

called TRF-like factor or BCDFII, are the same as those of cyotkine, which is now called interleukin 6 (2). Therefore, for me, interleukin 6 is a gift from patients with tuberculosis (Fig. 1). I moved to Kumamoto University Medical School as an Associated Professor of the late Professor Kaoru Onoue in 1980 and there I continued purification and characterization of this factor. Then I moved to Osaka University as an Associate Professor at Professor Kishimoto's lab in early 1984 and finally succeeded in its purification and determined its N-terminal 14 amino acid sequence at the end of 1984. I had a prospective view for the next year and really enjoyed the New Year Holidays with a big dream. However, reality was much harder than I expected. Our several trials to clone the cDNA encoding this active molecule completely failed in 1985. This raised the doubt that the sequence we determined might be incorrect or it might be the sequence of the other proteins co-purified with the active molecule. This worry gave me severe arrhythmia, which disturbed my sleep at the end of 1985. A medical checkup showed that my arrhythmia was just psychogenic but not pathogenic. Then I decided to purify the molecule again utilizing 100 liters of newly obtained culture supernatants. We obtained several fragments of the purified protein and their partial amino acid sequences in March, 1986. Then I started cloning the cDNA. We used three probes corresponding to three selected fragments of the purified proteins. I believed that this way would be surer than the way I took using only one probe corresponding to the N-terminal amino acid sequences in 1985.
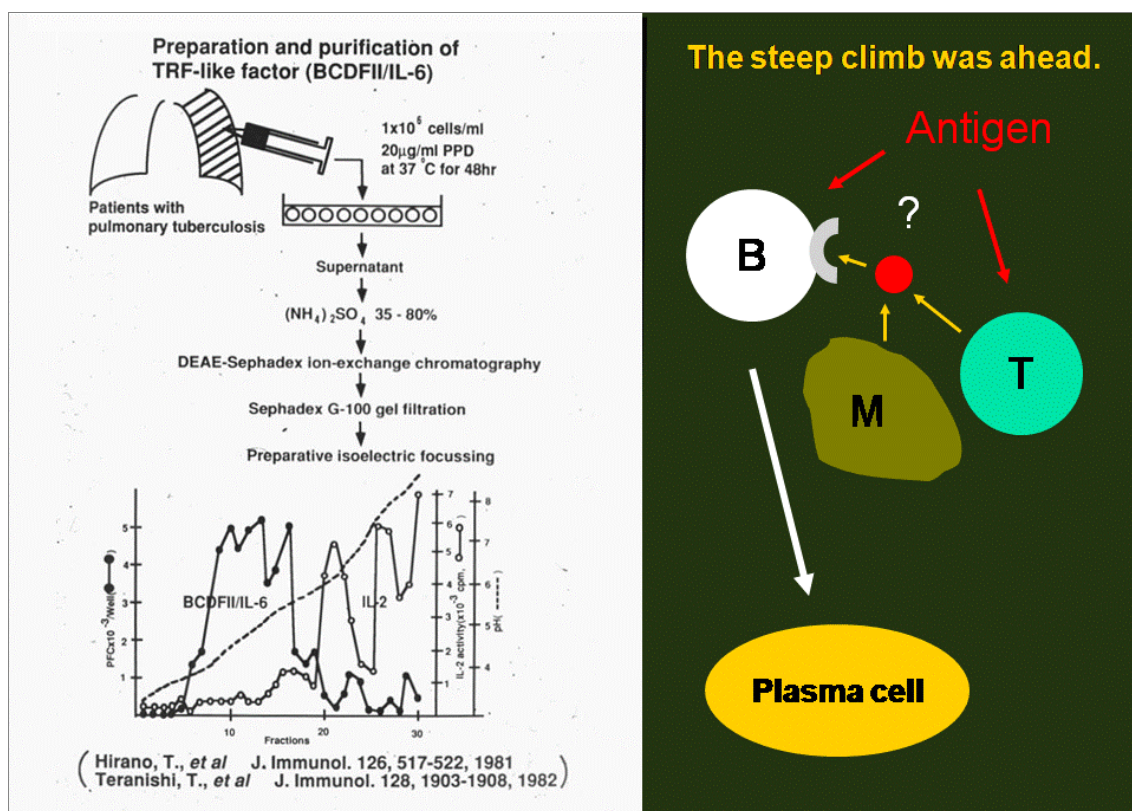

Figure 1. Interleukin6 is a gift from patients with tuberculosis. I worked in Osaka Prefectural Habikino Hospital where I saw many patients with tuberculous pleurisy and we found pleural effusion cells when stimulated with purified protein derivative (PPD) produced factors being able to induce immunoglobulin production in B lymphocytes in 1978. We started isolating these factors and we found one of active factors had similar biological activity and physicochemical properties with those of a cytokine, now called interleukin 6. Therefore, for me, interleukin 6 is a gift from patients with tuberculosis.

Standing at the top of the mountain

After 8 years of steep climbing, the summit suddenly came into view. 11 am Sunday morning May 25th 1986 I obtained the clone, which bound all three probes.

It was in my hand. It was not a dream. It was a reality.

I was very confident that I had finally cloned the cDNA encoding the molecule, which we called "BSF-2" at that time, which we previously called BCDF, BCDFII or TRF-like factor. Nucleotide 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. We published the result in the November issue of Nature, 1986 (3). To my surprise, the sequence we reported was found to be identical to that of interleukin 1-induced 26kDa protein reported by Hageman and Fiers in the Sept. issue of Eur. J. Biochemisty and that of interferon beta 2 reported by Zilberstein and Revel in the Oct. issue of EMBO J. These results revealed that all these molecules are identical. The January issue of Science, 1988 reported that orphan interferon had found a new home; Uncertainties about the role of interfero- β2 were being resolved as researchers found that it had numerous activities in the body's defenses. Furthermore, the plasmacytoma/hybridoma/myeloma growth factor and the hepatocyte stimulating factor which regulates the biosynthesis of a variety of acute-phase proteins, were also found to be identical to this factor. Therefore, the nomenclature meeting chaired by Dr. W. E. Paul, which was held in New York on Dec. 14, 1988 proposed the name of "interleukin-6" for this molecule (4). Thus, the name "interleukin-6" was born!

I could finally see an extensive prospect from the top of the mountain after more than 8 years steep climb since I began isolating the factor capable of inducing immunoglobulin production in B cells in 1978. We wished to isolate a factor acting on B cells to induce immunoglobulin production, calling this factor a variety of names, such as TRF-like factor, BCDF, BCDFII, BSF-2 and so on. However, once we cloned the factor, we saw that this factor, given a new name "interleukin-6 (IL-6)", acts on not only B cells, but also a variety of cells and tissues. It acts on hepatocyte to induce a variety of acute phase proteins; it activates osteoclasts to destroy bones; it is a growth factor for myeloma and plasmacytoma; it increases platelet, and it even induces fever and cachexia. IL-6 is now known to be a multifunctional cytokine that plays roles in the immune response, inflammation, hematopoiesis in the endocrine and nervous systems (Fig. 2) (4, 5).

