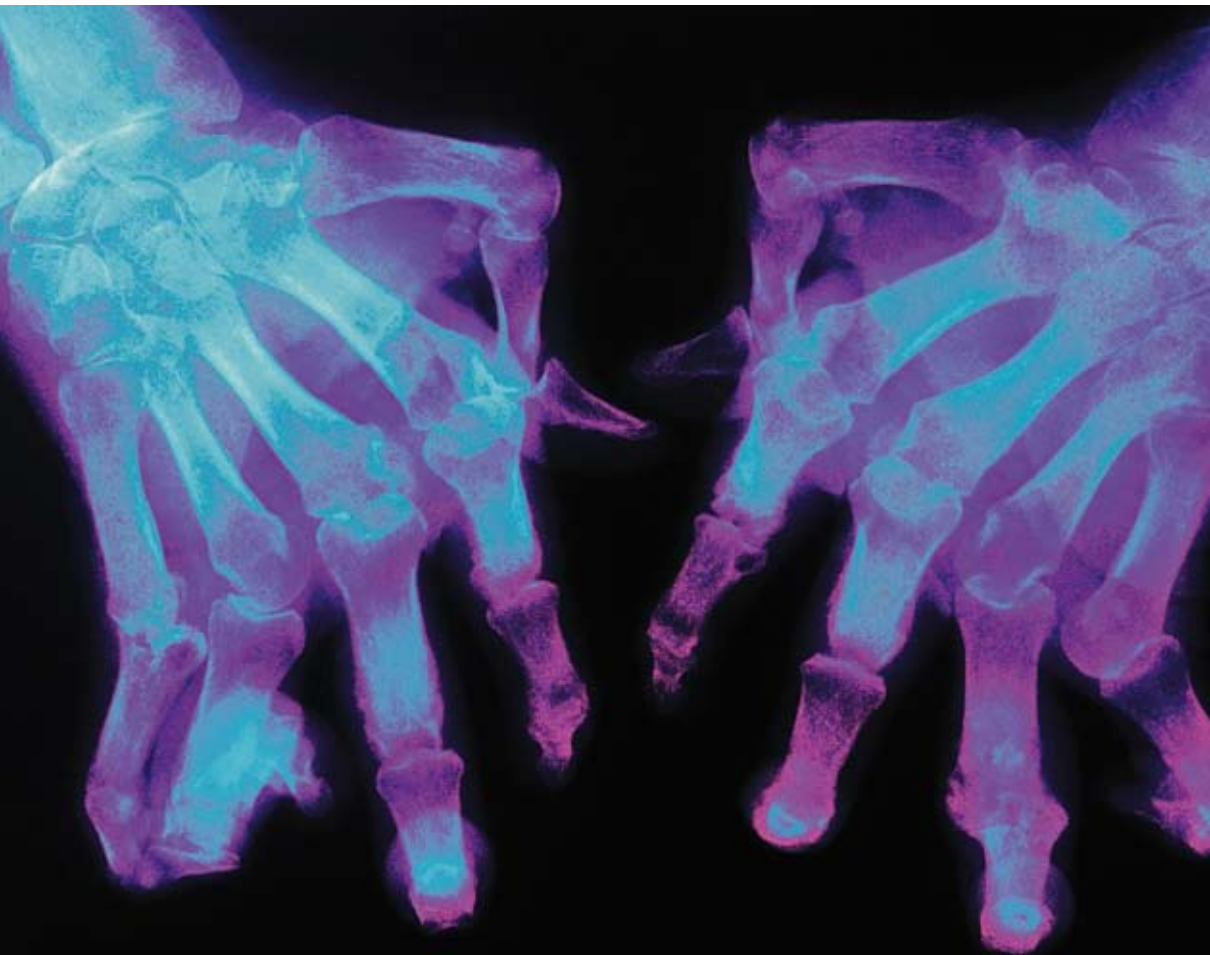




*We Innovate Healthcare*

# **Rheumatoid arthritis**

*When the body fights itself*







## Cover picture

Radiograph of the hands of a rheumatoid arthritis patient. The chronic inflammation that accompanies this autoimmune disease causes swelling, stiffness, loss of mobility, pain, and ultimately joint destruction.

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Pierre-Auguste Renoir's 1918 painting "The bathers" is now housed in the Musée d'Orsay in Paris. It is a sensual picture, typical of Renoir, joyful and idyllic. This is astonishing considering the circumstances in which it must have been painted: the painter was probably sitting in a wheelchair with the paintbrush strapped to his wrist because his fingers were no longer able to grip it firmly; he may even have needed an assistant to guide his painful hand, as by then Renoir had been suffering from rheumatoid arthritis for about 15 years.

Only a few decades ago a similar fate awaited most patients with this diagnosis. Pain, loss of mobility, and incapacity for work were almost inevitable stages in the relentless advance of rheumatoid arthritis. Fortunately, progression to these stages is no longer preordained: new drugs can largely prevent the disease from progressing, earlier diagnosis permits much earlier treatment, and long-term suppression of the disease has become a realistic objective.

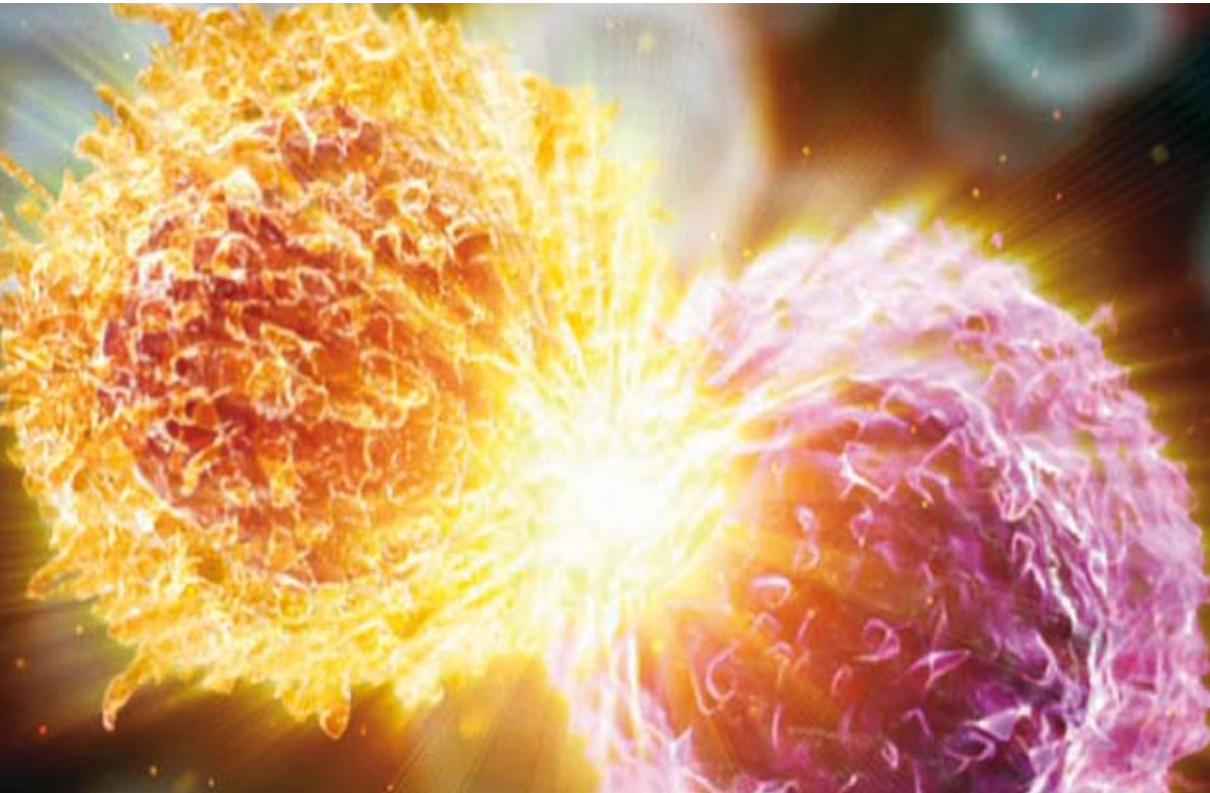
A decisive turning point in treatment is now clearly in sight: progress is being made at an ever-increasing rate now that rheumatoid arthritis is known to be an autoimmune disease. In fact, a large number of other chronic diseases are also attributable to a derangement of the immune system, or more precisely to an attack by the body on itself. These diseases, which range from diabetes through multiple sclerosis to rheumatoid arthritis, are now being intensively studied. And the resulting discoveries are enabling researchers to develop new techniques that permit earlier and better treatment of affected patients, since it is true of all autoimmune diseases that the earlier they are diagnosed, the better are the prospects for successful treatment.



