The Crafoord Prize 2009

This year's Crafoord Prize is awarded three scientists who have identified two key players in the immune system: the signal substances interleukin-1 (IL-1) and interleukin-6 (IL-6). These are essential to protect the body against invading viruses and bacteria. But elevated concentrations make the immune cells run amok and attack the body's own joints. New drugs that eliminate the effects of IL-1 and IL-6 can sometimes effectively inhibit the inflammation.

Discoveries paved way for effective drugs

Just some ten years ago rheumatoid arthritis, the commonest form of polyarthritis (see fact box at page 7), was a disease that could not be stopped. Many patients' joints slowly deteriorated and they were at risk of becoming severely disabled, at worst ending up in a wheelchair. There were treatments that slowed down the progress of the disease, but few people fully avoided its painful course.

For many of the world's tens of millions of sufferers from rheumatoid arthritis, the situation is now different. Thanks to new drugs known as 'TNF-alpha inhibitors', people previously confined to bed have gone back to work. Their joint pain has decreased or disappeared completely, and many former sufferers feel that they have got their lives back. In the year 2000, the Crafoord Prize was therefore awarded for the development of anti-TNF-alpha agents to combat rheumatoid arthritis.

But not all sufferers from rheumatoid arthritis obtain relief. For roughly a third, the situation remains almost the same as it was ten years ago. For some reason, the TNF-alpha inhibitors do not help them. The same applies to those affected by many other forms of polyarthritis.

New medical treatments based on the fundamental discoveries that resulted in this year's Crafoord Prize, on the other hand, afford hope that the above-mentioned groups, too, will be able to benefit. **Charles Dinarello** isolated IL-1 in 1977 and **Tadamitsu Kishimoto**, in cooperation with **Toshio Hirano**, isolated IL-6 in 1985. Both these proteins stimulate the cells of the immune system and are found in high concentrations in the joints of many people with polyarthritis. Drugs that mitigate the effect of IL-1 are already available, and a drug against IL-6 has been approved in Japan. Europe and the US will probably soon follow suit.

Lethal febrile substance a key player in the immune system

When, in the 1970s, Charles Dinarello and Tadamitsu Kishimoto began studying the immune system, a good deal was already known about the arsenal of white blood cells that protect the body against viruses, bacteria and other invading microbes. But the scientists knew less about the substances that cells release in order to communicate with one another, thereby making the system coherent. Today, we call these substances 'cytokines'; back in the 1970s they were still unknown 'factors'.

Charles Dinarello became intrigued by a factor referred to by the scientists as 'endogenous pyrogen', known to cause fever. As the researchers knew, it was remarkably potent. Only a tiny quantity was needed to induce fever in a rabbit, and in higher doses it was lethal.

In 1977, Charles Dinarello succeeded in isolating this pyrogen by exposing white blood cells of a certain type, monocytes, to killed bacteria. The result was that the monocytes released pyrogen in the ambient nutrient solution. Charles Dinarello succeeded in deriving pyrogen in its pure form, with no contaminants, from this liquid.

This paved the way for a series of experiments, in the course of which Charles Dinarello discovered that endogenous pyrogen, which later came to be known as 'IL-1', had been studied by many scientists in a wide range of fields. They had all given their various names to the substance that reflected what IL-1 was doing in their own respective experiments. But it was in fact one and the same substance.

Dinarello's results met, however, with scepticism among his fellow researchers. Could a single substance genuinely perform so many different functions in the body? Was the pyrogen used by Dinarello truly devoid of other substances? If there were contaminants from the monocyte included in the experiments, it might explain all the diverse effects.
It was not until the mid-1980s that Dinarello was able to prove his theories, and it was modern molecular biology that came to his aid.

Clean experiments gave clear answers

Modern molecular biology meant that the scientists learnt how to isolate genes and move them from one organism to another. They began to 'clone' proteins. Most genes are a blueprint for the appearance of a protein. The code in the genes is universal: bacteria, plants and human beings all have the same code. The scientists started to reap benefits from this. Having isolated the genes for human proteins, they inserted them into bacteria and transformed the bacteria into 'protein factories'. Accordingly, the researchers were suddenly capable of obtaining large quantities of a protein that previously, for example, had been present only in tiny amounts in the blood. They had laid the foundations for a completely new type of experiment.

Dinarello isolated the gene for IL-1 during 1984. He placed the gene in bacteria, inducing them to produce IL-1 instead of monocytes. This assured him that the effects he saw were not due to contaminating substances from the monocyte. On the other hand, the scientists discovered that there were two different kinds of IL-1, and dubbed these 'IL-1α' and 'IL-1β'.