Anyway, once a ligand, such as IL-6 was cloned, it was not so difficult for us to isolate its receptor in the late 1980s. In fact, we cloned the cDNA encoding IL-6 receptor utilizing expression cloning, which was just introduced by Brian Seed and his colleagues (6). Then we cloned a signal transuding receptor subunit, gp130 (7). We showed that IL-6 receptor is composed of two subunits, one is an IL-6 specific subunit, alpha chain and the other is a signal transducer, gp130. (see Fig. 4). Furthermore, it was found that gp130 is not only a receptor subunit for IL-6, but also a signal transducer for other cytokines, such as IL-11, OSM, LIF, CT-1, CNTF, IL-27 and so on. The view I saw at the top of the mountain was far beyond my

expectation. However, this was not the end of the story but just a beginning. Later, I saw another view from the top of the next mountain.
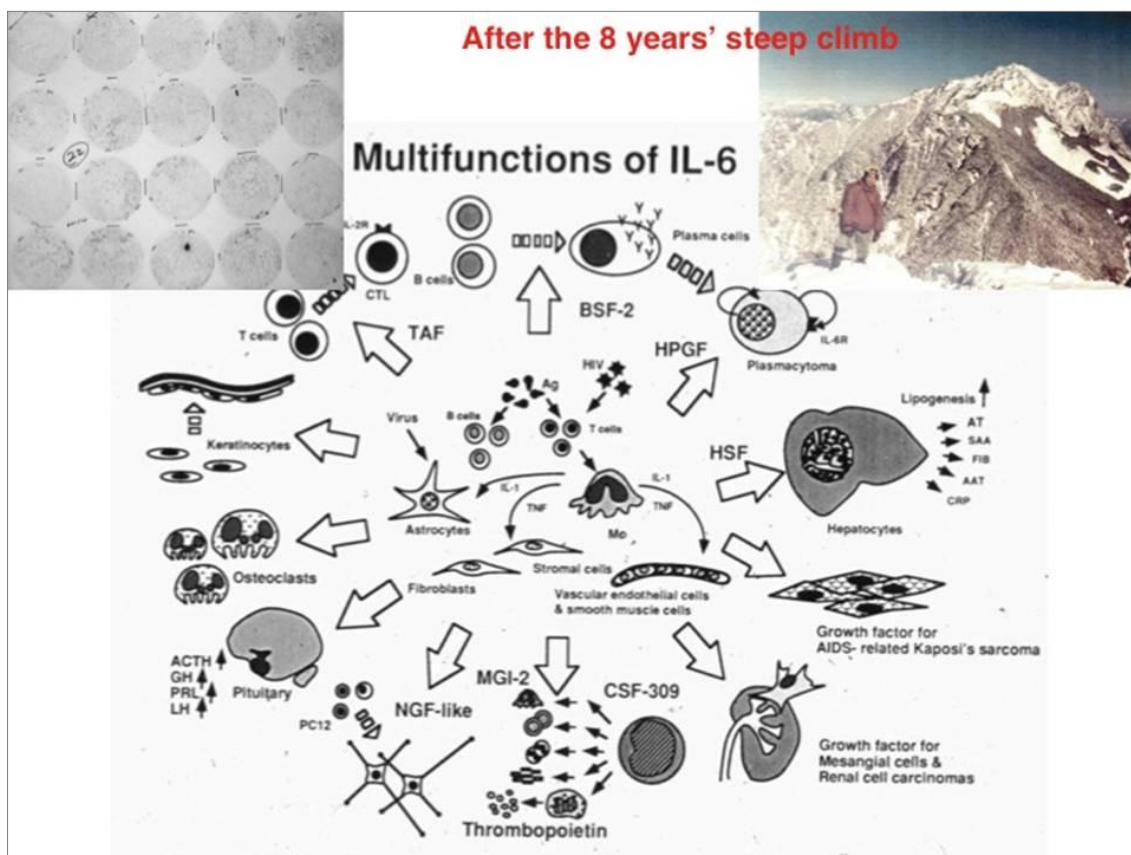

Figure 2. The name of "interleukin 6", which has multiple functions, was born.

Our study together with other studies showed that BSF-2 was identical with interferon-β2, 26kDa protein, plasmacytoma/hybridoma/myeloma growth factor and hepatocyte stimulating factor. The nomenclature meeting proposed the name of "interleukin-6" for this molecule in 1988. Thus, the name of "interleukin-6" was born. IL-6 is now known to be a multifunctional cytokine that plays roles in the immune response, inflammation, hematopoiesis, in the endocrine and nervous systems.

A beautiful view came next.

Patients with cardiac myxoma show a variety of autoimmune symptoms, such as hypergammaglobulinemia, the presence of autoantibodies and an increase in acute phase proteins, all of which disappeared after the resection of the tumor cells, suggesting that cardiac myxoma cells may induce autoimmunity. We found that cardiac myxoma cells produce IL-6 (8). This was the first suggestive evidence indicating that IL-6 might be involved in autoimmune diseases. Important findings came next. We found that a large amount of IL-6 is present in the synovial fluids of patients with RA (9), suggesting the involvement of IL-6 in RA for the first time. RA is a chronic polyarthritis and one of the autoimmune diseases. Both genetic and environmental factors are involved, but its etiology is unknown. Since patients with RA show a variety of symptoms, such as polyclonal plasmacytosis accompanied with production of rheumatoid factor, increase of acute phase proteins, enhanced bone resorption activity and increase of platelet and so on, all of which do not apparently have any relationship with each

other. However, if one considers the multiple functions of IL-6, this puzzle would be resolved. This led me to speculate that the cause, which triggers the dysregulation of IL-6 gene expression or abnormal IL-6 function is intimately related to the cause inducing RA (5, 10).