I.

## The over-zealous body

*It's a nightmarish scenario: our body equips itself with the most sophisticated defence systems to cope with every conceivable enemy – and then suddenly attacks itself. When the immune system mistakes self for non-self, problems are inevitable. Autoimmune diseases are therefore among the most important areas of research in modern medicine.*



## **Immune systems and autoimmune diseases**

It is only when our immune system makes a mistake that we realise just how much it achieves every day. This happens, for example, when a microorganism manages to slip silently and unchallenged through the many barriers that have been erected against it – and as a consequence we become ill. Or, conversely, when our defence systems suddenly treat a part of our own body as if it were an intruder – and we likewise become ill. The common outcome of these two situations provides a key to understanding the basic function of the immune system, namely to distinguish one's own body ("self") from everything that is foreign ("non-self").

This distinction is far less clear than one might think. For example, we could certainly distinguish a portrait of ourself from that of someone else at a glance. But would we also immediately recognise a close-up photograph of a little toe as being that of our own little toe? Or a close-up of our ear? A photo of the internal lining of our arteries? Since the immune system cannot afford to leave anything to chance, it adopts a strategy enabling it to recognise any foreign molecule, even those it has never encountered before.

## **No need to fear science fiction**

Let's imagine a scene from a hypothetical science fiction film: a bacterium or a virus that has spent the past 200 million years under the Antarctic ice sheet comes to the surface as a result of a drilling operation. No human being has ever been exposed to this potentially dangerous microbe. Will it therefore wipe out humanity? In all likelihood no – because every one of us is to some extent forearmed against foreign invaders.

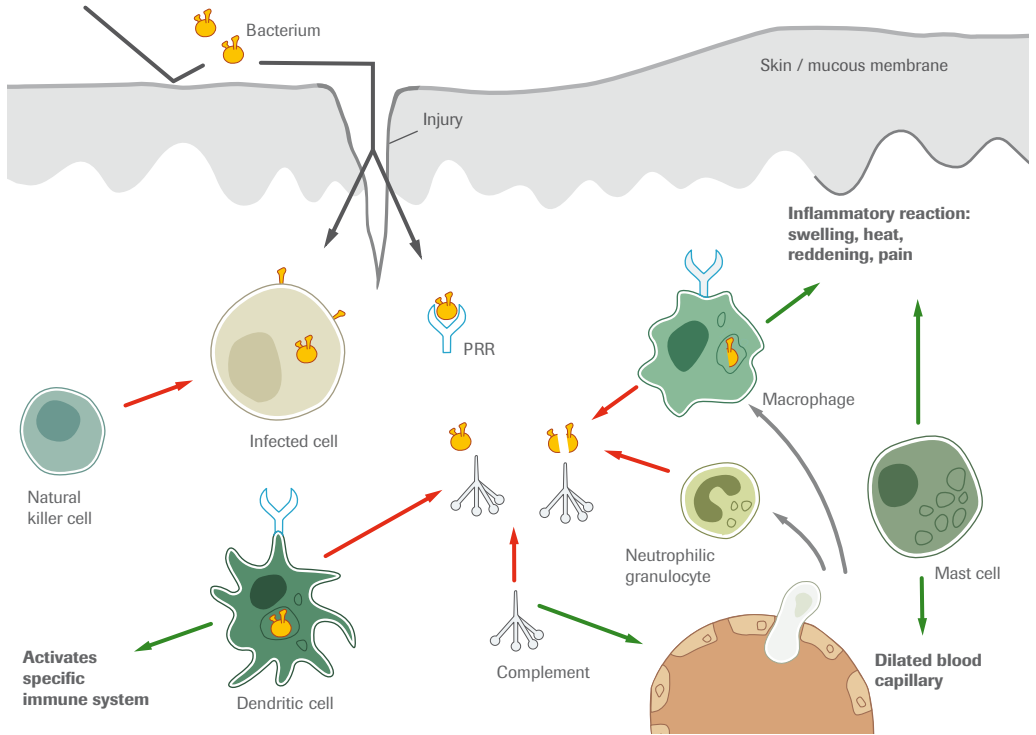
It makes scarcely any difference to our immune system whether a microorganism has spent millions of years sheathed in ice or been circulating in our own neighbourhood since time immemorial. Whatever the chemical structure of an intruder, somewhere in our body circulating immune cells specifically equipped to attack precisely that microorganism are to be found. It may take the body a while – up to two weeks – to locate these cells and prepare them to strike, but during this time the non-specific immune system holds the line.

## **Guarding the front line: the nonspecific immune system**

Our body's first line of defence is directed against anything that could be dangerous to us in any way. This includes an enormous number of microorganisms. Our skin and mucous membranes form an initial physical barrier. In addition, our body surface is thickly coated with harmless bacteria that defend their exposed position against new arrivals, to our benefit as well as their own.

Furthermore, our saliva, tear fluid, and other bodily secretions contain proteins known as defensins that can inhibit and even kill bacteria and viruses.

However, if our body surface is injured, the hypothetical bacterium from the Antarctic ice sheet could easily overcome this barrier (Fig. 1). Even then, however, we would not necessarily be at risk, because just below the body surface we possess a second



Against everybody and everything – the nonspecific immune response

Fig. 1

The nonspecific immune system combats microorganisms which – for instance as a result of an injury – have overcome the body’s first mechanical barrier, composed of skin or mucous membrane. The above illustration shows how the most important components of the nonspecific immune system act against intruding foreign bodies (red arrows) and how they further stimulate the immune system (green arrows). In this process soluble or cell-bound pattern recognition receptors (PRRs) recognise typical molecular structures of micro-

organisms. Phagocytic cells (macrophages, dendritic cells, and neutrophils) ingest foreign substances and break them down. Complement binds to foreign structures and facilitates their ingestion and destruction. Natural killer cells recognise changes on the surface of infected cells and kill them. Signalling substances from activated complement, mast cells, macrophages, and other cells bring about an inflammatory reaction, i.e. dilation of vessels and an influx of immune cells into the infected tissue.

nonspecific line of defence based on a broad arsenal of soluble and cell-bound recognition molecules known as pattern recognition receptors (PRRs). These bind preferentially to certain biological structures that are found only in microorganisms. These include components of the bacterial cell wall and free genetic material such as is often present during viral invasion. As soon as such structures appear in the body, PRRs are on the scene – either in soluble form or on the surface of specialised phagocytic (devouring) cells known as macrophages, neutrophils, and dendritic cells. Via PRRs, these cells bind to, engulf, and ingest the foreign bodies in a process known as phagocytosis.

**Beneficial inflammation:  
the complement system**

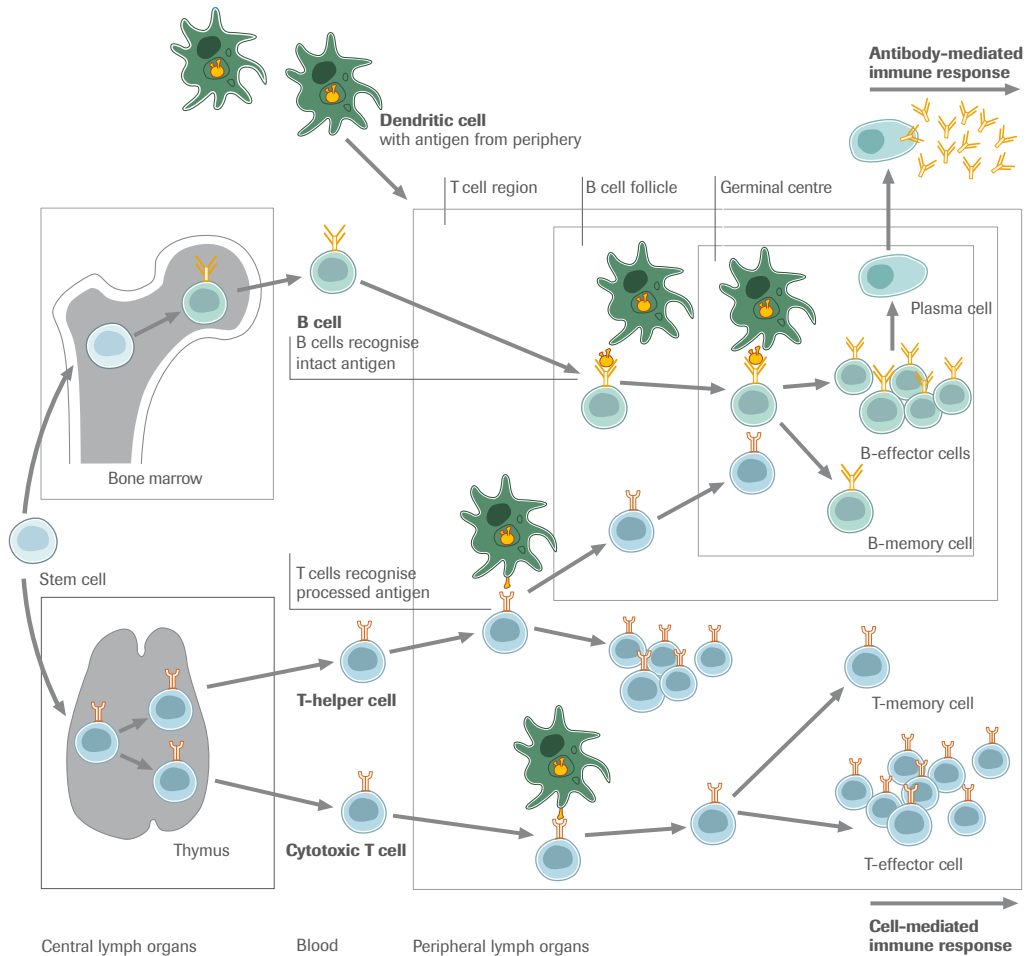
Another important component of nonspecific immunity is the complement system. On contact with a microorganism or PRRs bound to a microorganism, this group of about 20 proteins initiates a chain reaction at the end of which the foreign structure is lined with a coat of complement proteins. This sets off a series of highly effective defence measures:

