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INTERLEUKIN-6: From Basic Science to Medicine—40 Years in Immunology

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■ **Abstract** This essay summarizes my 40 years of research in immunology. As a young physician, I encountered a patient with Waldenström's macroglobulinemia, and this inspired me to study the structure of IgM. I began to ask how antibody responses are regulated. In the late 1960s, the essential role of T cells in antibody production had been reported. In search of molecules mediating T cell helper function, I discovered activities in the culture supernatant of T cells that induced proliferation and differentiation of B cells. This led to my life's work: studying one of those factors, interleukin-6 (IL-6). To my surprise, IL-6 turned out to play additional roles, including myeloma growth factor and hepatocyte-stimulating factor activities. More importantly, it was involved in a number of diseases, such as rheumatoid arthritis and Castleman's disease. I feel exceptionally fortunate that my work not only revealed the framework of cytokine signaling, including identification of the IL-6 receptor, gp130, NF-IL6, STAT3, and SOCS-1, but also led to the development of a new therapy for chronic inflammatory diseases.

EARLY DAYS

I was born in Japan in 1939, in the southern, rural part of Osaka prefecture. Being an only child, my parents cherished me too much, turning me into a spoiled child: You can perhaps trace this element in me even now! In 1946, I entered primary school near our home and remained in my hometown until graduating from high school at the age of 18. School was peaceful and idyllic, with plenty of freedom and no competition.

What first sparked my interest in medicine? It was Dr. Hideyo Noguchi. As a child, I read his biography and was very impressed by his life and achievements. I dreamed of becoming a medical researcher in the United States like him. After finishing at the local high school, I entered Osaka University Medical School, whose hospital had a strong reputation within Osaka prefecture.

ENCOUNTER WITH MY MENTOR

After a two-year premedical course at Osaka University, I joined the medical school in 1960. I found the lectures in basic medicine, such as anatomy, biochemistry, and pharmacology, boring; they only mentioned facts that we had to memorize. There was certainly no scientific pursuit involved, no question "Why?"

In my fifth year at medical school, Professor Yuichi Yamamura, a professor of biochemistry at Kyushu University, was appointed chairman of the department of medicine at Osaka University. He had graduated from Osaka University Medical School during World War II and served as a doctor in the navy. He discovered that dead mycobacterium could induce cavities in the lungs of rabbits that had been sensitized with BCG, the first demonstration of the involvement of delayed-type hypersensitivity in tuberculosis cavity formation. He went on to identify several cell surface components responsible for the induction of delayed-type hypersensitivity. This led to his life's work: cancer immunotherapy using cellular components of mycobacterium, and from there, the study of innate immunity.

I was excited by Professor Yamamura's lecture on immunological diseases, such as systemic lupus erythematosus. It prompted me to consider many interesting problems. Why does the immune system produce antibodies against the body's own DNA? Why do patients show such a wide variety of symptoms? In response to his lecture, I decided to enter his department and engage in immunology research. It was not only the contents of the lecture that influenced me; I also began to regard Professor Yamamura as my lifetime mentor in medicine.

STUDIES ON IgM

In a ward of the hospital, I met a patient with Waldenström's macroglobulinemia, a disease characterized by increased levels of monoclonal IgM. This prompted me to investigate the molecular structure of IgM. In the mid-1960s, one of the hottest topics in immunology was the structure and function of immunoglobulin molecules. IgM was one of the molecules that particularly attracted immunologists' attention because it loses antibody activities such as hemagglutination upon reduction and alkylation. At that time, Metzger and Small had just published papers claiming that IgM was composed of five subunits with two heavy chains and two light chains (1, 2). In contrast, studies by my supervisor, Dr. Kaoru Onoue, suggested instead that IgM antibody had only five high-affinity antigen-binding sites and each subunit had only one high-affinity site (3).

I purified human IgM from a patient's serum and analyzed its structure by papain and pepsin digestions. I obtained ten Fab papain fragments as well as five $(Fab')^2$ peptic fragments. By employing activated papain without reducing reagents, I succeeded in isolating large Fc fragments with molecular mass of 320 kDa, in which five Fc fragments were linked together with disulfide bridges (4). These results

confirmed that IgM was composed of five IgG-like subunits, linked together by disulfide binds between the Fc portions. All these features were later confirmed directly when IgM was visualized by electron microscopy (5). By employing monoclonal IgM with anti-DNP activity, Ashman and Metzger detected ten antigen-binding sites per IgM molecule. With hindsight, the detection of only five binding sites by Onoue was due to heterogeneous affinity of antibody molecules.

Several years ago, I gave a lecture on IL-6 at a medical school in the United States. A graduate student approached me afterwards and said, "I read a paper on molecular structure of IgM in the 1960s authored by T. Kishimoto. Is he your father?" I was pleased to hear this, realizing over again that my research in immunology has spanned 40 years.

B CELL GROWTH AND DIFFERENTIATION FACTORS

In 1970, I went to the United States as a postdoctoral fellow in Dr. K. Ishizaka's laboratory at Johns Hopkins University in Baltimore. It was my first visit to the United States. In the early 1970s, there were still enormous differences between Japan and the United States in every aspect of life. For instance, one dollar was worth 360 yen at that time. My stipend as a "joshu" (equivalent to postdoc in the United States) at the university was 58,000 yen (less than $200) per month. But in the United States, I earned $1000 per month, five times more than the amount I was used to in Japan. There were also big differences in equipment and laboratory instruments. Over the past 30 years, this situation has completely changed. In some cases, the stipend of a postdoctoral fellow in Japan is better than in the United States, and in the leading laboratories, the equipment and instruments are often of higher quality, too. As a result, the younger generation of Japanese students is not as eager to study abroad as my own generation. It is a pity that our young people tend to be less academically "hungry." When I was in the United States, the young Asian scientists there were mostly Japanese, and I think Japanese scientists made a major contribution to the development of the life sciences in America in the 1960s.