I was appointed as a Professor of Osaka University Medical School on November 1st, 1989. In early 1990s, I proposed a working hypothesis of possible mechanisms involved in certain autoimmune diseases, chronic inflammatory proliferative disease (CIPD) like RA where IL-6 is suggested to play a role (5,10). In the hypothesis illustrated in Fig. 3, the constitutive activation of a set of transcription factors, such as NF-kB, is the central factor governing the onset as well as the progression of the disease. In the initial phase, the activation of the transcription factors is induced by a variety of stimuli, including infection, stimulation with foreign materials, and injury. This initial stimulation induces inflammation that activates a set of transcription factors,
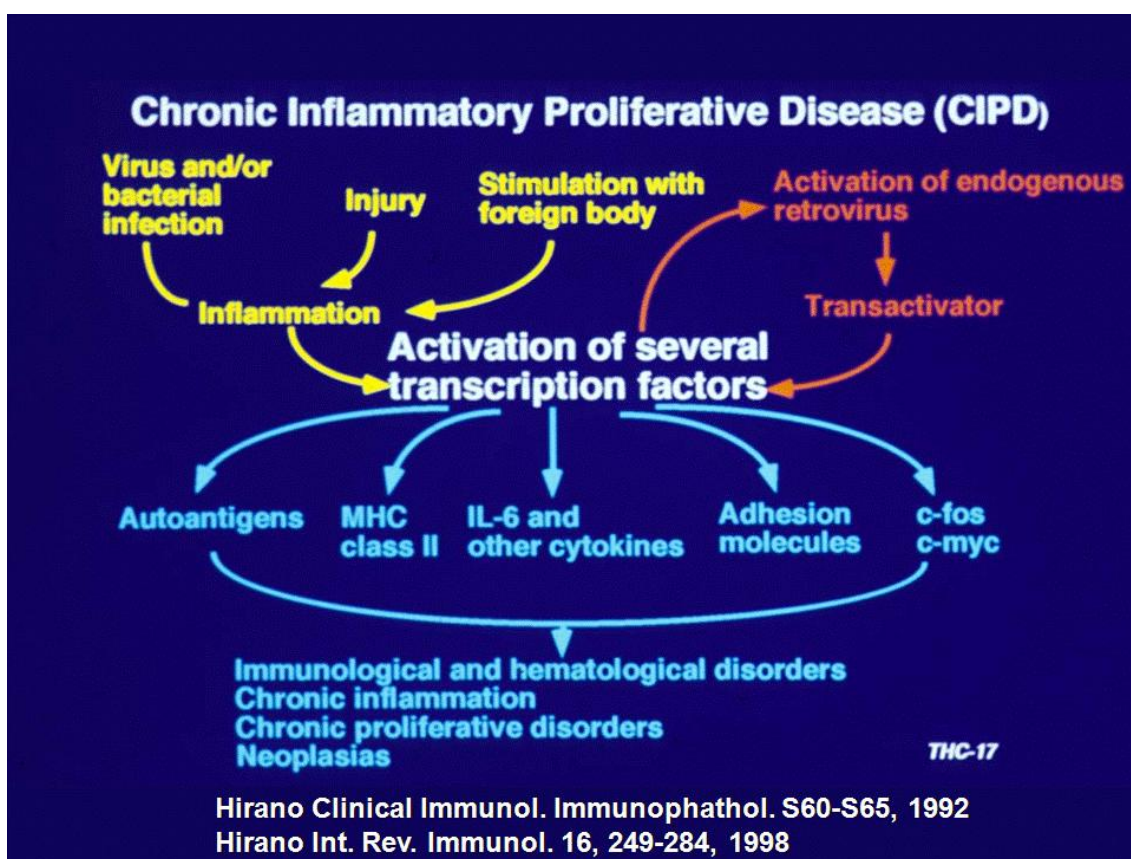

Figure 3. A working hypothesis of possible mechanisms involved in autoimmune diseases, chronic inflammatory proliferative disease (CIPD) like RA. The constitutive activation of a set of transcription factors, such as NF-kB, is the central factor governing the onset as well as the progression of autoimmune diseases and CIPD. In the initial phase, the activation of the transcription factors is induced by a variety of stimuli, including infection, stimulation with foreign materials, and injury. This initial stimulation induces inflammation that activates a set of transcription factors, leading to the expression of various genes encoding cellular proteins, such as IL-6 and other cytokines, MHC molecules, adhesion molecules, giving rise to the activation of immune system. Since the first phase could be induced in non-immune cells or tissues, this hypothesis suggested the interaction between the non-immune and immune system plays a critical role in autoimmune diseases and CIPD.

leading to the expression of various genes encoding cellular proteins, including IL-6 and other cytokines, MHC molecules, adhesion molecules, various autoantigens, and several transcription factors. Thus expressed, autoantigens could be recognized by autoreactive T-cells in the context of the MHC molecules. These activated autoreactive T-cells also induce the inflammatory response. Since the first phase could occur in non-immune cells or tissues, my hypothesis indicated that the interaction between the non-immune system and immune system plays a critical role in autoimmune diseases and CIPD.

To understand the molecular mechanisms of IL-6 actions, we investigated signal transduction pathways through the IL-6 receptor. We showed IL-6 induces two major signal transduction pathways, the Stat3 signal and the SHP2/Gab/MAPK signal in a manner dependent on the YxxQ motif and the Y759 of gp130, respectively (11-14). Then, I wished to clarify the in vivo roles of each of the two signals of gp130. For this, we generated a series of knock-in mouse lines in which the gp130-mediated SHP2 or Stat3 signal is selectively disrupted (15). To make the SHP2 signal-deficient mice (F759 mice), we mutated Y759 of gp130 to phenylalanine. F759 mice show enhanced STAT3 activation through gp130 since Y759 is required for SOCS3-mediated negative feedback mechanisms. The most intriguing finding is that F759 mice, which show enhanced STAT3 activation by IL-6, spontaneously develop RA-like joint disease (F759 arthritis) (Fig. 4) (16). This is the first definitive evidence showing that IL-6 is critically involved in spontaneous autoimmune disease and shows that an abnormal IL-6 signal can induce autoimmune disease like RA. F759 arthritis is late onset, symmetrical and progressive like human RA. F759 mice show a variety of immunological abnoramlities, including hypergammaglobulinemia, production of autoantibodies, increase of memory activated T cells and so on. Then, we have tried to clarify molecular and immunological mechanisms. Both genetic and environmental factors are involved in autoimmune diseases. In F759 mice, point mutation of gp130 leading to the enhanced activation of STAT3 by IL-6 is one of the examples of the genetic factor. Then we asked a question: does HTLV-1 infection as an environmental factor have any effect on F759 arthritis? Iwakura and his colleagues showed that HTLV-1 env-pX transgenic mice, a model of HTLV-1 infection, developed arthritis in certain genetic backgrounds. Then we asked if HTLV-1 has any effect on arthritis in C57BL/6 backgrounds. We found HTLV-1 pX enhanced F759 arthritis (17). F759 mutation enhances STAT3 activation by IL-6, while HTLV-1 activates NF-kB, suggesting that both STAT3 and NF-kB are involved in F759 arthritis. Thus F759 mice are a new animal model of RA.