- > The coating of complement promotes phagocytosis of the microorganisms.
- > Certain components of complement attack and penetrate bacterial cell walls.
- > Activated complement proteins and signalling substances released by immune cells increase the permeability of local vessels and in this way bring about a typical inflammatory reaction.

The infected region starts to swell, redden, and become warmer. All this helps to create an ideal working environment for the immune cells that are now migrating into the area: at higher temperatures phagocytic cells are more easily able to digest microorganisms, whereas the optimum temperature for the growth of intruders is generally lower. Fever and inflammation are therefore highly appropriate physical reactions to infection. Many microorganisms, viruses in particular, protect themselves against nonspecific immunity by hiding within the body's cells. This defence mechanism of microorganisms is countered by natural killer cells, which recognise infected cells on the basis of an altered pattern of surface molecules and destroy them.

**Recognition of invaders:  
the specific immune system**

While the bacterium from the Antarctic ice sheet is busy defending itself against advancing phagocytic cells, our specific



All faces are familiar – the specific immune system

Fig. 2

The specific immune system becomes active only when the nonspecific immune system signals that microorganisms have penetrated the body. This message is conveyed not only by signalling substances produced in the inflammatory reaction, but above all by dendritic cells, which ingest microorganisms and transport them to nearby lymph organs. There they act as antigen-presenting cells to activate the B and T cells of the specific immune system that are being produced constantly in the bone marrow and thymus, respectively.

Activated B cells reproduce after contact with an antigen, mature into plasma cells, and then produce massive amounts of specific antibodies. T-helper cells divide and help to activate more B and T lymphocytes. Cytotoxic T cells form effector cells that migrate around the body in order to render infected bodily cells harmless at the site of infection. T and B memory cells accelerate the reaction on renewed contact with antigen (modified from Goodnow et al., 2005).

immune system is already working to develop a custom-made immune response (Fig. 2). This has two arms, namely the humoral immune response, at the centre of which are B cells which produce antibodies that bind to specific foreign structures, and the cell-mediated immune response, in which cytotoxic T cells bind to and eliminate microorganisms.

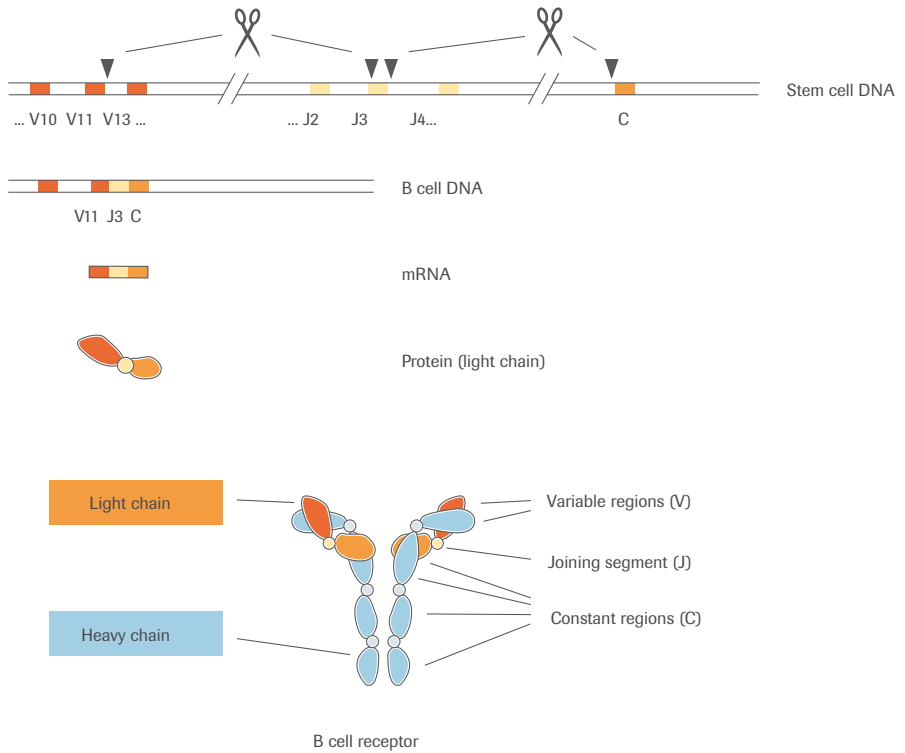
These two types of cell, also known as B and T lymphocytes, are among the body's white blood cells. New B and T cell precursors are constantly undergoing maturation in the bone marrow. Each such cell bears a receptor for a specific foreign structure – an antigen – on its surface. This multiplicity of receptors arises during the maturation of the cells via a very creative interaction between the body and its genetic information (Fig. 3). At the end of a complex genetic process, every human being possesses a set of cells which, between them, have receptors for almost every conceivable chemical structure – even for the chemical structures of a bacterium extracted from the Antarctic ice sheet. However, the existence of such a vast number of different receptors creates a risk that endogenous, i.e. the body's own, structures will also be attacked, and this is precisely what happens in autoimmune diseases.

### **Mechanisms of immunological tolerance**

Newly formed lymphocytes thus have to undergo a rigid process of selection to eliminate all cells that could attack endogenous structures. This process of learning not to react to specific antigens is known as immunological tolerance. In the case of B cells it occurs in the bone marrow, whereas T cells migrate into the thymus for this purpose. At these sites the maturing lymphocytes learn to tolerate endogenous structures before moving on to the spleen and the lymph nodes.

But how does our body manage to train every single lymphocyte to tolerate the unbelievable number of endogenous structures that are present in the body? This task is undertaken mostly by dendritic cells, which also play a role in nonspecific immunity. These cells are able to ingest and break down almost any kind of microorganism or endogenous structure and present the resulting fragments to the cells of the immune system like a set of specifications.

During peaceful periods dendritic cells constantly gather endogenous material, e.g. from dead cells, and transport it into the lymph organs. Using long processes known as dendrites, they feel their way in all directions and make contact with passing lymphocytes (Fig. 4). When a T or B lymphocyte binds via its receptor to an endogenous antigen, it is either eliminated by



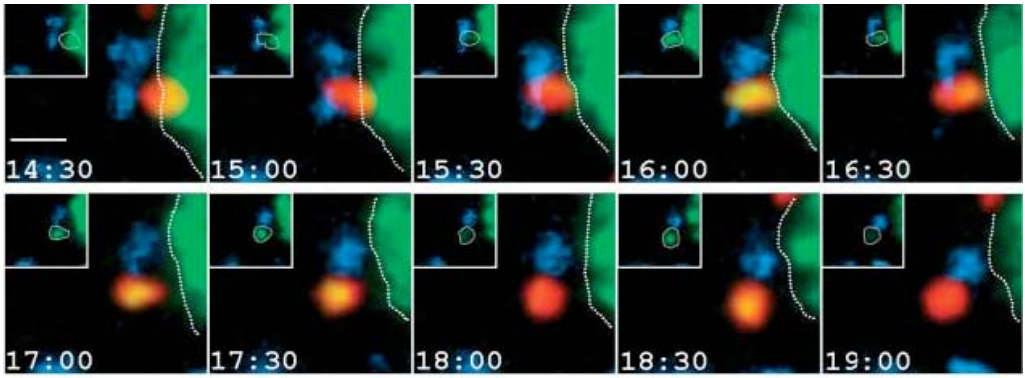
To accept or reject – the difficulty of choosing an antigen receptor

Fig. 3

New lymphocytes, each of which bears a different antigen receptor, are produced on a continuous basis in the bone marrow and the thymus. The immune system maintains this multiplicity of antigen receptors by means of a sophisticated system of genetic recombination, shown here using the example of a B cell receptor. This receptor – the structure of which is almost identical to that of the antibody that the cell will later be able to produce – consists of two identical light and two identical heavy peptide chains that are joined together in a “Y” shape.