In 1967, Dr. Ishizaka identified a new immunoglobulin, IgE, which mediates allergic reactions (6). Johns Hopkins invited him from Children's Asthma Research Institute in Denver, and I joined his group, first as a postdoctoral fellow and later as an assistant professor in 1970. In 1968, interactions between T and B cells in the antibody production were made clear by J.F.A.P. Miller and H. Claman (7, 8). Immunology was rapidly moving from immunochemistry toward cellular immunology. Dr. Ishizaka and I both decided to turn in this direction. We had research backgrounds in immunochemistry, and Dr. Ishizaka was interested in the regulation of IgE production. We thus tried to develop an in vitro culture system in which IgE was produced, as well as IgG and IgM. We measured the amounts of antibodies quantitatively using the immunochemical method. For this purpose we chose rabbit lymphocytes because we were both familiar with rabbit

immunoglobulins from our previous studies on IgM and IgE. This system had two disadvantages. First, inbred strains were not available. Second, identification and separation of T and B cells were very difficult, even though anti-Ig columns could be applied for the separation of B cells. Nevertheless, an advantage with the rabbit system was that it was easy to establish an in vitro system for IgE antibody production using the Marbrook method. By using mesenteric lymph node cells primed with DNP-conjugated Ascaris antigen or ragweed antigen, we succeeded in inducing in vitro anti-DNP IgE as well as IgG antibody production by stimulating a DNP-homologous carrier (9).

Armed with this in vitro system, we went on to demonstrate the presence of soluble factors that enhance antibody production. When DNP-Asc primed lymphocytes were cultured with a DNP-heterologous carrier, such as DNP-bovine γ-globulin (DNP-BGG), there was little or no induction of anti-DNP IgG and IgE. By adding the culture supernatants obtained from Asc-primed lymphocytes stimulated with Asc antigen, IgG and IgE antibody production was augmented, which indicated to us the presence of factors responsible for B cell stimulation. Schimpl & Wecker (10) reported the presence of similar factors from alloantigen-stimulated murine T cells. They called these factors T cell replacing factor (TRF).

Dr. Ishizaka and I reported these results in 1973 (11–13). It was a memorable paper for me, because it set me on my life's work studying IL-6. The paper also contained the important suggestion that the factor(s) that enhance the IgE response might be different from those enhancing the IgG response. Culture supernatants from lymphocytes primed with BGG in complete Freund's adjuvant (CFA) augmented the IgG response but not IgE, whereas supernatants from cells primed with ragweed antigen in aluminum hydroxide gel, a very good adjuvant for the IgE response, could induce very high IgE production. Today, every immunologist would interpret this result as Th1 versus Th2 and γ-interferon (IFN) from Th1 versus IL-4 from Th2. In 1973, however, we could not offer a plausible explanation. Speculating that the factor(s) might have Ig-binding activity, we tried to absorb the activity by Ig-conjugated column but were unsuccessful. I am just happy now that our early experimental results in the 1970s already suggested the presence of isotype-specific factors.

In the 1970s, nobody working in immunology was interested in antigen nonspecific factors, or so-called cytokines. People even neglected any activities (or what was going on) in the culture "soup." At that time, most immunologists were instead attracted by so-called "antigen-specific factors." Many experimental results showed that the factors had H-2 restriction and their activity was absorbed with anti-H-2 antibody or antigenic determinants. Now we know this is not true. History teaches us the importance of accurate experiments: We should not be biased by the fashionable ideas of influential scientists.

At that time, I did not believe in the presence of so-called antigen-specific helper or suppressor factors. In fact, I was confident that even the antigen-specific response of lymphocytes would follow the standard theory of cell biology. In the early 1970s, Ishizaka's group elucidated the triggering mechanism of histamine

release from basophils: The crosslinkage of high-affinity IgE receptors activates the signal cascade of mast cells (14). I thought that this principle could be applied to the B cell responses; the crosslinkage of antigen receptors on the surface might activate B cells to become responsive to nonspecific T cell factors. My hypothesis was this: The interaction and crosslinkage of antigen receptors of B cells with antigens should be antigen-specific, whereas the factors could be antigen-nonspecific.

To prove this hypothesis, I attempted to induce Ig-production in B cells with anti-Ig and T cell factor(s). It was successful (15). Once again, I owed this success to the use of rabbit lymphocytes. In murine lymphocytes, negative signals through Fc receptors were strong, and Ig-production could not be induced. In the 1970s and 1980s, only studies with murine lymphocytes were widely accepted, so our studies were not generally appreciated. But later, Parker et al. managed to induce Ig in murine B cells by employing anti-Ig-conjugated beads that block negative signals through Fc receptors (16). With this event, our own studies were finally recognized and cited in the field.

The experimental design using anti-Ig plus T cell factor was also used to great effect by W.E. Paul's group (17, 18). This led them to the discovery of BSF-1, later renamed IL-4.

DISCOVERY OF IL-6

After my four years of study in the United States, I returned to the department of medicine in Osaka University chaired by Professor Yamamura, and began to investigate B cell growth and differentiation factors, which led to my life's work, IL-6. At this point, we switched our experimental system from rabbit lymphocytes to human cells for two reasons: (*a*) Monoclonal and homogeneous cells, such as transformed B cell lines or leukemic cells, were available; and (*b*) we could easily separate T and B cells by exploiting cell surface markers. We established an in vitro culture system of human peripheral lymphocytes in which polyclonal IgM and IgG production was induced by pokeweed mitogen (PWM) stimulation. We demonstrated that T cell factor(s) released from mitogen-stimulated T cells reconstitute Ig production in PWM-stimulated B cells (19). This confirmed the presence of factors that affect the antibody production of human B cells, just as previously observed in rabbit lymphocytes.