Using studies of bone-marrow transplantation and various knock-out strains, we demonstrated that F759 arthritis is CD4 T cell-dependent, and interestingly, the gp130 F759 mutation is necessary in cells of a non-hematopoietic origin. In response to IL-6 stimulation, these non-hematopoietic cells from F759 mice show an enhanced production of T-cell survival factor, IL-7, leading to the activation of CD4 T cells by homeostatic proliferation. This homeostatic proliferation of CD4 T cells is important for the development of F759 arthritis (18). Thus our results show that the interaction between the non-immune system and immune system plays a critical role in causing autoimmune F759 arthritis and suggested the important role of the non-immune system in causing autoimmune diseases in general (see Fig. 5). It was recently found that IL-6 together with TGFβ induces TH17, which has been considered to play a pivotal role in
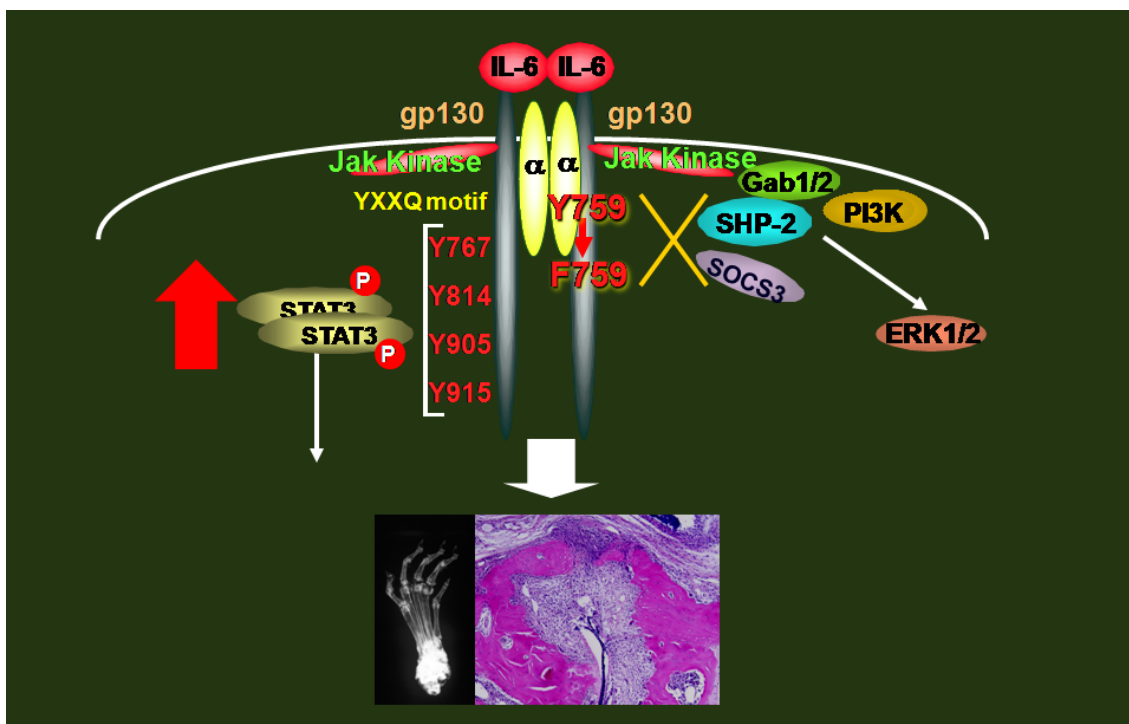
Figure 4. Dysregulation of IL-6 signaling spontaneously induces RA-like arthritis with age. IL-6 receptor is composed of two subunits, one is an IL-6 specific subunit, alpha chain and the other is a signal transducer, gp130. IL-6 induces two major signaling pathways, one is mediated by STAT3 and the other SHP2/GAB through tyrosine residues of go130 with YxxQ motif and tyrosine 759 of gp130, respectively. F759 mice, which express mutated gp130 (Y759F) defective of SOCS3-mediated negative feedback, spontaneously develop RA-like arthritis with age.

causing autoimmune diseases and inflammation, indicating that IL-6 is located upstream of IL-17. In fact, we showed that gp130 and Stat3 in T cells are essential for TH17 development (19). In addition, we found that IL-6 is not only an IL-17 inducing factor, but also a target gene of IL-17 in non-immune cells including fibroblast cells. Importantly, IL-6 is a critical downstream target gene of IL-17 for F759 arthritis. An intriguing finding we made was that IL-17-induced IL-6 gene expression through NF-kB activation is augmented in the presence of IL-6. This synergistic induction of IL-6 is mediated through an interaction between NF-kB and STAT3. IL-6 can induce TH17 cells to produce IL-17 and therefore once an IL-6 positive feedback loop is initiated in non-immune cells, enhanced IL-6 production results in the enhanced TH17 development, giving rise to further enhanced production of IL-6. We named this positive loop the "IL-6 amplifier" (see Fig. 5) (20). More importantly, "IL-6 amplifier" in type1 collagen positive non-immune tissues is required for autoimmune arthritis in F759 mice.

"IL-6 amplifier" is induced by the interaction between the immune system and non-immune system where both NF-kB and STAT3 activation has occurred. "IL-6 amplifier" is enhanced in F759 mice where IL-6-mediated STAT3 activation is enhanced due to the lack of negative feedback through SOCS3. Consistent with this scenario, HTLV1-p40Tax capable of activating NF-kB enhances F759 arthritis. We showed that MOG-specific TH17-induced experimental autoimmune encephalomyelitis (EAE) is dependent on STAT3 in type 1 collagen positive non-

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immune tissue. Collectively, we hypothesized that any event, including antigen-specific T cells, virus infection, injury and physical stimulation capable of activating IL-6 amplifier through either STAT3 activation or NF-kB activation or both plays a critical role in causing autoimmune diseases. This scenario might be applied in general autoimmune models (Fig.5). Thus, I would like to remind you of my old hypothesis (see Fig. 3), which I proposed in the early 1990s and suggested that the interaction between the non-immune system and immune system through the activation of several transcription factors is critically involved in autoimmune diseases and chronic inflammatory proliferative diseases.
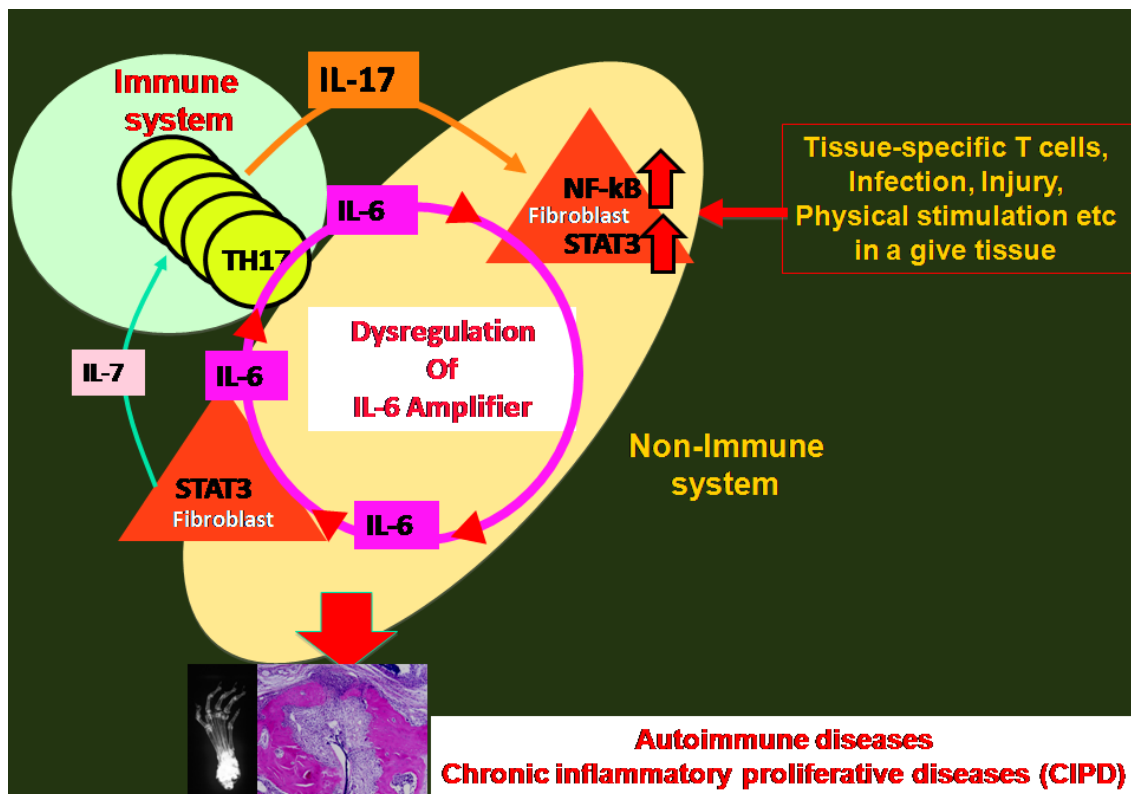

Figure 5. The interaction between the **non-immune and immune system** through an **"IL-6 amplifier" plays a role in autoimmune diseases and CIPD.** "IL-6 amplifier", which is dependent on NF-kB and STAT3 plays an important role in causing F759 arthritis. It is speculated that any event, any reagent, any pathogen, capable of chronically activating either or both NF-kB and STAT3 plays a role in RA and this scenario might be applied in autoimmune diseases and CIPD in general.