Bone marrow stem cells have a number of different gene segments that can act as blueprints for both the light and the heavy chain. During their development B cells

randomly select some of these gene segments from the available stock and combine them into a complete gene (in the example shown above, the gene for the constant region (C) is combined with segments J3 and V11). Additional variety results from the fact that the cells make minor errors when combining gene segments and variations thus occur at the sites at which the gene segments are joined. The resulting gene is then translated to form a protein. The receptor produced in this way has to demonstrate whether it has any functional errors and whether it will attack the body’s own structures. In either of these cases the B cell can reject the gene and replace it by means of a new round of recombination.



The antigen bazaar – how immune cells patrol the lymph organs

Fig. 4

A teeming mass of cells in a lymph node: countless B and T lymphocytes wander past antigen-presenting dendritic cells, always on the lookout for the right binding partner for their antigen receptor. The long processes of the dendritic cells reach out in all directions to cover a broad area. Like shoppers strolling through a bazaar, the lymphocytes pause for a moment here, linger somewhat longer there, and even

gather into larger groups if they come across a dendritic cell with a particularly well-fitting or interesting item. In these images a B cell (red) is seen leaving the bloodstream (green) and then immediately coming into contact with a dendritic cell (blue). This appears to have a suitable antigen ready, as the B cell reacts with an activation signal, visible as a yellow flash<sup>1</sup>.

programmed cell death or else made to undergo another round of genetic recombination and in this way given a second chance to produce a harmless receptor. Another possibility is for the lymphocyte to lose its ability to produce an immune response, e.g. by transferring its receptor from its surface to its inside, where it cannot bind to autoantigens. In addition, some auto-reactive T cells can mature into regulatory T cells and thereby keep other T cells that recognise the same autoantigen under control.

**The battle begins:  
the immune response**

When the nonspecific immune system detects foreign material, dendritic cells sound the alarm and deposit special additional “costimulatory” receptors on its surface. Once this has happened, any passing B or T lymphocyte that has a receptor that

<sup>1</sup> (from Qi et al., 2006, “Movie S2” film sequence available at [www.sciencemag.org/cgi/content/full/312/5780/1672/DC1](http://www.sciencemag.org/cgi/content/full/312/5780/1672/DC1))

fits the foreign antigen has found its mark: the costimulatory receptor stimulates it to reproduce, and its multitude of descendants can then migrate throughout the body in order to render the antigen harmless.

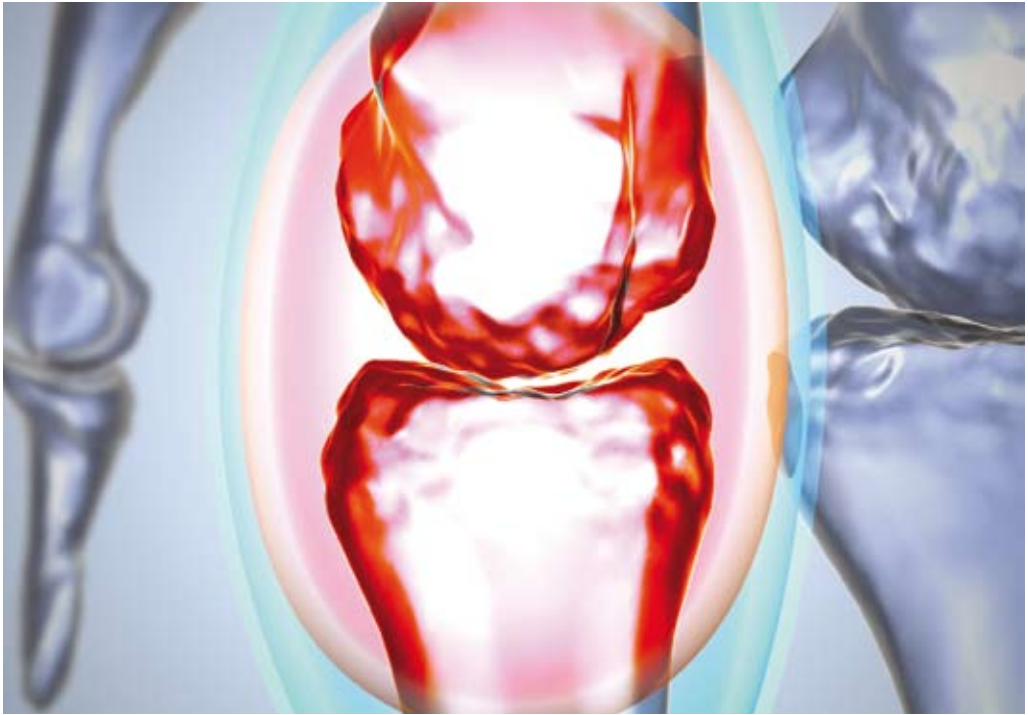
In this way B cells are converted into plasma cells and secrete large amounts of antibodies into the blood. These bind to the microorganism and mark it for destruction. Since the antibodies have two identical binding sites, they can bind simultaneously to two microorganisms and cause bacteria, for example, to clump together into immobile masses. Cytotoxic T lymphocytes, by contrast, kill infected cells on the spot. Finally, T-helper cells regulate the other immune cells and help dendritic cells or antigen to activate them.

Between 20% and 50% of all newly formed lymphocytes bear receptors that are directed against endogenous antigens, i.e. autoantigens. Via the mechanisms described above, these cells are mostly either removed at once in the bone marrow and thymus or else rendered harmless later in the lymph nodes or spleen. However, there are always a few “autoreactive” T and B cells that slip through this filtering process and come to circulate in the blood and lymph. Inappropriate activation of these leads to autoimmune disease (Fig. 5), a situation in which the immune system attacks endogenous structures as if they formed part of an intruder. Three to eight percent of the population are affected by some kind of autoimmune disease, the effects of which vary according to which autoantigen is being attacked.

In rheumatoid arthritis, for example, antibodies and immune cells attack the lining membrane of joints and cause chronic, painful joint inflammation and insidious destruction of cartilage and bone (see Chapter II). Other autoimmune diseases manifest themselves as hormonal disturbances, neurological illness, or kidney damage. Common to the more than 60 autoimmune diseases that are known is the fact that despite their many similarities, their principal causes remain unknown – and that they are still incurable.

### **Common autoimmune diseases**

In type 1 diabetes mellitus the immune system destroys the insulin-producing beta cells of the pancreas. This results in an insulin deficiency that necessitates administration of exogenous insulin. This form of diabetes, which often develops at an early age, is therefore also known as insulin-dependent diabetes mellitus. In the more common non-insulin-dependent form of the disease (type 2 diabetes mellitus), by contrast, the pancreas pro-



Rheumatoid arthritis – the straw that breaks the camel's back

Fig. 5

Unfortunately, the astonishing efficiency with which our immune system can render foreign molecules of whatever origin harmless goes hand in hand with a significant risk to our own body. Our immune system, despite its many and varied control mechanisms, is not infallible. This is apparent from the fact that up to eight percent of the population suffer from one of the more than sixty autoimmune diseases, including rheumatoid arthritis, that are known to exist. In general, an autoimmune disease will develop only if a number of random events occur in the presence of various factors that favour the development of the disease concerned. In this respect autoimmune diseases are similar to cancers, in which uncontrolled cell growth is made possible only by an interaction between random mutations, environmental factors, and individual genetic predisposition.

Large-scale studies have shown that individual genetic changes increase the likelihood of a person developing an autoimmune disease only by a factor of 1.1 to 1.7. This means that the additive effect of a number of changes is required. Also important are environmental influences including microorganisms, nutritional deficiencies, and smoking, which can precipitate autoimmune diseases in predisposed individuals. Conversely, pregnancy and its associated hormonal changes can inhibit the immune system and cause temporary remission of an existing autoimmune disease.

The finger joint shown above is inflamed and swollen. Joint mobility and strength are also sharply reduced compared with healthy joints.

duces insulin but the cells of the body lose their capacity to respond to it.

Multiple sclerosis is due to the formation of antibodies to the myelin sheath that surrounds nerve fibres in the central nervous system. This results in neurological deficits including visual disturbances, problems with balance, and pins and needles in the extremities. The disease has a relapsing course and becomes worse after infections, which put the immune system in a state of alarm and apparently also stimulate autoimmune phenomena.