When the researchers injected these IL-1 proteins into people and animals, the effects were immediate and powerful. The subjects became feverish and began to shiver, while incurring joint pains and headaches. If IL-1 was injected straight into the joints, the subjects temporarily got the same symptoms as those suffered by patients with polyarthritis. (However, all symptoms disappeard afterwards.)

Rival to IL-1 became a new drug

In 1984, the same year that the gene for IL-1 was isolated, scientists found a related substance. It proved to be a rival that counteracts the functioning of IL-1 in the body. Today, this related substance is known as the 'IL-1 receptor antagonist (IL-1ra)'. In the body, it competes with IL-1 to bind to special receptors found on the surface of cells that react with IL-1.

When, for example, IL-1 binds to receptors on brain cells that govern thermoregulation in the body, a signal is transmitted that makes the body temperature rise. When IL-1ra sticks to the same receptor, on the other hand, nothing happens. IL-1ra merely blocks the receptor, preventing IL-1 from becoming attached.

In animal experiments in which researchers have eliminated the IL-1ra gene, mice have developed a disease that resembles rheumatoid arthritis. When the mice were unable to make IL-1ra, IL-1 was free to exert its effects. For example, IL-1 stimulates the production of various substances, including nitrogen oxide that wears down the joints. IL-1 also inhibits production of collagen, a protein that imparts strength and elasticity to bone and cartilage, thereby preventing natural repair from taking place.

The above-mentioned experiments helped the scientists to realise that IL-1ra would act as a drug against various rheumatic and autoimmune diseases in which the concentration of IL-1 in the body is much too high. By injecting IL-1ra, the researchers thought, it would be possible to eliminate the effects of IL-1.

Today, 25 years after the discovery, IL-1ra is registered as a drug for the treatment of rheumatoid arthritis. There are also preliminary experiments indicating that IL-1ra can curb inflammation in gout. Moreover, the drug has recently been shown to be effective against certain forms of febrile diseases, such as Still's disease in adults. The drug also seems to help some children who suffer from juvenile chronic arthritis.

As an alternative to IL-1ra there is also now, in the experimental stage, an antibody that binds to IL-1 and eliminates its effects. The new antibody has recently been tested on humans and appears to be especially effective against polyarthritis in children.

Kishimoto fascinated by enigmas of the immune system

Tadamitsu Kishimoto's lifelong interest in the immune system was kickstarted back in the mid-1960s. He was then a medical student in his fifth year at Osaka University, finding the lectures about immunological diseases utterly captivating. The teaching professor, Yuichi Yamamura, talked about such diseases as SLE, a rheumatic disease in which, for some unknown reason, the body starts to produce antibodies against its own genetic material. This brings about numerous symptoms, such as fatigue, fever, joint pain, weight loss, skin rashes, hair loss, mouth sores and hypersensitivity to sunlight. Why does this happen? How can it have so many different effects? These and similar questions captured Kishimoto's interest and he soon joined Yamamura's research team.

After obtaining his PhD on the structure of a particular antibody, Kishimoto left for the US in 1970. There, he embarked on a new line of research and began investigating the 'factors' released by helper T cells. Helper T cells serve a kind of coordinating and commanding role in the immune system, dispatching various substances that signal to other cells in the system how they should act.

Kishimoto showed that one of these substances activates the 'B cells', as they are called, that produce antibodies. On the surface of B cells there are antibodies, specially designed to

recognise and bind to foreign viruses, bacteria and other microbes that invade the body. The system is superbly designed: every B cell makes its own distinctive type of antibody and, since all antibodies differ slightly from one another, the body's B cells can trace millions of unknown substances. All this prevents intruders from making their way in without detection.

Kishimoto discovered that two different signals are required to activate B cells. A foreign substance, such as a bacterium, must be caught by an antibody on the surface of a B cell, and at the same time the B cell needs stimulating with a factor from the helper T cells. This together causes the B cells to mature and start producing large quantities of antibodies, which are released from the cells into the bloodstream. There, they circulate and attach themselves to the foreign microbes. The antibodies serve as flags to other parts of the immune system, such as macrophages and neutrophiles, which perform the task of neutralising foreign substances. The antibody signals that it has encountered something that must be destroyed (see the graphic illustration on page 5).

The dual signal revealed by Kishimoto exists so that exactly the right B cells are activated. Once the immune system gets going it is extremely powerful, and the regulation must therefore be rigorous.

Factors in flux

At that time, in the late 1970s and early 1980s, researchers had found a range of factors that activated or subdued the immune system in various ways. A variety of names had been given to these factors. In an overview article, Kishimoto wrote that 'it was evident that there were more names than factors to be named'. It was not until the scientists, using the new molecular biology, started cloning the various factors (see above) that sorting them out became feasible.