How extensive the view from a mountain top is!

Now, I am standing on top of the mountain and I can finally enjoy the view. 37 years ago when I graduated Osaka University Medical School in 1972 I could not have imagined it. I have discovered one of essential factors involved in the immune system, IL-6. It is the multifunctional cytokines which are involved not only in the immune system, but also in inflammation, hematopoietic system, nervous system and even in early development of our body. 37 years ago, I did not imagine how autoimmune diseases could develop and how RA occurs. Now I can see part of these mechanisms and how autoimmune diseases develop and how IL-6 is involved. Dysregulation of the IL-6 signaling pathway spontaneously induces autoimmune arthritis in mice (F759 arthritis). Furthermore, we found the presence of an "IL-6 amplifier" which is

critically involved in F759 arthritis. These facts led me to speculate that any event, any reagent, any pathogen capable of chronically activating either or both NF-kB and STAT3 plays a role in RA and this scenario might be applied to autoimmune diseases in general. Anti-IL-6 receptor antibody treatment, which inhibits IL-6 function, has been found to be beneficial for many patients. Thus our basic studies during the last 30 years contributed toward an understanding of the immunological mechanisms of RA and paved the way to develop a new drug beneficial for patients with RA and hopefully other autoimmune diseases and inflammatory diseases.

Finally, I would like to acknowledge many people. First of all, I acknowledge the late Professors Yuichi Yamamura and Kaoru Onoue, Professor Sohei Kondo, Dr. Albert A. Nordin and Professor Tadamitsu Kishimoto all of whom are my mentors. I also express my special thanks to Professors Kiyoshi Takatsu, Tadatsugu Taniguchi and Takeshi Watanabe for their valuable advice and encouragement. I also wish to thank many friends and colleagues, Drs Tetsuya Taga, Kiyoshi Yasukawa, Katsuhiko Yamasaki, Tsuyoshi Teranishi, Shizuo Akira, Atsushi Muraguchi, Hitoshi Kikutani, Kazuyuki Yoshizaki, and Yoichiro Iwakura. Finally I acknowledge the many people in my laboratory, including 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, Hideyuki Ogura, Yukihisa Sawa and Masaaki Murakami and many other colleagues.

I am very grateful to the Royal Swedish Academy of Sciences and the Crafoord Foundation. Finally, I would like to express my sincere thanks to Ms. Elin Mellqvist for her perfect arrangement of the Crafoord Days 2009.

Toshio Hirano
Lund 13 May 2009

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The Crafoord Prize Lecture held by Laureate in polyarthritis 2009, Tadamitsu Kishimoto

Interleukin 6: from bench to bedside – 40 years in immunology

I would like to express my sincere appreciation to the Royal Swedish Academy of Sciences and the members of the Crafoord Foundation for awarding me with this prestigious prize.

I am honored and delighted that my work on IL-6 is so highly appreciated and that the results of my studies have been applied to the treatment of rheumatoid arthritis and several autoimmune inflammatory diseases. In this lecture, I will briefly review my life's work on IL-6, from its discovery to the development of anti-IL6R antibody for the treatment of autoimmune diseases.

The picture shown in Figure 1 was taken 6 years ago and at that time this boy was 5 years old and suffering from juvenile idiopathic arthritis (JIA). He had a high fever every day, joint pain and swelling as well as hepatosplenomegaly. His growth had completely stopped when he became sick. However, 18 months after treatment with anti-IL6R antibody, all symptoms had disappeared and his heights increased by 18cm. The question is why the blockade of IL-6 signals could have such a dramatic therapeutic effect.

Figure 1. Boy suffering from juvenile idiopathic arthritis, before and 18 months after treatment with anti-IL6R antibody.

T cell factor(s) for antibody production

In 1968, the role of T and B lymphocytes in antibody production was discovered by J. Miller and H. Claman. They found that B lymphocytes produce antibodies but not without the presence of T lymphocytes.

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I speculated that T cells must produce certain factors which induce growth and differentiation of B cells, such as B cell growth factors (BCGF) and B cell differentiation factors (BCDF).

In the early 1970s, I was a postdoctoral fellow in the laboratory of Dr. K. Ishizaka, who discovered IgE (1967), at Johns Hopkins University. Together we discovered the presence of such activities in the culture suspernatants of activated T cells (Fig.2). Interestingly, our results also indicated the presence of distinct factors for IgE and IgG responses. At that time, we could not explain this phenomenon on a molecular basis but later the presence of TH1 and TH2 T cell subsets for IgG and IgE responses was discovered by T. Mossman and colleagues.
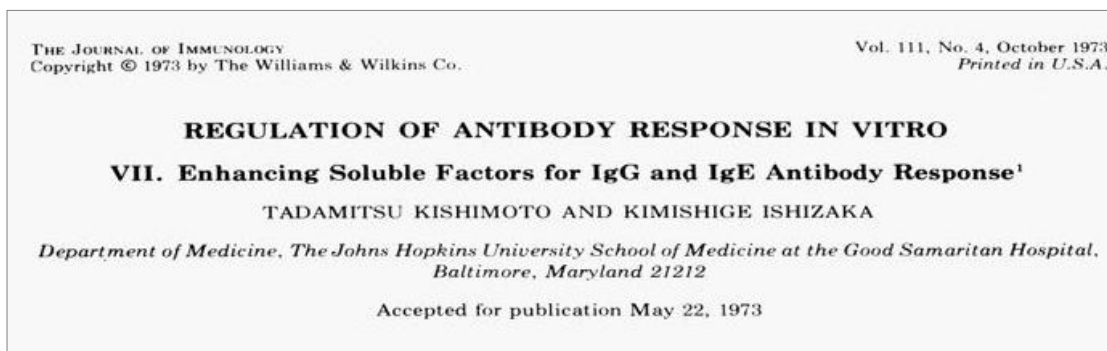

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REGULATION OF ANTIBODY RESPONSE IN VITRO

VII. Enhancing Soluble Factors for IgG and IgE Antibody Response[1]

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Accepted for publication May 22, 1973

Figure 2. The paper with Ishizaka was published in 1973 in The Journal of Immunology.