Many rheumatic diseases are due to autoimmune phenomena (see Chapter II). Rheumatoid arthritis, for example, affects more than 20 million people worldwide. Juvenile idiopathic arthritis (JIA) has similar manifestations and occurs, by definition, in children under 16 years of age. The relatively rare condition of Wegener's granulomatosis is accompanied by inflammation of the blood vessels and as a result can impair the function of various organ systems including the skin, the eyes, the kidneys, and the lungs. In systemic lupus erythematosus the immune system produces antibodies against the body's own genetic material (DNA) and associated proteins. The resulting immune complexes cause bouts of inflammation throughout the body, especially at sites where the immune complexes are deposited, such as the skin, the kidneys, and the joints. In this regard this disease is similar to rheumatoid arthritis, which is likewise characterised by deposition of immune complexes, though mostly in joint spaces.

### Approaches to treatment

Autoimmune diseases can be difficult to treat because the complex interaction between the nonspecific and the specific immune system involves a multitude of different signalling molecules and cells. Only very recently have we acquired much concrete information on what disturbances lead to autoimmune diseases and what bodily structures are attacked in individual autoimmune diseases. Chronic autoimmune diseases, in particular, are among the most difficult diseases to treat. Until only a few years ago the treatment of autoimmune diseases was based essentially on large-scale suppression of the immune system (immunosuppression) and the use of anti-inflammatory agents. This approach, however, suffers from the disadvantage that it weakens the body's overall resistance. More recent approaches therefore seek to identify central sites of action at which the immune system can be inhibited in more specific fashion.

One very promising approach is to block individual signalling molecules that play a role in the immune reaction. For example, a new generation of immunotherapeutic agents is based on monoclonal antibodies that recognise and block the action of cytokines (see Chapter III).

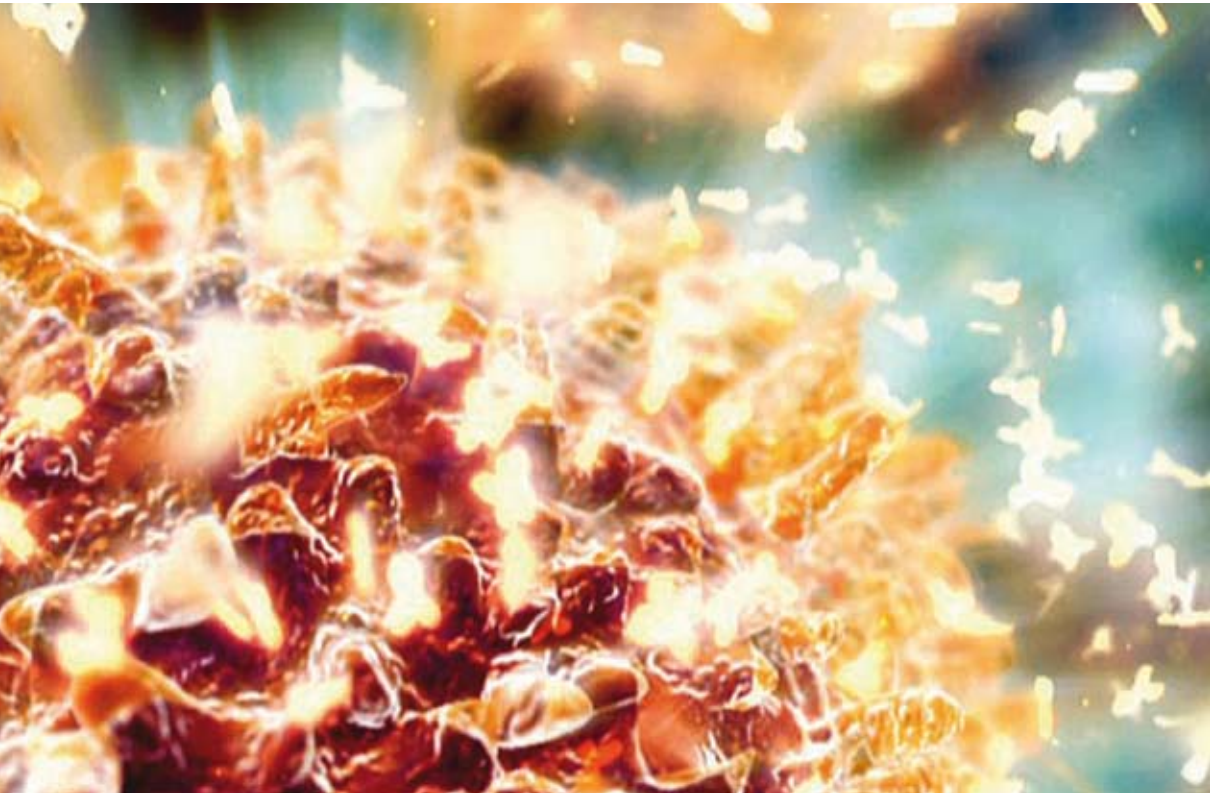
An important precondition for this approach is precise identification of the disease, ideally at an early stage (see Chapter IV). If attacked early, many autoimmune diseases may be curable, whereas in chronic disease the malfunction of the immune system is often so firmly established that lifelong treatment is required. Even in chronic disease, however, there is hope, as shown by the successes that have been achieved with anti-CD20 antibodies. In this form of treatment a high proportion of the body's activated B cells are specifically eliminated, leaving stem, pro-B, and plasma cells unaffected. Thus, the destroyed cells can be completely replaced within a few months. This approach, which amounts to a kind of "reset button" for the antibody response, has considerable potential for use in the treatment of systemic lupus erythematosus and multiple sclerosis, and is already available for the treatment of rheumatoid arthritis.

In order to identify additional targets for such highly specific forms of treatment, it is essential that the appropriate autoimmune diseases be understood in as much detail as possible. Advances in immunological research, especially in the last few years, have created an immense knowledge base and spurred on the development of new immunotherapeutic agents. The market potential for targeted therapeutic approaches is considerable, especially as a substantial proportion of the population stands to benefit from new developments of this kind.

II.

## Pain with every movement

*Rheumatoid arthritis does not spare the famous – the painter Auguste Renoir and the film director Alfred Hitchcock both had the disease, and so does the actress Kathleen Turner. In the past few years, however, scientists have gained deep insights into the causes and natural history of rheumatoid arthritis. And by so doing they have laid the foundations for better diagnosis and treatment.*



## Rheumatoid arthritis and its consequences

For Steve Robson, a customer services engineer from Bedford, England, an encounter with a jackhammer marked the start of a long tale of woe. He used the jackhammer for a few hours while on assignment for his company. “The next morning I woke up with a terrific pain in my left hand”, he recalls. “I took painkillers for a few weeks, tried to rest my hand, and had a couple of days off work to see if that would stop the pain, but it didn’t. The pain seemed to get worse and worse.” It took a whole year full of uncertainty and visits to doctors until it was established that Steve Robson – a man just 35 years old with a physically demanding job, a passionate football player, and a father of two young children – had rheumatoid arthritis.

And he is by no means an isolated case: in Europe and the USA about one percent of the population suffers from this autoimmune disease; women are three times more commonly affected than men and often develop their first symptoms while still middle-aged. Typical symptoms are painful, swollen finger joints, and the symptoms rapidly become worse. Even the simplest everyday tasks suddenly become enormously difficult: shoelaces are unreachable, drying the dishes is out of the question, and it takes hours even to have a shower. “I couldn’t grip anything, couldn’t lift or pick things up”, remembers Steve.



“Suddenly I couldn’t grip anything, couldn’t lift or pick things up” – Steve Robson, customer services engineer, a rheumatoid arthritis sufferer.

Anyone who has shaken the hand of a rheumatoid arthritis sufferer will not easily forget the effects this simple action can have: in many patients even the slightest contact causes pain. “Some patients don’t go to church any more, just to avoid the handshakes,” says Prof. Randall Stevens, Group Leader Pharma Development, Roche Medical Sciences, Nutley, USA. And: “As a doctor, when I shake the hand of a patient, I know how they are doing.”