Fortunately, we happened to meet a chronic B cell leukemia (B-CLL) patient whose leukemic cells were responsive to anti-Ig and a cell-free supernatant of PHA-stimulated T cells (PHA-sup). Neither anti-Ig nor PHA-sup alone induced any IgM secretion (20). This confirmed our previous finding with rabbit lymphocytes, i.e., that two signals, crosslinkage of Ig receptors and T cell factor(s), are required for the activation and differentiation of B cells into antibody producing cells. Further characterization of the factors raised the possibility that at least two distinct kinds of factor(s) were present: one for growth of anti-Ig-stimulated B-CLL cells, and the other for differentiation into Ig-producing cells.

While we were studying human T cell factors, Morgan and colleagues (21) reported that a cell-free supernatant from PHA-stimulated T cells induces continuous growth of human T cells. On the basis of this result, Gillis & Smith (22) succeeded in the long-term propagation of human and murine cytotoxic T cells. In light of this, it was clear that we should next test whether all these factors reported on B cells and cytotoxic T cells were the same or different from each other.

In 1976, Dr. R.A. Good, president of the Sloan-Kettering Cancer Institute, visited Osaka for an annual meeting of the Japanese Cancer Association. I took Dr. and Mrs. Good to Kyoto for some sightseeing. During the short visit, Dr. Good appeared more interested in my ongoing experiments than in sightseeing! He kindly invited me to spend a couple of months every summer at the Sloan-Kettering. This gave me a great opportunity to screen a number of transformed or neoplastic B and T cell lines that were available at the Sloan-Kettering: B cell lines responsive to T cells factors, and T cell lines that secrete T cell factors. During my stay at the Sloan-Kettering, I identified several transformed B cell lines that produced Igs responding to T cell factor(s). One of these was the famous CESS cell line, which was later used as the indicator cells in the cloning of IL-6. I also found a subclone of a T cell line, CEM, which could be used as parental cells for generation of human T cell hybridomas. By establishing T cell hybridomas, we demonstrated the presence of two different kinds of B cell stimulatory factors: one for the growth of anti-Ig-stimulated B-CLL cells, and the other for Ig-induction in B-CLL cells or CESS cells (23).

Using murine lymphocytes, W.E. Paul, M. Howard, and their colleagues (24) suggested the presence of a B cell–specific growth factor (BCGF). They used a short-term B cell costimulator assay with anti-IgM. In their study, proliferation of anti-IgM-stimulated B cells was augmented by adding a culture supernatant of a mitogen-stimulated murine thymoma cell line (EL-4). They showed that this activity is different from IL-2 because it was not absorbed by an IL-2-dependent cytotoxic T cell line. The factor was later purified to homogeneity by their group as BSF-1, and its cDNA was cloned by Dr. T. Honjo's group. It is now called IL-4. The presence of a second BCGF activity was also reported by several groups, including K. Takatsu's and S. Swain's (25, 26). This factor, which they called B151 TRF or (DL)BCGF, induced growth and IgM production in the murine B cell line, BCL_1. Later, this factor was cloned and named IL-5.

From the late 1970s to the early 1980s, a number of factors were reported, but none of them were purified and their cDNAs were not cloned. Many questions were waiting to be answered. For instance (*a*) Do B cell–specific growth and differentiation factors really exist that are distinct from the growth factor for cytotoxic T cells (IL-2)? (*b*) If they do, how many factors are there? (*c*) Is B cell–specific growth factor different from B cell differentiation factor? In the early 1980s, the field of immunology was flooded with, and hampered by, complicated nomenclatures. It was evident that there were more names than factors to be named!

Under such circumstances, Dr. W.E. Paul and I organized a meeting to consider the nomenclature of these substances when the International Congress of

Immunology was held in Kyoto in August 1983. At the meeting, participants agreed that factors that had been functionally and chemically well characterized should be given a formal designation, namely B cell stimulatory factor (BSF) followed by a consecutive number (i.e., BSF-1, BSF-2. . .). On the basis of the available data, the 20 kDa mouse BCGF, studied by W.E. Paul's group, was designated BSF-1, while our human B cell differentiation factor (BCDF), which induces Ig-induction in CESS cells and B-CLL cells, was named BSF-2. However, these designations did not last long, because within a few years all the cDNAs encoding BSFs were cloned. In 1985, I wrote a review article for the *Annual Review of Immunology* entitled "Factors Affecting B Cell Growth and Differentiation" (27). In the conclusion, I wrote, "The isolation of several B cell stimulatory factors and the cloning and sequencing of their genes should be accomplished in the near future. And the activation mechanism of B cells may be revealed at molecular level by employing theoretically pure recombinant BSFs." Within a year, in 1986, the cDNAs encoding BSFs had been cloned. This led me to an entirely new world, which I had never expected. BSFs were not merely B cell stimulatory factors!