Fortunately, I could identify a B lymphoblastoid cell line called "CESS", which produces IgG upon stimulation with T cells or T cell-derived factors (Fig.3). By employing this cell line, Dr. T. Hirano and I could isolate cDNA for one of the B cell stimulating factors which is now known as IL-6.
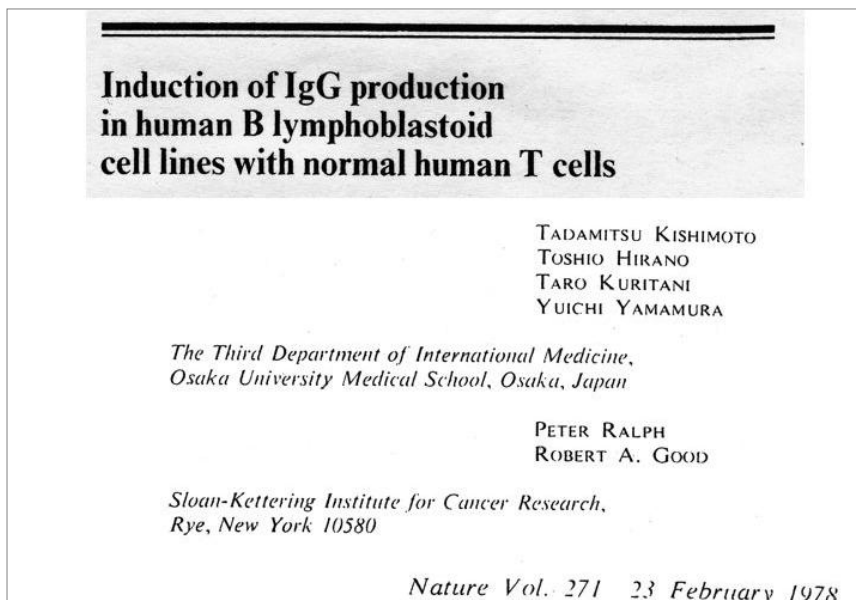

Figure 3.

Induction of IgG production in human B lymphoblastoid cell lines with normal human T cells

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Nature Vol. 271 23 February 1978

Pleiotropic activity of IL-6

Surprisingly, the use of a c-DNA, recombinant IL-6 and anti-IL6 antibody has made it clear that this molecule had been studied under many different names as depicted in Table 1.

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One of these names was plasmacytoma growth factor in mice, and, in fact, Eµ-IL6 transgenic mice prepared in a BL6 strain showed polyclonal growth of plasma cells in spleen and lymph nodes. When we introduced a Balb/c genetic background in these transgenic mice, monoclonal and transplantable plasmacytomas were induced. This confirmed seminal studies performed by M. Potter et al

Interleukin-6

B cell stimulatory factor 2 (BSF-2)

Interferon β2 (IFN β2)

26kD protein

Hybridoma plasmacytoma growth factor (HPGF)

Hepatocyte stimulating factor (HSF)

Table 1.

in which an intraperitoneal injection of paraffin oil in Balb/c mice generated inflammatory granulomas producing plasmacytoma growth factor and typical plasmacytomas.

IL-6 had also been studied under the name of hepatocyte stimulating factor (HSF) which induces various acute phase proteins including C-reactive protein (CRP), β2-fibrinogen, amyloid protein, haptoglobin, hemopexin and so on. In specially prepared IL-6 gene deficient mice a significant reduction was observed in the acute phase reaction induced by the intramuscular injection of terpentile oil, which confirmed that IL-6 functions as HSF.

IL-6 receptor system and gp130
The next question we tackled was how the signal of IL-6 could be transduced into the cells and stimulate pliotropic activities. We first isolated the receptor for IL-6 in 1988, but this did not have any unique sequence to account for the signal transduction. During the following couple of years, however, most cytokine receptors were isolated, including those for IL-2, IL-4, interferon, erythropoietin, growth hormone and others, and it was found that all these cytokine receptors showed a similar tertiary structure and formed a family, the so-called "cytokine receptor family".

Our study of the IL-6 receptor revealed a unique cytokine receptor organization. Binding of IL-6 with the 80Kd IL-6 receptor was seen to induce the interaction of another cell surface polypeptide chain with MW.130Kd called gp130. Interestingly, subsequent studies performed by several laboratories including our own revealed that gp130 functioned as a receptor component for several cytokines other than IL-6, such as CNTF (cilliary neurotropic factor) in the brain, LIF (leukemia inhibitory factor), Oncostatin M, IL-11 and cardiotropin in the heart (Fig.4). Gp130 was expressed in almost all tissues and cells, some of which did not express 80Kd IL-6R, and this could explain the pleiotropy and redundancy of the IL-6 family of cytokines. Later it was shown that the unique structure of the IL-6 receptor system, consisting of the specific 80Kd receptor and a 130Kd common signal transducer, was found in most cytokine receptor systems, such as γ-chain for IL-2, -4, -7, -9, -15 and β-chain for IL-3, IL-5, GM-CSF. The discovery of gp130 established the concept of a unique cytokine receptor system consisting of a specific receptor and a common signal transducer which could explain the redundancy and pleiotropy of cytokine functions.

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Figure 4.

Signal transduction from the cell surface to nuclei

Stimulation of cells with IL-6 induced tyrosine phosphorylation of intracytoplasmic proteins, which indicated that a certain unknown typrosine kinase was activated, although gp130 does not possess a kinase domain in its intracytoplasmic portion.

We found a conserved sequence known as box1 in the intracytoplasmic portion of most cytokine receptors including gp130. A novel tyrosine kinase designated as the JAK family was identified by J. Ihle and his colleagues.

We also isolated a new transcription factor, initially called APRF (acute phase responsive factor), but now known as STAT3, which was activated by JAK, formed a dimer and t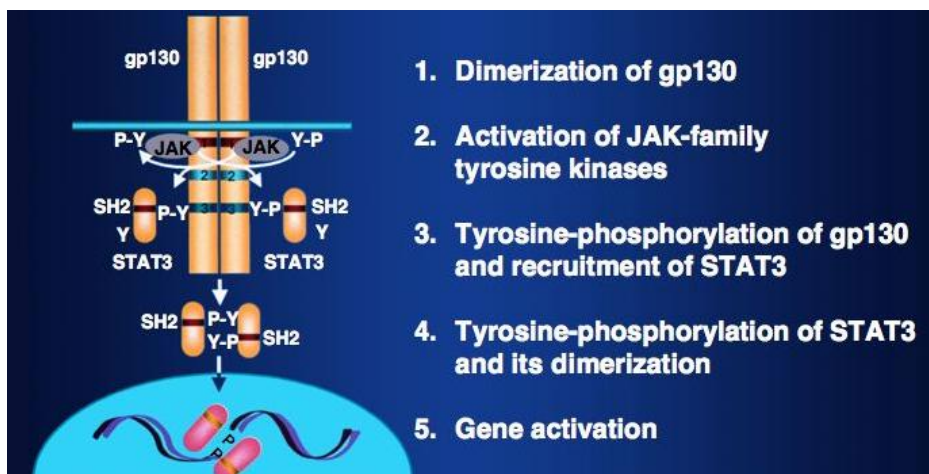ranslocated into the nuclei. Thus, the whole picture of IL-6 signal transduction, from cell surface to gene expression, was identified (Fig.5).

Figure 5.

Negative regulation of the IL-6 signals by SOCS

Cytokines such as IL-6 are essential for life but its constitutive overproduction is often involved in various diseases, which accounts for negative regulatory mechanism in the IL-6 signaling system. We discovered a molecule initially called SSI (STAT-induced STAT inhibitor) but now SOCS (suppressor of cytokine signals). SOCS is one of the target genes of the JAK-STAT signaling pathway which binds with JAKs to inhibit their activity and thus negatively regulates the signals.