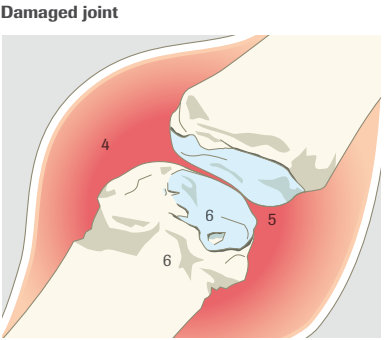
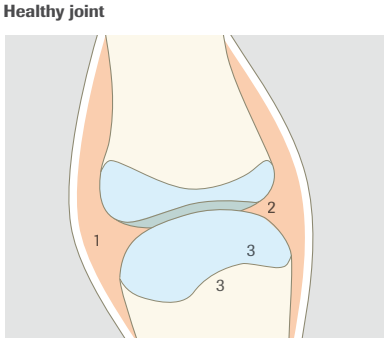
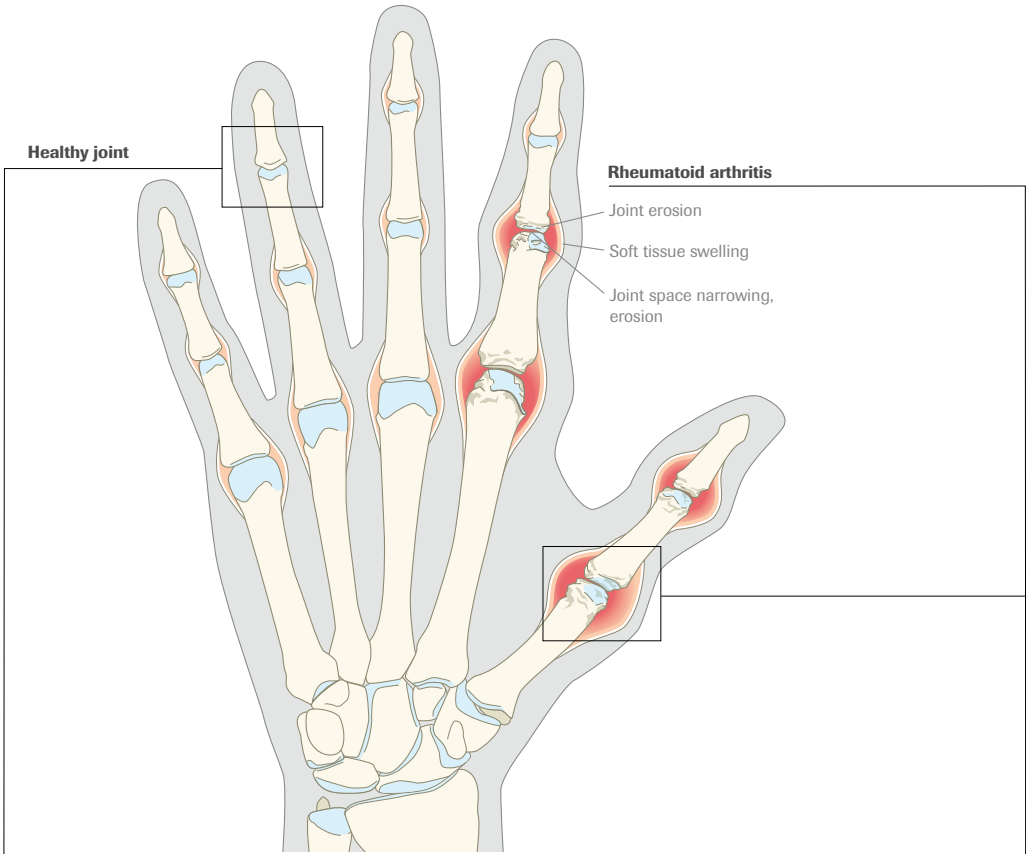
### **Attack on the joints**

The popular name “rheumatism” is in some ways misleading. This everyday term covers a broad range of painful conditions of joints and other bodily parts. Rheumatism is commonly believed to affect mostly elderly people, and in fact joint symptoms due to wear and tear often – but by no means always – appear only at an advanced age. On the other hand, many sufferers do not conform to this picture, since quite a few forms of “rheumatism” have their onset at an early age. Rheumatoid arthritis, the disease from which Steve Robson suffers, is a very widespread example of this.

The tormenting pain that accompanies this disease results from chronic inflammation of the lining membrane of joints and gradual destruction of bone and cartilage. In most cases the disease starts in the finger joints and then spreads throughout the body. After only a short time in many cases, any movement of the limbs causes pain and the wrists and finger joints become stiff, especially in the mornings. Along with tenderness, heat, and redness, the characteristic features of the disease include joint swelling and deformities. Thankfully, the final stage of rheumatoid arthritis, which is characterised by severely deformed wrists and ankles, has become less common due to advances in the diagnosis and treatment of the disease.

### **When the body attacks itself**

The symptoms of rheumatoid arthritis are due to a progressive autoimmune disease (see Chapter I) in which the affected person’s immune system mistakenly attacks parts of his own body, in this case the lining membrane of joints (synovial membrane) and joint cartilage. But it doesn’t stop there: “Rheumatoid arthritis is a systemic disease, it is not just the joints”, says Prof. Randall Stevens. “Patients may have anemia, fatigue, and/or rheumatoid nodules, which can be anywhere in the body. They may have vasculitis with damage to skin and the eye. And the risk of heart attack or stroke is increased more than twofold.” The precise cause of the inappropriate attack by the immune system remains unclear; however, it is known that a combina-



- 1 No inflammation, no swelling
- 2 Normal joint movement
- 3 Normal cartilage and bone

- 4 Severe inflammation, swelling
- 5 Loss of joint movement and normal function
- 6 Cartilage and bones are destroyed

Rheumatoid arthritis attacks our joints: healthy and damaged joints in comparison

Fig. 2

tion of genetic susceptibility and environmental factors including smoking contribute to the development of the disease. The molecular details of the disease are likewise only partially understood, despite the fact that over the past few decades advances in our knowledge have radically improved the possibilities for treating rheumatoid arthritis. For example, many of the immune cells and signalling substances that play a role in this disease have now been identified; we know that the body produces autoantibodies and immune cells that bind to endogenous molecules and initiate an inflammatory reaction; and the damage to cartilage and bone has been linked to this immune reaction, among other things.

Many of the processes that occur in this disease occur in similar fashion in healthy individuals. In patients with rheumatoid arthritis, however, the whole process becomes self-perpetuating and – unlike in healthy individuals – does not subside, but rather intensifies. In recent years researchers have identified a variety of cells and signalling substances that keep this inappropriate immune reaction going – and in so doing have opened up many new possibilities for intervening in and breaking this vicious circle. Central to these efforts is the new class of medicines known as biologics, or biopharmaceuticals. These are presented in detail in Chapter III.

**Life expectancy greatly reduced**

Thanks to increased knowledge of the factors that underlie the disease, it is also clear that Steve Robson's encounter with the jackhammer acted only as a precipitant of pain, whereas the actual cause of the pain proved to be a longstanding autoimmune disorder. "I think the description that Steve gave is very graphic, but it's actually not that uncommon", says Professor Marc Feldmann, Director of the Kennedy Institute of Rheumatology, Imperial College London. "Trauma increases vascular permeability and might also release antigens. So I think it is by no means impossible that the trauma started the cytokine release that gave Steve the acute symptoms. However, we can be certain that the disease process had already been present for many months, or perhaps even years. People have looked at blood samples from blood donors for anticitrulline antibodies, which are typical of rheumatoid arthritis, and there are many people who have these antibodies ten years before diagnosis." Once the first symptoms appear, the disease often progresses rapidly: the inflammation of the joints fails to subside, but instead becomes self-perpetuating and spreads to other organs. "Despite advances in treatment, the disease often has devastating

consequences”, says Randall Stevens. After only a year, one in ten patients is no longer able to work, and after two years early destructive changes can be seen in the joints of most patients. After five years half of all patients are significantly disabled. “Without proper treatment rheumatoid arthritis is very progressive. There is a severe shortening of life expectancy of anything up to 15 years”, adds Marc Feldmann. This, together with increasing incapacity for work, has severe social consequences: in 1998 the economic cost of the disease was estimated at 1.5 billion euros in the United Kingdom alone. More than 20 million people suffer from the disease worldwide, making rheumatoid arthritis one of the most common autoimmune diseases – and one that imposes immense medical and social costs.