INTO MOLECULAR IMMUNOLOGY

Cloning of IL-6

In early 1980s, my mentor, Professor Yamamura, was elected president of Osaka University. He established the Institute for Molecular and Cellular Biology as a research center for molecular biology, and he invited Professor Yoshio Okada, who discovered cell fusion by Sendai virus in the 1950s, to be a director of the Institute. I joined him in 1983. This move enabled me to accomplish more molecular-oriented research into B cell factors. We invited Dr. Tadatsugu Taniguchi from Tokyo to the institute as a professor. At that time, he was only 35 years old and the youngest professor at Osaka University. He had succeeded in cloning the cDNA for IL-2 in 1983 (28), in the face of tough international competition. This was the first cloning of a cDNA for an interleukin. The field of "factor(s)" was heading inexorably to the molecular level. Because I was trained as a medical doctor, I had no background in molecular biology, having never been involved in cDNA cloning or DNA sequencing. The early 1980s were therefore difficult years for us.

T. Honjo, who had done beautiful work on Ig isotype switching in the 1970s, moved into the field of interleukins. At a meeting held in late 1985 in Japan, he reported that his group had succeeded in isolating the cDNA encoding BSF-1, later called IL-4. The paper was published in early 1986 (29). I was very much surprised and shocked. Indeed, I felt that I had been completely defeated. Dr. Honjo also reported, together with Dr. Takatsu, the cloning of the cDNA for (B151) TRF, later called IL-5, at the International Congress of Immunology in Toronto in the summer of 1986.

It was really hard for us to isolate a cDNA for BCDF/BSF-2 because we did not have experience in the field of molecular biology. After several years of struggling

with repeated failures, I, together with T. Hirano and colleagues, finally obtained what we wanted in June 1986: the cDNA for BSF-2, later called IL-6 (30). I appreciated the technical assistance and advice of Dr. T. Taniguchi. We submitted a paper to *Nature*, but at that time I was worried that our molecule might be the same as T. Honjo's TRF/BCGF II, and hence our paper would be rejected. In spite of my worry, both papers, his and ours, were published in the same issue of *Nature* in November (31). The two molecules were fortunately different from each other.

While trying to isolate the cDNA for BSF-2, we noticed that the same activity was observed in cardiac myxoma cells (32, 33). Cardiac myxoma is a benign heart tumor originating from the atrium. Patients with cardiac myxoma exhibit a wide variety of autoimmune and inflammatory symptoms, including autoantibodies, fever, joint pains, and anemia. All these symptoms disappear after surgical removal of the tumors. We found that cardiac myxoma cells produced a large amount of BSF-2 detectable by our antibody against purified BSF-2. With this result, I thought the molecule we were studying might contribute to the pathology of autoimmune diseases and play an important role not only in B cell immunology but also in various diseases. Perhaps I could attribute this kind of instinct to my medical training.

Biology of IL-6

Cloning of the BSF-2/IL-6 cDNA revealed that this molecule had in fact been studied under several different names by numerous labs. It seemed that IL-6 might have various interesting biological activities not limited to B cell immunology. It had been regarded as a novel interferon, but studies with recombinant IL-6 and anti-IL-6 antibody demonstrated that this was not the case (34).

As mentioned earlier, structural studies of immunoglobulin (Ig) molecules were hot topics in immunology in the 1950s and 1960s. Every immunologist wanted to have murine monoclonal Igs. M. Potter succeeded in generating murine plasmacytomas by simply injecting mineral oil into the peritoneal cavities of BALB/c mice (35). This achievement accelerated the structural and genetic studies on immunoglobulins. Intraperitoneal injection of mineral oil induced granulomas that produced plasmacytoma growth factor. One year after IL-6 was cloned, the partial amino acid sequence of plasmacytoma growth factor was determined, and it turned out to be mouse IL-6 (36). Indeed, transgenic expression of IL-6 in BALB/c mice induced monoclonal, transplantable plasmacytomas (37), confirming that IL-6 functions as a plasmacytoma growth factor. In addition, it turned out that human myeloma cells were responsive to IL-6, and some myeloma cells also produced IL-6. In most cases, bone marrow stromal cells produced a large amount of IL-6, which might be responsible for the generation and expansion of multiple myelomas in the bone marrow (38). I remember vividly how much I was excited by the fact that IL-6 functioned as a myeloma/plasmacytoma growth factor.

Another excitement for me at that time was the discovery that IL-6 functions as a hepatocyte stimulating factor (HSF) (39, 40). It was already known that acute

inflammation is accompanied by changes in concentration of many plasma proteins, a decrease in albumin and increased levels of many so-called acute phase proteins, including C-reactive protein (CRP), fibrinogen, serum amyloid protein, and haptoglobin. Indeed, this observation had been applied to various laboratory tests for the diagnosis of disease. Because inflammation, injury, or cancer in other parts of the body results in the increased synthesis of acute phase proteins in the liver, some had suggested the existence of hormone-like mediators, termed HSF. Using recombinant IL-6 and anti-IL-6 antibody, Gauldie, Heinrich, and colleagues (39, 40) confirmed that HSF was in fact IL-6. This opened up a new field studying cytokines and disease. Later, generation of IL-6 knockout mice by G. Köhler and his group showed that IL-6 was an essential molecule for antiviral antibody responses, as well as for the induction of acute phase reaction (41). We were getting almost daily requests for IL-6 cDNA protein and antibody from immunologists all over the world, and most weeks we learned fascinating new information about IL-6 function.