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Overproduction of IL-6 and diseases

In spite of the presence of such a negative feedback mechanism, constitutive overproduction of IL-6 is responsible for the pathogenesis of various inflammatory diseases. Just after isolation of the IL-6 gene in 1986, we noticed that cardiac myxoma, a benign heart tumor, often produced a large amount of IL-6. We realized that this could explain various inflammatory symptoms of patients. We also noticed that the synovial tissues of the joints of patients with rheumatoid arthritis constitutively produced a large amount of IL-6 (Fig.6).

Patients with Castleman's disease showing multiple lymph node swelling with massive infiltration of mature plasma cells suffered from severe inflammatory symptoms, such as high fever, anemia, increase in acute phase proteins and hyper-γ-globulinemia. We detected constitutive production of IL-6 in the affected lymph nodes of the patients, while their sera showed high concentrations of IL-6, which could explain the inflammatory symptoms of the patients.
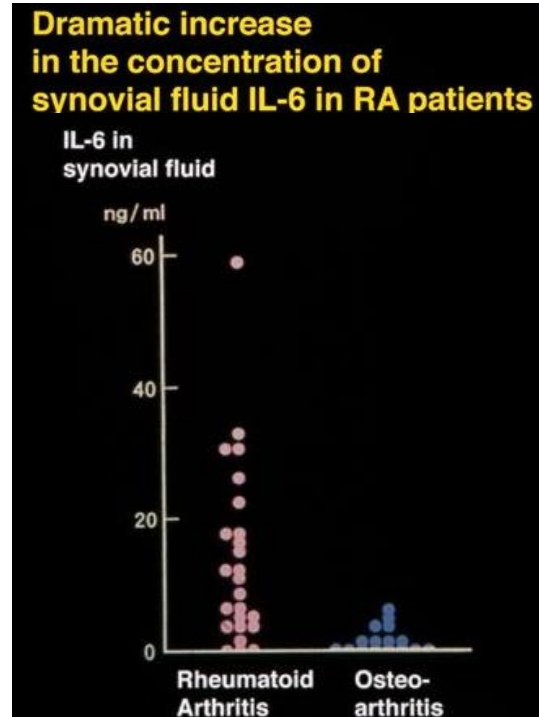

Figure 6.

7) Treatment with anti-IL6 receptor antibody

Not only IL-6 but also the soluble IL-6 receptor is enhanced in patients with such inflammatory diseases, so that the complex of IL-6 and soluble IL-6R stimulates gp130 and induces inflammatory signals. Whilst treating these patients we therefore attempted to block the IL-6 signals induced by the interaction of IL-6 and IL-6R as well as the neutralization of the soluble receptors by establishing the antibody against the receptors, known as the anti-IL6R antibody (Fig.7). This antibody has been humanized and with the label Tocilizumab and the trade name
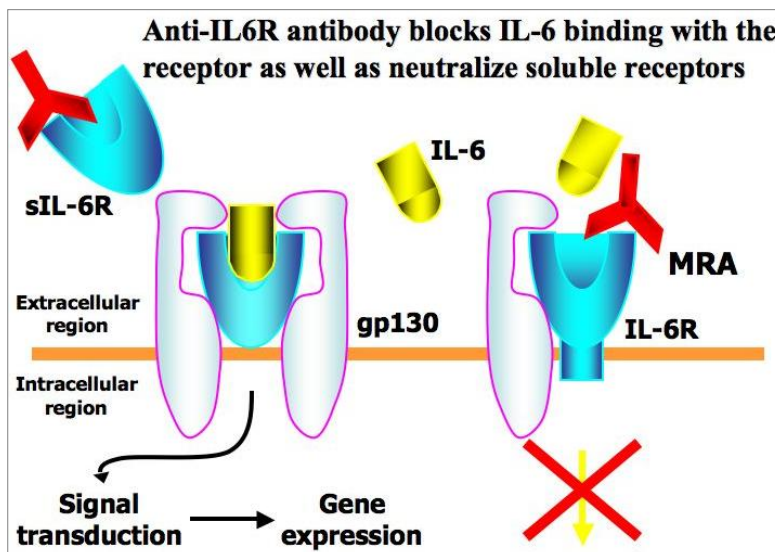

Figure 7

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Actemra. It was approved in April 2008 in Japan and in January 2009 in Europe.

More than 10 years ago this antibody was first used for the treatment of a patient with Castleman's disease. After its administration to a patient suffering from high fever, the fever went down, CRP dropped to zero and hemoglobin increased. A Phase II clinical study with 30 patients in Japan confirmed its effectiveness for Castleman's disease. All laboratory test results including CRP, SAA, hemoglobin, albumin, IgG and cholesterol showed normalization.

In an experimental animal model of rheumatoid arthritis, that is, antigen-induced arthritis, IL-6 gene-deficient mice did not show any significant inflammation in the joints, indicating that IL-6 was an essential effector molecule for the induction of arthritis. As a follow-up to these basic studies, a Phase III trial involving rheumatoid patients was completed in Japan in September 2005. As shown in Fig.8, the patients showed significant improvement of symptoms and ACR (American College of Rheumatology) improvement scores 20, 50, and 70 were 89%, 70%, and 47%, respectively. As shown in Fig.8, an important point in this trial was that this was monotherapy without the use of any other anti-rheumatic drugs such as methotrexate. The most important point of this therapy was that bone absorption and joint destruction could be completely prevented (Fig.9). The culture of the synovial cells from the patients combined with their peripheral mononuclear cells demonstrated that multinuclear osteoclast generation was completely inhibited by the addition of the anti-IL6R antibody.
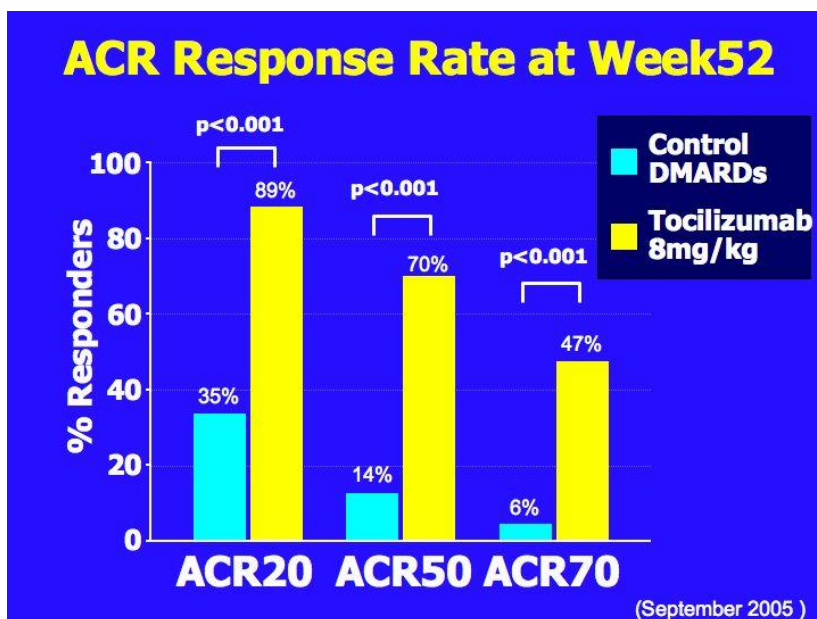

Figure 8

Interestingly, a Phase III clinical trial with a group of anti-TNF unresponsive patients showed a significant positive response to the anti-IL6R antibody, suggesting that the mechanism of the therapeutic effect of anti-IL6R and TNF inhibitors may be different.