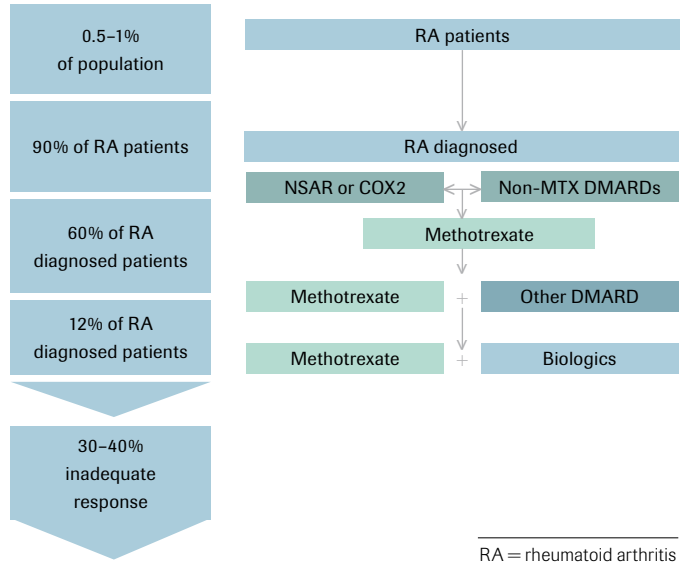
### **Biologics permit effective treatment**

In the midst of his misfortune, Steve Robson also had some good fortune. When he became unable to perform the more manual components of his work, his employer found an office job for him. Nevertheless, his degree of mobility progressively deteriorated. Despite treatment, the pain in his joints became worse and worse. “I used to do sport quite a lot, played football at a decent level, and enjoyed golf. But with the onset of rheumatoid arthritis, my little son had to help me in the morning by tying my shoelaces”, he recalls. “The last three years of pain made me want to close the books. On a couple of occasions, if I could have pressed a button to end it, I would have ended it, without a doubt.”

“The problem with all the current oral treatments is that the majority, that is at least 60 percent, of patients do not respond, and of those who do respond, most do so for only a short period of time”, says Feldmann. For a long time the only available weapons for use against rheumatoid arthritis were analgesics, anti-inflammatory drugs, and heat application. These brought some relief from the acute pain, but had scarcely any effect on progression of the disease. Such an effect was hoped for from a group of drugs known as DMARDs (disease-modifying anti-rheumatic drugs), long-term use of which can slow down the rate of joint destruction, at least in early stages of the disease. This group initially included gold salts, sulfasalazine, and chloroquine and was later supplemented by methotrexate and leflunomide. “However, the term ‘disease-modifying’ is actually misleading,” says Feldmann, “because apart from methotrexate, these drugs did very little to modify disease. But most importantly, patients on drugs of this type do not feel good. They still suffer to a significant extent from the original symp-

toms and then they have new symptoms brought on by these drugs.”

The situation changed only with the advent of biologics, i.e. bio-engineered protein drugs that interfere with diseases at the molecular level. An early example of biologics is a group of com-



Current treatment options for rheumatoid arthritis

Fig. 3

pounds known as TNF inhibitors. These are genetically engineered antibodies directed against the cytokine TNF-alpha, one of the many signalling molecules that regulate the inflammatory process. Using these new drugs, Feldmann and his colleague Maini achieved good results in the treatment of rheumatoid arthritis and Crohn’s disease from 1992 and in the treatment of other autoimmune diseases some time later. “Another important point about the new biologics compared to small molecules is that they are relatively nontoxic, provided you use them in the right way. The patients feel very much better”, says Feldmann. Combined administration of methotrexate and TNF inhibitors has therefore rapidly established itself as the gold standard in the treatment of rheumatoid arthritis.

“While TNF blockade plus methotrexate is progress, there are still very significant unmet medical needs”, says Feldmann. For one thing, about a third of patients treated with TNF inhibitors

fail to respond to treatment or cannot tolerate it. In these cases new alternatives such as anti-B-cell agents or IL-6 receptor antagonists often offer the only hope of effective treatment. And of the patients who tolerate TNF inhibitor treatment and respond to it, more than two thirds benefit only to a limited extent: “On average, if you look at patients’ joint counts when they go into clinical trials, it’s about 25, 28 joints”, says Stevens. “If they get a 70 percent improvement in their arthritis, they still have eight joints that are active. That’s enough disease to get you into the next clinical trial.” These patients are by no means cured, though they may have experienced a substantial overall improvement in their symptoms.

For another thing, it has been found that the new medications work best when taken in combination with other drugs. Unfortunately, however, TNF inhibitors cannot be combined at will with other biologics, since in many cases this can result in dangerous side effects. Here again, there is a need for new drugs in order to provide new combination therapies. Another point at issue is the price of the present standard treatment: “The cost of these therapies does lead to limited use, it does lead to rationing, and one way this problem is being addressed is by having more drugs competing”, says Feldmann.

In this regard some progress has been made in the past few years: new biologics that are presently undergoing clinical trials or are already on the market have in some patients proved to be even more effective than TNF inhibitors (see Chapter III). One patient who benefited from these new possibilities was Steve Robson.

### **Effective treatment improves quality of life**

Two years after the diagnosis of rheumatoid arthritis was made, Steve Robson’s joints were already severely affected and he had to have a hip replacement. In December 2003, with a replacement for his right and left shoulder already planned, Steve started participating in a clinical trial on the anti-B-cell agent rituximab. “I went in the morning and was told the infusion of the drug would take about six to eight hours. You could lie on the bed or you could sit in a chair and read a book or watch TV.” At home later that same day, Steve saw some cups and plates in the sink. “So I decided to do some washing up, which was a strange thing to do. I hadn’t managed to wash up in a long time. As I was washing up, the strength in this hand had returned. You had to be there to see it: I was just holding this cup in my left hand, and I think I could have actually broken it in half with the strength I had.” This rapid effect was probably due to the



Joints damaged by rheumatoid arthritis

Fig. 4

The disease process starts with inflammation of the lining of the joint - the synovium - and is followed by destruction of the underlying cartilage, and then the bone itself.

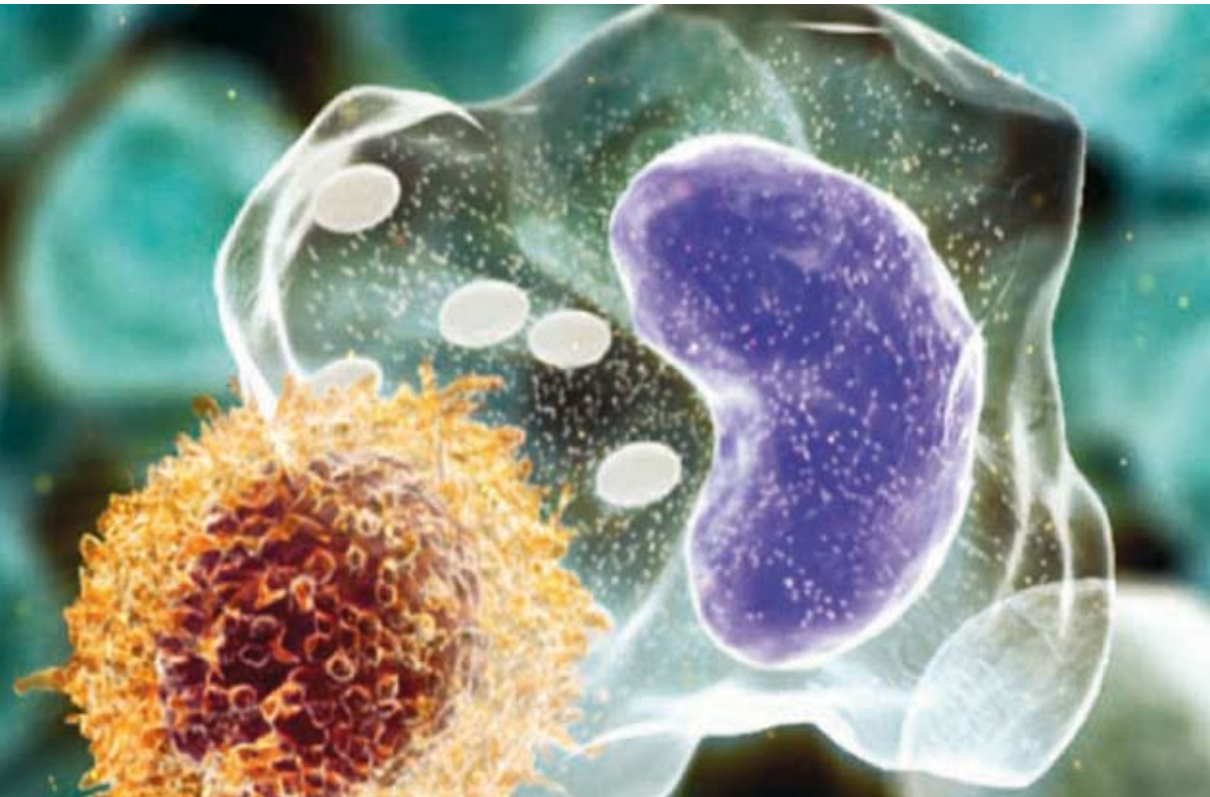
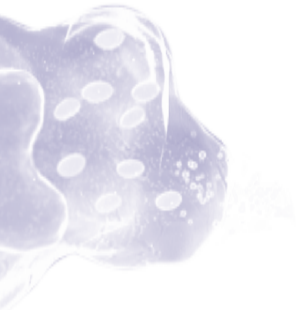
glucocorticoids which at that time were still being given in addition to the actual study drugs in order to eliminate the possibility of a negative reaction to the infusion - a precaution which has since become unnecessary. Nevertheless, Steve's senses were not deceiving him, and a few months after the treatment he was aware of a sustained and noticeable reduction in pain. His sleep had improved and he was increasingly able to look after himself. The effect of the two infusions of rituximab (administered two weeks apart) lasted for over a year. In February 2005 he received a second course of treatment with the drug. This provided him with another year with no significant disability. "So I think that's a very dramatic description of what

effective therapy can do in terms of letting people get on with their lives. And I think that's what the research enterprise wants to do: let patients have their lives back, both in quality as well as, obviously, in quantity", says Marc Feldmann. Steve Robson is once again able to get by without analgesics, play with his children, and tie his own shoelaces. And when he says goodbye, you can tell by his handshake that things are going well for him.