IL-6 Receptor and gp130

Despite this excitement, I decided not to expand our studies on the activities of IL-6 on various tissues and cells but moved directly to the subcellular level: receptors, signaling molecules, and gene expression. At that time, absolutely nothing was known about cytokine signal transduction. I particularly wanted to reveal the signaling pathway of cytokines "from surface to nucleus" by using IL-6 as a model. In the mid-1980s, none of the cytokine receptors, except for IL-2 receptor α chain (TAC), had been identified at the molecular level. The number of cytokine receptors on the cell surface is usually on the order of 10^2 to 10^3, a hundred times less than that of hormone or growth factor receptors. Seed & Aruffo (42) developed a high efficiency COS cell expression vector, and shortly afterward we succeeded in isolating the cDNA for IL-6 receptor by employing this method. This was the first example of the cloning of a cytokine receptor. It had an Ig-like domain at the N-terminal but no unique sequences in any other portions. It had a very short intracytoplasmic portion and did not have kinase domains. These features made it unlike an authentic "receptor" at that time, and *Nature* did not want to publish it, but *Science* kindly accepted it in 1988 (43). In the following two years, most cytokine receptors, including interferon receptors, IL-2 receptor β chain, and erythropoietin receptor, were isolated using the same or similar methods. The results were very interesting. They each showed very similar tertiary structures and thus comprised a large family of cytokine receptors. Even receptors for growth hormone and prolactin belong to this family.

Our excitement continued. We prepared an antibody against IL-6 receptor which could precipitate the 80 kDa protein. Interestingly, when T. Taga precipitated the IL-6 receptor following IL-6 stimulation of cells, another protein of 130 kDa was always coprecipitated (44). Without stimulation, only the 80 kDa band could be detected. I was thrilled with this result and speculated that the IL-6 receptor

consisted of two polypeptide chains, 80 kDa and 130 kDa, and that IL-6 stimulation would trigger association of the two chains. As the 80 kDa IL-6 receptor had a very short intracytoplasmic portion without any unique sequence, it was reasonable for us to speculate that the 130 kDa chain was responsible for signal transduction. We called this molecule simply gp130, because it was a cell surface glycoprotein of 130 kDa. Fortunately, in less than a year, we isolated a cDNA for gp130 by using anti-gp130 antibody (45).

Our excitement still continued. When we examined the expression pattern of gp130, we found it was expressed ubiquitously in all tissues, even in cells that lacked detectable expression of the 80 kDa IL-6 receptor (45). This result suggested to me that gp130 was not merely a component of the IL-6 receptor; perhaps it functioned as a common signal transducer for various cytokines. If many different cytokines shared the same receptor component, then we could easily explain the redundant activity of several cytokines.

This prediction turned out to be true. In the following years, we and others, including Dr. Yancopoulos, Dr. Metcalf, and colleagues, reported that ciliary neurotropic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OM), IL-11, and cardiotropin-1 (CT-1) all used gp130 as a component of their receptors (46–50). This explained why these cytokines had very similar activities. IL-6 was shown to inhibit the growth of a murine leukemic cell line, M1, and to induce differentiation into macrophages. Sachs and Metcalf also reported on factors called MGI-2 and LIF, respectively (51, 52), which exert a similar activity on M1 cells. MGI-2 turned out to be identical to IL-6, although LIF remained a distinct molecule. It became clear that LIF used gp130 as its receptor component, which explained why IL-6 and LIF show similar biological activities. The principle that cytokine receptors consist of two polypeptide chains, namely a specific receptor for each cytokine and a common signal transducer, turned out to be applicable to other cytokine receptor systems, too. For example, IL-3, IL-5, and GM-CSF use a common β component as their receptors. Interleukins for the growth and development of lymphocytes, IL-2, IL-4, IL-7, IL-9, and IL-15, use a common γ-component that was originally identified as a component of IL-2 receptor. I think the discovery of gp130 may be one of my most important achievements, for it introduced a completely new concept into the cytokine receptor field.

MAP Kinase-NF-IL6 Pathway

IL-6 is produced not only by T cells, but also by a panoply of cells including macrophages, fibroblasts, synovial cells, endothelial cells, glia cells, and keratinocytes. IL-6 expression was induced by a variety of stimuli, including cytokines such as IL-1, tumor necrosis factor (TNF), and platelet-derived growth factor (PDGF). Bacterial and viral infection and microbial components such as lipopolysaccharide (LPS) were also potent inducers of IL-6.

All the above findings led me to study the mechanisms of the IL-6 gene expression. One year after the isolation of the IL-6 cDNA, S. Akira and his colleagues

cloned the IL-6 gene and searched the promoter regions of the gene responsible for the regulation of IL-6 expression. We determined a novel 14 bp dyad sequence motif in the IL-6 promoter region that conferred IL-1-induced IL-6 expression. Next, we identified a nuclear factor binding to this motif, NF-IL6 (53). The cDNA of NF-IL6 was cloned, and shortly afterwards it became clear that it contains a region highly homologous to C/EBP, a rat liver nuclear factor with a leucine zipper structure.

Interestingly, although C/EBP was constitutively expressed, NF-IL6 was only expressed upon stimulation with various inflammatory signals such as LPS, IL-1, TNF, and even IL-6. Particularly in the liver, various stimuli, including IL-6, strongly induced NF-IL6 but not IL-6. This indicated that NF-IL6 might be responsible for the induction of acute phase proteins. Indeed, NF-IL6 was bound to the IL-6-responsive elements in the promoter regions of acute phase genes. These results showed that C/EBP and NF-IL6 were involved in negative and positive acute phase reactions, respectively: C/EBP is constitutively expressed and regulates the albumin gene, whereas NF-IL6, which is induced by inflammatory signals including IL-6, positively regulates the expression of the acute phase genes. We studied the transcriptional activation of NF-IL6 to elucidate the signal transduction pathway through gp130. Phosphorylation of NF-IL6 at threonine 235 by a Ras-dependent MAP kinase was shown to be essential for transcription factor activity (54). Although we had clearly identified one of the pathways of signal transduction through gp130, this was not so exciting because a Ras/MAP kinase cascade had been already described in the signaling of various growth factors.