As I showed in Fig.1, anti-IL6R treatment had a dramatic therapeutic effect on a patient with JIA. In Phase II trials with 14 patients, CRP and ESR went down and fever episode was stabilized, while all laboratory test findings became normalized. A double-blind, placebo-controlled, Phase III trial reported in Lancet in 2008 confirmed the efficacy of this antibody for

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JIA. Systemic onset of JIA is not responsive to TNF inhibitors. Moreover, a significant portion of anti-TNF unresponsive RA patients were shown to be responsive to the anti-IL6R antibody, suggesting that the mechanisms for these anti-cytokine therapies to exert their effects may differ.
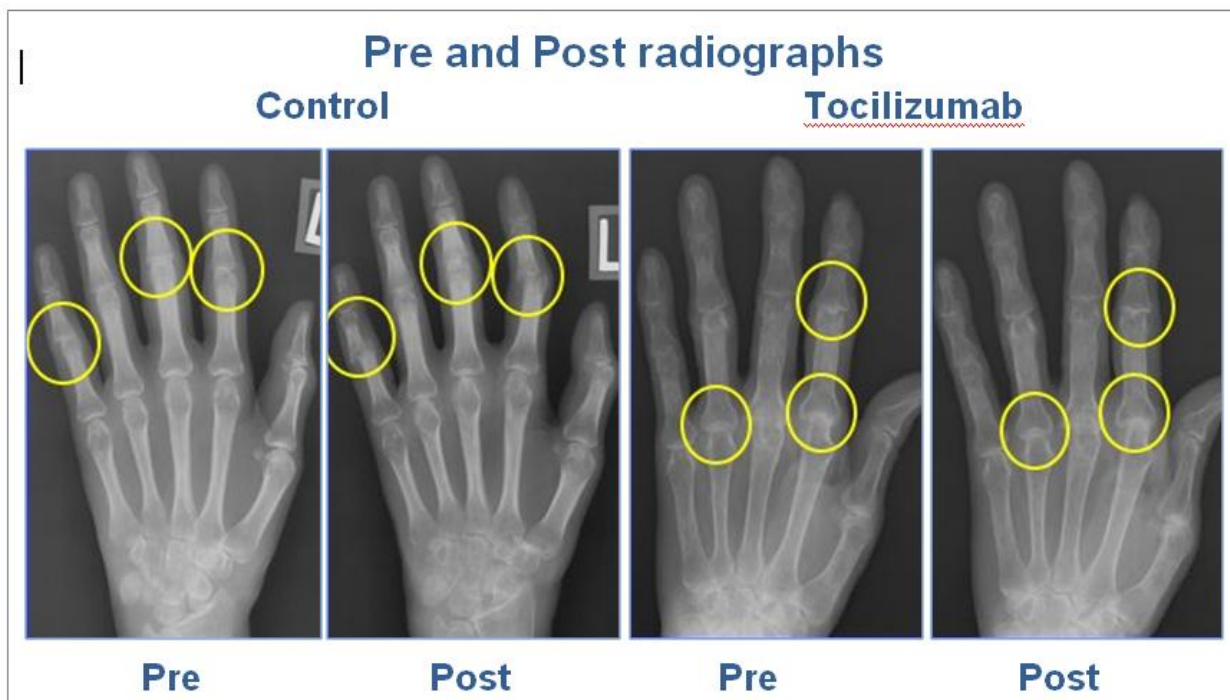

Figure 9.

In RA patients undergoing anti-IL6R therapy, serum IL-6 levels gradually decreased to normal levels. Anti-IL6R antibody can block the IL-6 signal but not neutralize IL-6, which strongly suggests that a blockade of the IL-6 signal could normalize the immune disorders present in these autoimmune diseases.

Recently, IL-6 has been found to be essential for the induction of one of the T helper subsets, TH17, which may be involved in the pathogenesis of autoimmune diseases (Fig.10).

Further basic as well as clinical studies on IL-6 and anti-IL6R antibody can be expected in the near future to contribute to the elucidation of the pathogenesis of various autoimmune diseases including RA and JIA.
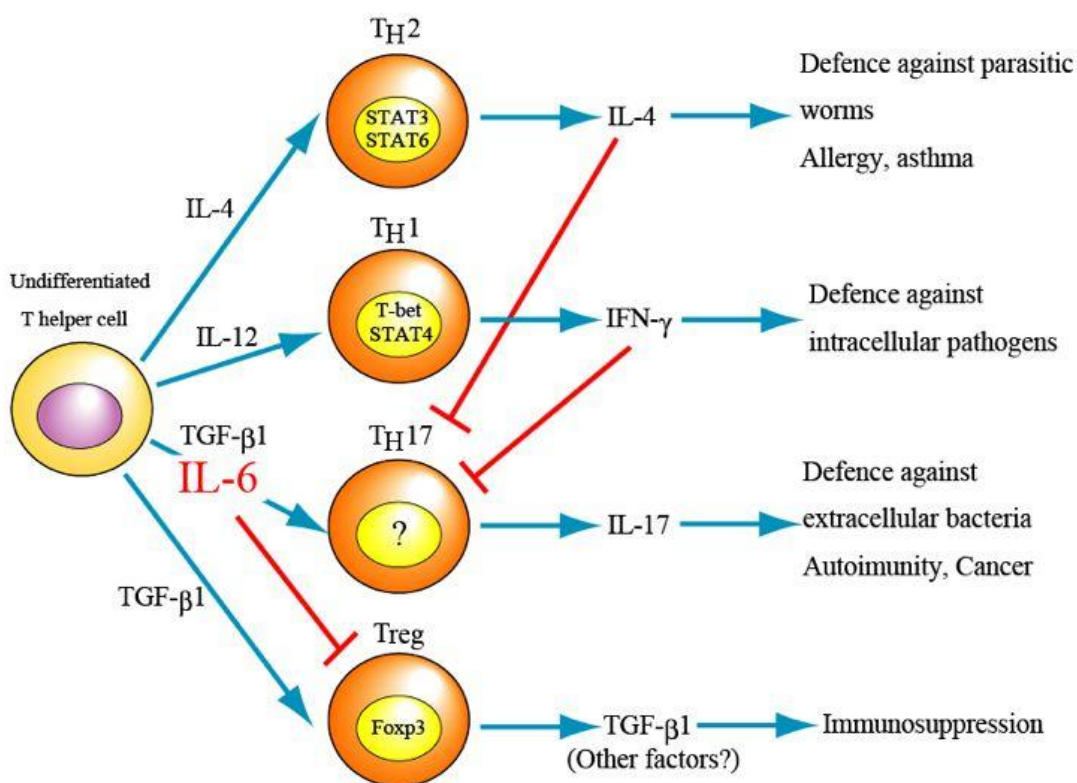
Figure 10.

/Tadamitsu Kishimoto
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