Kishimoto, now back in Osaka, was a trained doctor but not well versed in molecular biology. He suddenly found himself overtaken by his colleagues, who were isolating numerous genes for different factors in the immune system. Kishimoto began to feel under pressure. He wanted to be the first to clone the factor he was studying. This factor was later to be named 'IL-6'.

This year's third Crafoord Prize laureate, Toshio Hirano, now enters the picture. Hirano had a background in molecular biology, also worked in Japan and had been studying IL-6 since 1981. When he moved to Kishimoto's laboratory they were able, in 1986, to clone IL-6 and derive the protein in its pure form. Just as Charles Dinarello had done with IL-1, Kishimoto and Hirano now revealed that many researchers had studied IL-6 under other names and that the factor had numerous other functions in the body.

Clues from a benign tumour

At the time when Kishimoto and Hirano were attempting to find the gene for IL-6, they discovered that a benign cardiac tumour, myxoma, produced IL-6 in large quantities. Patients with myxoma get symptoms resembling those of rheumatic diseases: fever and joint pain, for example. But as soon as the tumour is surgically removed the symptoms vanish.

Monocytes are white blood cells that mature into macrophages. They are attracted to the inflammation by cytokines, such as IL-1 and TNF-α.

Macrophages, a key driver of inflammation, release large amounts of IL-1, IL-6 and TNF-α, which activate other white blood cells. Macrophages also clean up the inflamed area by ingesting microbes and dead cells.

Helper T cells perform a kind of coordinating command role in the immune system, releasing a series of cytokines that activate other immune-system cells.

B cells produce antibodies. In rheumatoid arthritis they make 'autoantibodies', which mistakenly assail the body itself instead of invading microbes.

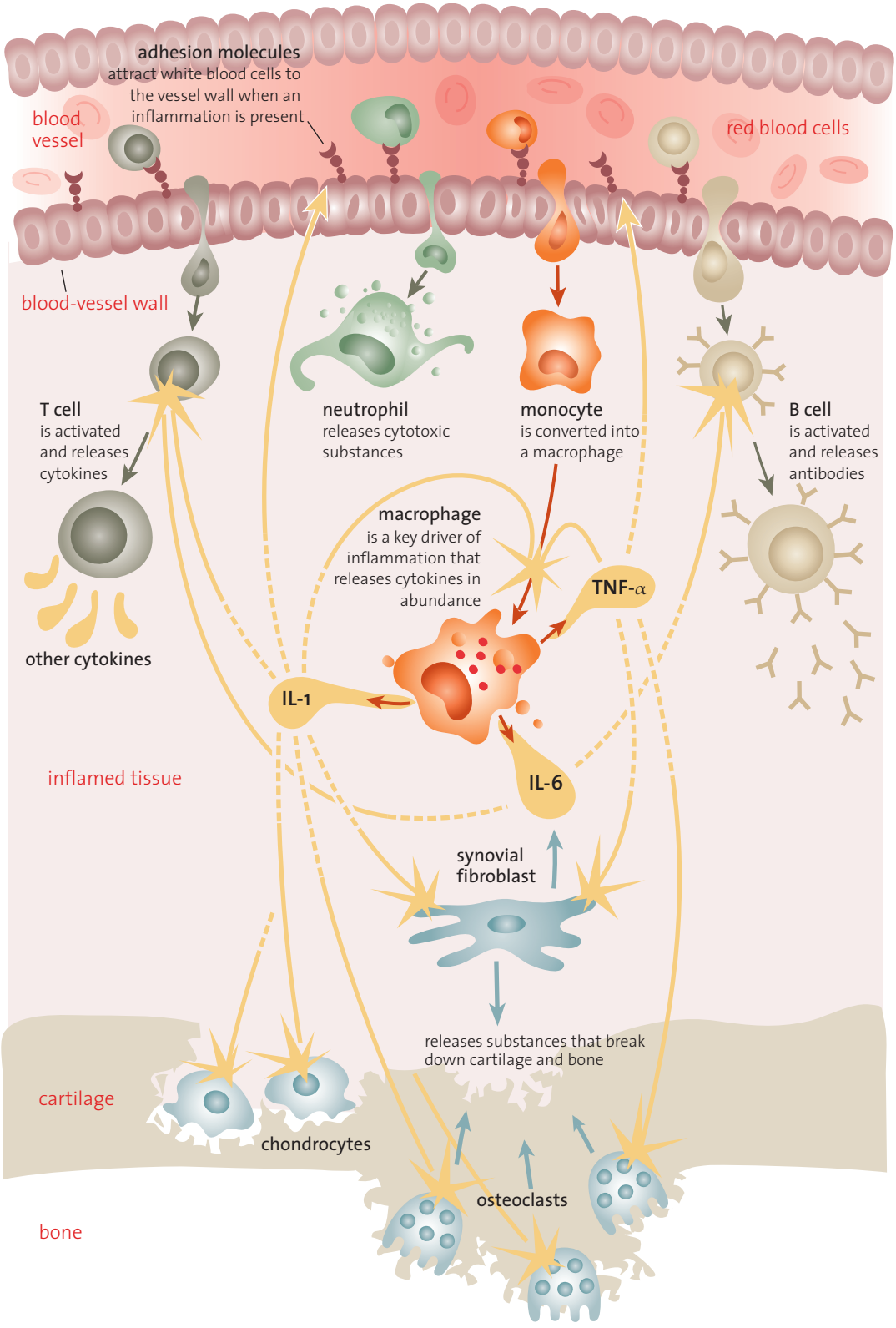
Neutrophils are short-lived white blood cells that ingest whatever comes their way, such as the remains of dead cells or bacteria. They also release oxygen radicals and other tissue-damaging substances.