JAK-STAT Pathway

As mentioned above, none of the cytokine receptors have tyrosine kinase domains in their cytoplasmic regions. Nevertheless, dimerization of the receptors can activate tyrosine kinase activity in cells. We had previously noticed that various members of the cytokine receptor family contain similar sequences of about 60 amino acids in their membrane-proximal cytoplasmic regions. In particular, 8 amino acid stretches in these regions were strongly conserved. We termed these stretches Box1, and speculated that they might bind specific intracytoplasmic tyrosine kinases (55, 56). Although we searched extensively for tyrosine kinases that would bind to the dimerized gp130, we were beaten in this race by J. Ihle's group (57–59). They identified JAK family tyrosine kinases as the major players downstream of the receptor in cytokine signal transduction.

Acute phase gene expression is the most suitable model for the study of signal transduction through gp130. Two types of IL-6 responsive element, type 1 and type 2, were present in the promoter regions of the acute phase genes. Type 1 IL-6 responsive element bound members of the C/EBP family, including NF-IL6 that we had already identified (53). A nuclear factor, which bound to Type 2 IL-6 responsive element, was identified by P. Heinrich and colleagues (60). They called this nuclear factor acute phase responsive factor (APRF). IL-6 stimulation

induced tyrosine phosphorylation of APRF within minutes, and the phosphorylated APRF was translocated into the nucleus. This process was strongly reminiscent of the activation of interferon-stimulated gene factor 3 (ISGF3) following IFN stimulation. Therefore, we hypothesized that APRF was a target of JAK tyrosine kinases and an important downstream component of signal transduction through gp130. S. Akira and his colleagues set out to clone the cDNA for APRF. It was a tough competition with P. Heinrich. I thought that Heinrich's group had almost done it. However, Akira's group succeeded in isolating the cDNA sooner than I had expected. They injected recombinant IL-6 into mice intravenously and isolated livers within minutes. They used approximately 8000 mice and a large amount of recombinant IL-6. These reagents would have cost about $2 million if we had purchased them commercially, but instead they were kindly donated by Ajinomoto Co. Ltd.

Our cloning of APRF revealed that it had a high degree of homology to the p91 subunit of the ISGF3 family involved in IFN signaling (61). APRF was tyrosine phosphorylated and translocated to the nucleus in response to IL-6 in hepatocytes. Tyrosine phosphorylation of APRF was also observed in response to other cytokines (LIF, OM, CNTF, CT-1, and IL-11) whose receptors share gp130, but not in response to IFN. In contrast, p91 was not phosphorylated in response to IL-6. From these results, we surmised that several different p91-related factors were present, and selective activation of those factors might explain the diversity of cellular responses to different cytokines. Later this was confirmed by the isolation of different p91-related factors, now called STATs (from STAT1 to STAT6). In February 1994, just after cloning the APRF cDNA, I went to the United States and learned that J. Darnell's group had already cloned a novel p91-related factor named STAT3 and submitted the paper to *Science*. We submitted our paper to *Cell*, asking the editor to publish it simultaneously with Darnell's paper in *Science*. Fortunately, both their paper and ours appeared in April 1994 in *Science* and *Cell*, respectively (61, 62).

Negative Feedback Pathway, SOCS

After the isolation of STAT3, we were naturally led to search for other members of the STAT family. Using the monoclonal antibody that we had generated against a sequence motif found in the SH2 domain of STAT3, we screened a murine thymus cDNA library and isolated about 20 new genes. One of them encoded the molecule that we originally called SSI-1 (STAT-induced STAT inhibitor) (63). Dominant-negative STAT3 could inhibit the IL-6- or LIF-induced SSI-1 expression, indicating that this was one of the target genes of STAT3. Moreover, overexpression of SSI-1 could inhibit LIF- or IL-6-induced M1 differentiation. When SSI-1 and JAK-1 were coexpressed in COS cells, SSI-1 interacted with JAK-1 and inhibited its kinase activity. We concluded that SSI-1 must be involved in the negative feedback regulation of cytokine signals. At that time, the Hilton, Nicola, and Metcalf group at the Walter and Eliza Hall Institute and Yoshimura at Kurume University each

cloned the same gene, and the three papers were published together in the same issue of *Nature* in June 1997 (64, 65). An Australian group named it SOCS, for suppressor of cytokine signals. Because this terminology represents the biological nature of this molecule more exactly than SSI, it has become the widely used nomenclature.

My investigation into how IL-6 signals are transmitted and regulated from "cell surface to the nucleus" is now almost complete. Our group has identified almost all the signaling components except for JAK tyrosine kinase, including IL-6, IL-6 receptor, gp130, NF-IL6, STAT3 (APRF), and SOCS (SSI). Our group at Institute for Cellular and Molecular Biology at Osaka University was most active in the late 1980s and early 1990s. In those times, more than 30 students and research fellows were working there, and interesting results emerged almost weekly. Indeed, someone in the United States once commented, "We can never compete with Kishimoto's Army!"

Other Projects Besides IL-6

Besides IL-6-related studies, several interesting projects were going on in our laboratory at that time. H. Kikutani's group was studying IgE binding receptors, and they identified the B cell marker CD23 as FcεRII (66, 67). At that time, the existence of many B cell markers, such as CD19, CD20, and CD21, were known, but their function or natural ligands were not. An exception was CD21, which functioned as receptor for a complement fragment, C3d and Epstein-Barr virus. Thus, CD23 was the second marker whose function was disclosed. Moreover, they identified two different species of FcεRII, i.e., FcεRIIa and IIb, which had different structures in their N-terminal intracytoplasmic portions. Interestingly, FcεRIIa expression was limited to a certain stage of B cells, but FcεRIIb was expressed on B cells, monocytes, and eosinophils, and their expression was regulated by IL-4 (68).

T. Nagasawa and H. Kikutani identified a new cytokine involved in pre–B cell development, called PBSF (SDF-1) (69). The essential role of PBSF/SDF-1 in B lymphopoiesis was confirmed by the preparation of the gene-deficient mice (70). An interesting story emerged when Nagasawa isolated a receptor for PBSF/SDF1 (CXCR4) (71). He noticed that its sequence was a murine homolog of fusin, the human immunodeficiency virus I entry coreceptor. This suggested to us that inhibitors of CXCR4 might be created as efficient drugs for AIDS. However, when he generated the CXCR4 knockout mice (72), they were embryonic lethal, raising potential safety problems with such drugs. Interestingly, these mice were defective in the large vessels supplying the gastrointestinal tract, indicating the existence of a new signaling system for organ vascularization.

We also identified a novel molecule for bone development (73). Komori, in our laboratory, was also studying B lymphopoiesis. To see how a transcription factor, cbf1, would function in the lymphoid development, he prepared the knockout mice as usual. To our surprise, knockout mice died just after birth without breathing.

He noticed that the mice did not have any bone, completely lacking ossification. In other words, cbf1 plays an essential role in osteogenesis. The result contributed to the subsequent identification of the gene responsible for a dominantly inherited disease, cleidocranial dysplasia. Komori went into the field of bone research and has been publishing interesting papers ever since.

BENCH TO BEDSIDE

In 1991, when the activities of our laboratory at the Institute for Cellular and Molecular Biology were at their peak, I was asked to be chairman of the department of medicine. My mentor, Professor Yamamura, who used to hold the post, had passed away in 1990. Having entered that department in 1965 after graduating from the medical school, I had a nostalgic feeling toward the department, but I initially declined the invitation, wanting to continue my basic studies. However, the medical school faculty persisted in their attempts to persuade me, and finally I accepted the invitation. In an article that appeared in *Science* in 1990, I read an impressive passage: "With science having largely demystified the 'witchcraft' of immune response, immunologists are turning to the next challenge: putting their new knowledge to clinical use in taming pathological immune responses. Successes are still mostly on the horizon." I felt it was the appropriate time to try to apply our basic studies on IL-6 to human disease.

I had been at a similar crossroads one year earlier. I was invited to Harvard Medical School, which offered me a chaired professorship in the department of medicine. Professor Tosteson, dean of the school, said to me, "The most excellent graduate students and postdoctoral fellows come to Harvard from all over the world, and you can do your research with them. You may not be able to make such an environment in Osaka." While I was wavering between going to Boston and staying in Osaka, Professor Yamamura, who was seriously sick at the time, told me not to go. He urged me instead to work for Japanese science and young Japanese scientists. So, after experiencing several difficult choices in my life, I finally moved to the department of medicine as chairman.

While studying IL-6, I had noticed that this molecule is involved in various diseases, including chronic inflammation and hemopoietic malignancies. For example, cardiac myxoma cells produce large amounts of IL-6, which explains various symptoms of the patients. In 1988, we reported the constitutive overproduction of IL-6 by synovial tissues of rheumatoid patients (74). This well explains all the symptoms seen in patients. We also found an abnormal overproduction of IL-6 in patients with Castleman's disease (75). Affected lymph node cells overproduced IL-6, which explained symptoms such as high fever, anemia, acute phase reactions, hyper γ-globulinemia, secondary amyloidosis, and massive plasma cell infiltration into affected lymph nodes. Later, it turned out that a Kaposi's sarcoma associated herpesvirus [Human Herpes Virus 8 (HHV-8)] was a causative agent, and a viral genome could be detected in Castleman's lymph nodes (76). The HHV-8 genome

encoded a viral IL-6 that directly binds human gp130 to stimulate the production of endogenous IL-6 (77). From these findings in patients with cardiac myxomas, rheumatoid arthritis, and Castleman's disease, we expected that the blockade of IL-6 and its receptor interactions would provide a new therapy for these diseases.

On the basis of these experimental and clinical results, we started to develop an anti-IL6 receptor blockade therapy. Together with Chugai Pharmaceutical Co. Ltd. and the MRC Collaborative Center in London, mouse monoclonal antibody binding human IL-6 receptor was humanized by complementarity-determing region (CDR) grafting. This antibody was applied to treat seven patients with multicentric Castleman's disease, with the approval of our institute's ethical committee and the patients' consent (78). Immediately after administering the antibody, fever and fatigue disappeared, while anemia, as well as serum levels of C-reactive protein, fibrinogen, and albumin started to improve. After three months of treatment, hyper γ-globulinemia and lymphadenopathy were remarkably alleviated, as were renal function abnormalities in patients with amyloidosis. The pathophysiologic significance of IL-6 in Castleman's disease was thus confirmed, and blockade of IL-6 signal by anti-IL-6 receptor antibody was shown to be a potential new therapy for IL-6-related diseases. The phase II clinical trial with 28 Castleman's patients was done in 2002, and the antibody showed a significant effect on all patients. As Castleman's disease is an orphan disease, the antibody for this disease will be on the market in the beginning of 2005.