IL-1 and TNF-α attract white blood cells from the blood vessels to the inflamed tissue, and activate a range of cells. In addition, IL-1 raises body temperature.

IL-6 keeps the inflammation going by activating several different types of immune cell, and also contributes to a rise in body temperature. In the liver, IL-6 triggers production of 'acute-phase proteins', one effect of which is to exacerbate the inflammation.

Rheumatoid arthritis

A simplified depiction of the roles of IL-1, IL-6 and TNF in joint inflammation



Synovial fibroblasts are a type of connective-tissue cells that turn aggressive in rheumatoid arthritis. For example, they release IL-6 and substances that break down cartilage and bone.

Chondrocytes are cells that normally promote cartilage growth. IL-1 and TNF-α destroy chondrocytes or pervert them so that they break down cartilage instead.

Osteoclasts are cells that break down and resorb bone tissue.

Illustration: Airi Iliste

This discovery meant that Kishimoto and Hirano began to suspect that IL-6 is implicated in autoimmune diseases—those in which the immune system goes wrong and turns against the body itself. In 1988 Kishimoto and Hirano also found overproduction of IL-6 in the joints of patients with rheumatoid arthritis, confirming their suspicions. In the following year, Kishimoto and Hirano also reported that patients with Castleman's disease, with its symptomatic enlargement of lymph glands and overactive immune system, produced abnormal quantities of IL-6.

New targeted drugs

From these discoveries, Kishimoto and Hirano drew the conclusion that an IL-6 'blockade' could counteract rheumatoid arthritis and Castleman's disease. The patients developed an antibody that binds to the receptor for IL-6 on the surface of various cells. Just like IL-1ra, the antibody carries out this binding without transmitting the signal that IL-6 would have triggered.

The first studies of the new antibody were performed on patients with Castleman's disease. Seven patients, aged 33–59, were treated. As if by magic their fever and tiredness ended, and after three months' treatment the patients' blood values were considerably better. Some patients had already, before the study, had problems with their kidneys as well, but the treatment improved their kidney function as well.

Today this drug is approved in Japan for treating Castleman's disease and rheumatoid arthritis, and is also close to attaining approval in Europe and the US alike. A major study of nearly 1,200 sufferers from rheumatoid arthritis recently demonstrated a very good effect when the treatment is administered in combination with Methotrexate, a cytotoxic agent. After a year, nearly half the patients had become symptom-free. Many of them were people who had not been helped by TNF-alpha inhibitors. The drug also appears to be effective in treating certain types of severe arthritis in children.

Facts about polyarthritis

• *Polyarthritis* is a collective term for rheumatic diseases that involve inflammation of several joints in the body.

• The commonest form of polyarthritis is *rheumatoid arthritis*. Tens of millions suffer from rheumatoid arthritis. The immune system identifies joints in the sufferer's own body as foreign, and breaks them down.

• *Gout* is a form of polyarthritis in which uric acid crystallises and is deposited in the joints. The immune system reacts to the crystals and inflammation results.

• People with *psoriasis*, a chronic skin disease, can also get inflamed joints. This is known as *psoriatic arthritis*. The joints of the back are sometimes also affected.

• *Bechterew's disease* (also known as spondylarthritis) also causes inflammation of the back joints. This disease usually affects men and the first symptoms commonly appear before the age of 40.

• *Reactive arthritis* sometimes arises after intestinal or urinary infections. The disease can be caused by fragments from bacteria that have lodged in the joints.

• People who suffer from the rheumatic disease *SLE* (*Systemic lupus erythematosus*) can also get polyarthritis.

• *Still's disease*, which is primarily a febrile disease, sometimes leads to polyarthritis.

• *Juvenile arthritis* (or juvenile rheumatoid arthritis) is a collective name for several different types of polyarthritis that affect children and adolescents.

LINKS AND FURTHER READING

Review articles

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Scientific articles

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The cloning of IL-6:
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