After the successful experimental therapy of Castleman's patients, we tried to apply the same antibody for the treatment of rheumatoid patients. To investigate the direct role of IL-6 in the development of rheumatoid arthritis, IL-6-deficient mice were backcrossed for eight generations into C57BL/6 mice, and histological manifestations were compared between wild-type and IL-6-deficient mice following the induction of antigen-induced arthritis (79). Wild-type mice developed severe arthritis, whereas IL-6-deficient mice displayed little or no arthritis. The expression of TNF mRNA in the synovial tissues in IL-6-deficient mice was comparable to that of wild-type mice, even though no arthritis was observed in the former. Recently, S. Sakaguchi and colleagues reported that deleting the IL-6 gene in his SKG mice, which develop rheumatoid arthritis owing to the mutation of the T cell signaling pathway, resulted in the complete protection from the development of the disease, whereas 20% of TNF-α-deficient SKG mice still developed the disease (80). All these basic studies encouraged me to apply anti-IL-6 receptor therapy to patients with rheumatoid arthritis.

In 2001 and 2002, phase II trials had been completed in Japan with 164 patients, and in Europe with 359 patients. The results were comparable with or better than anti-TNF antibody or soluble TNF receptor therapies (81). The incidence of a 20% improvement in disease activity according to the American College of Rheumatology criteria (ACR20) was 78% in the Japanese phase II trial. The incidences of 50% and 70% improvement in disease activity (ACR50 and ACR70) were 40% and 16%, respectively. In long-term trials of over 15 months, ACR20, 50, and 70 reached 88%, 67%, and 42%, respectively. After long-term administration,

the serum IL-6 level gradually decreased and reached an undetectable level in some patients, which suggested that anti-IL-6 receptor therapy was not simply anti-inflammatory but might affect the fundamentals of the immune system. At present, large-scale phase III trials are being carried out in Japan by Chugai Pharma Co. Ltd. and in Europe and the United States by Roche Co. Ltd. The results of these trials will be available in 2005 and 2007, respectively.

Another exciting clinical result was observed in the treatment of systemic onset juvenile idiopathic arthritis (sJIA). sJIA is a severe multi-organ disease, which is accompanied by symptoms such as spiking fever, skin rash, arthritis, pericarditis, hepatosplenomegaly and growth retardation. A high dose of corticosteroids is the only medication that suppresses the disease activity, and anti-TNF therapy is not very effective. Phase I/II trials of 11 children were carried out by Professor Yokota in Yokohama City University. After administration of the antibody, high-grade or quotidian fever subsided and arthritis improved quickly in all the children, accompanied by the normalization of all the laboratory tests, including CRP. Eighteen months after treatment, we observed that one of the children grew taller by 18 cm. IL-6 inhibits the signaling pathway provided by growth hormone, and so the result confirmed that the growth retardation observed in sJIA was due to the overproduction of IL-6. Long-term administration normalized the serum IL-6 levels in several children, which again indicated that IL-6 might fundamentally restore the immune disorders in sJIA. At the moment, we do not understand the pathogenesis of sJIA. Because the effects of our antibody treatment on sJIA patients were so dramatic, further studies into changes in immune function and gene expression in the patients before and after treatment might provide important insights into the pathogenesis of the disease. If so, our studies "from laboratory to clinic" will again move "from clinic to basic studies."

FROM UNIVERSITY PRESIDENT TO COUNCIL FOR SCIENCE AND TECHNOLOGY POLICY

In June 1997, I was elected president of Osaka University. I felt greatly honored to be president of my alma mater, one of the most prestigious universities in Japan. However, for a while I could not decide whether to accept the position, for several reasons. A big research grant ($3 million per year for five years) had recently been awarded to my laboratory in April. At that time, I really wanted to concentrate on my research because it was getting very exciting. I agreed to be president on the condition that I could still be involved in research. But I soon realized that it was almost impossible to combine the two roles. Our studies on SOCS were lagging very much behind those of the Melbourne group. I realized that good research required concentration. As president, I devoted myself to the development of Osaka University as a more internationally competitive research-oriented university. I had established two new graduate schools, for frontier bioscience and information science. All ten faculties, including natural as well as social and human sciences, had been reorganized as graduate schools, to which undergraduate courses were

attached. The annual budget reached almost $1 billion, and the quantity of competitive research grants was increased.

My six-year term as a president ended in August 2003, and I was very much looking forward to being back in the laboratory. I thought that I might still catch up with the front line. I was donated $5 million by Chugai Pharma Co. Ltd. to establish my laboratory in the graduate school of frontier biosciences. As a professor of immunology, I restarted my laboratory with several faculty, fellows, and graduate students. Our aims are to reveal the mechanism whereby blockade of IL-6 signaling could show such a significant curable effect on JIA or RA. It was not due to a simple anti-inflammatory effect, as I have already discussed. My studies originated in B cell immunology, developed into molecular immunology, and moved into clinical medicine, and are once again going back to basic immunology and molecular biology.

When I had just restarted my laboratory, I was asked to be a member of the Council for Science and Technology Policy in the Japanese government, chaired by the prime minister. Again, I hesitated to accept but was persuaded that it was a very important task upon which the future of Japanese science depended. I am now mainly working at the cabinet office in Tokyo, three or four days a week, with one or two days in my laboratory at Osaka University. I would be happy if I could devote myself to creating better environments for young talented scientists in Japan.

Here I am closing the story of my 40-year history as a basic immunologist, a chairman of internal medicine, a university president and a policy maker in Japanese science. I think I was very lucky as a researcher because I found an interesting molecule, IL-6, with pleiotropic activity and an involvement in the pathogenesis of several diseases. Now, the results are going to be applied to patients with otherwise incurable diseases. Through these studies, many young scientists were trained to become internationally highly regarded researchers, including Drs. T. Hirano, H. Kikutani, S. Akira, and T. Taga. If I were embarking on my career again today, I think I would hesitate to follow the same path: I may not be so fortunate the next time!

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