### III.

## Hope from biotechnology

*Pain, loss of mobility, incapacity for work – until only a few decades ago patients with rheumatoid arthritis had to resign themselves to an enormous loss of quality of life. Only since the 1980s has it been possible, thanks to drugs borrowed from cancer therapy, to stop or at least slow progression of the disease. Now biopharmaceuticals are creating possibilities for completely novel forms of treatment – for the first time raising hope that the disease can one day be cured.*



## New therapies for rheumatoid arthritis

Patients don't decide to participate in clinical trials of new drugs simply as a matter of course. Most patients who take part in clinical trials do so because they are hoping for an improvement in their condition or alleviation of their symptoms. The case of Steve Robson was initially no different in this respect: rheumatoid arthritis had severely impaired his quality of life, the pain had become unbearable, and standard treatment seemed to be of no avail. However, the clinical trial in which he decided to participate was unusual in a number of ways: the active substance rituximab, a therapeutic antibody, was already well established for use in cancer therapy, the risks of treatment were limited, and the mechanism of action of the drug was known.



Targeted action

Fig. 1

Rituximab, a therapeutic antibody, binds specifically to the CD20 molecule on the surface of B cells, thereby putting these cells out of action.

So, having decided to participate, Steve Robson sat waiting with a book in his hands while an intravenous infusion was given over several hours and the drug spread throughout his bloodstream. There the well-camouflaged antibody mingled with the

countless endogenous antibodies that are always ready and waiting to bind to and inactivate invading microorganisms. Unlike endogenous antibodies, however, rituximab is directed against endogenous structures. This therapeutic antibody recognises selected B cells of the body's immune system, or more precisely the CD20 molecules that these cells bear on their surface. Rituximab's task is to combat B cells that have got out of control and in this way to stem the progression of autoimmune diseases such as rheumatoid arthritis.

It is crucially important that the drug should also penetrate into the inflamed, painful tissue of the joints. In Steve Robson's case, at least, it seems that many of the B cells were soon labelled with rituximab and thus marked for destruction, since in the days and weeks that followed the injection most of the active B cells disappeared from his blood. In this way the drug eliminated a central reason for the development and persistence of Steve Robson's illness.

### **Palliatives, not cures**

Only a few years ago such success would have been unthinkable. "In the 50 or so years from the 1930s rheumatoid arthritis patients did not have much hope on the horizon; the drugs that were available were essentially borrowed from our oncology colleagues or from the anti-infectives field", explains Dr Anthony Manning, Global Head Inflammation, Autoimmunity and Transplantation Research at Roche, Palo Alto, USA. The drugs concerned were certainly well known and tried and tested, however, they dealt only with the symptoms of the disease. Their analgesic and anti-inflammatory actions did not stop the disease from progressing. The same is true of traditional household remedies for joint pain such as heat application, mud baths, and autumn crocus extracts, and also of analgesic tablets containing substances such as aspirin and phenylbutazone.

Among the first substances to bring longer-term success against rheumatoid arthritis were gold salts. These were first used in the 1920s, and though their mechanism of action is still unknown, they are still prescribed, at least in the early stages of the disease. Substances commonly used to combat the symptoms of rheumatoid arthritis include anti-inflammatory agents such as non-steroidal anti-inflammatory drugs (NSAIDs) and COX2 inhibitors.

A genuine advance occurred in the 1980s with the introduction of "disease-modifying antirheumatic drugs" (DMARDs). As their name suggests, these substances were often able to significantly slow the rate of progression of the disease. On the other

hand, they could cause serious side effects. At first, therefore, they were generally used to replace symptomatic therapy only in advanced stages of the disease. Later, however, after studies had shown just how negative an impact rheumatoid arthritis has on life expectancy, they came to be introduced earlier. The most important representative of this group is methotrexate, a chemotherapeutic agent that was first used to treat cancer, albeit at much higher doses than those used in rheumatoid arthritis.

### **Biopharmaceuticals offer new options**

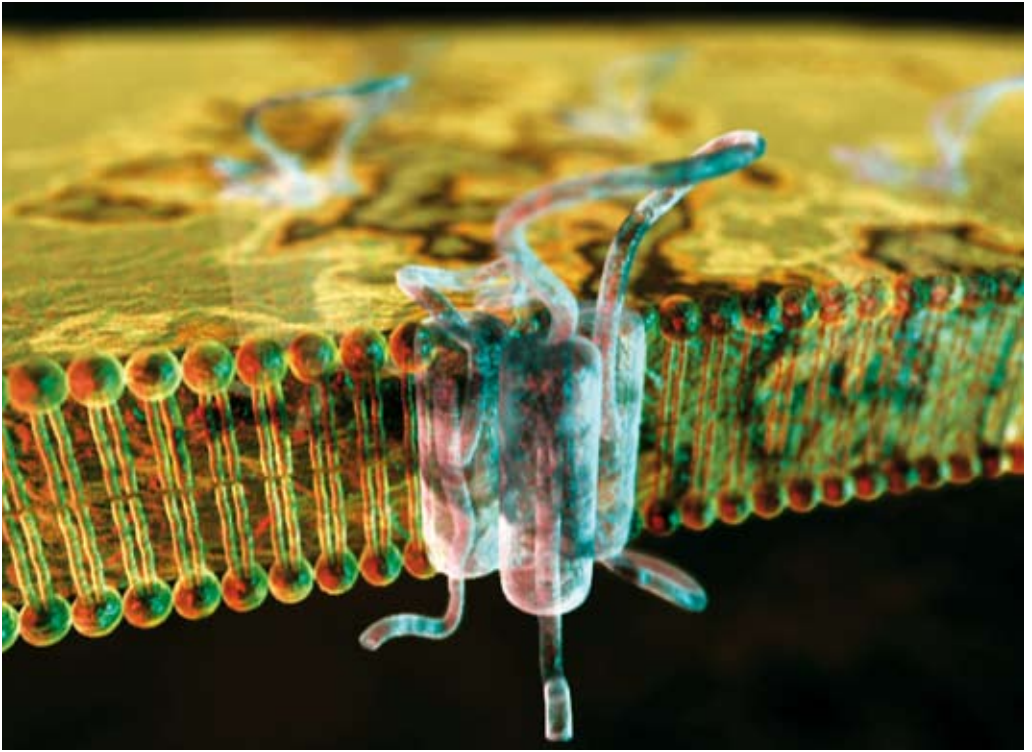
“In the 1990s progress in biotechnology led to a genuine revolution in rheumatoid arthritis therapy,” explains Dr Manning. The basic impetus for this came from science: “We set ourselves the task of learning more about the immune system and finding out exactly what goes crazy in autoimmune diseases.”

Over the next few years researchers succeeded in acquiring ever more knowledge about rheumatoid arthritis and other autoimmune diseases – and this knowledge then paid off. At the same time, advances in gene technology provided medicine with a completely new class of substances, namely biopharmaceuticals. These are relatively large biomolecules, mostly proteins, that are modelled on natural components of our bodies. Unlike broad-spectrum chemotherapeutic agents, for example, which inhibit cell growth in general, these substances are mostly highly specific in that they selectively target individual components of the immune system. This meant that for the first time there were genuine alternatives to DMARDs for the treatment of rheumatoid arthritis – alternatives that were similarly effective but considerably better tolerated.

### **The trailblazer: rituximab**

By deciding to participate in the study on rituximab after standard therapy for his rheumatoid arthritis had failed, Steve Robson became another patient to benefit from this development. By knocking out selected B cells, the drug removed a central pillar of the disease. All the other cells of Steve Robson’s body were unaffected, including B cell precursors and the antibody-producing plasma cells that are derived from B cells.

This mechanism of action has three decisive advantages. Firstly, the available stock of endogenous antibodies that are directly responsible for defending the body against invading microorganisms remains intact. Secondly, new B cells can be made because the B cell precursors survive the treatment. And thirdly, the memory cells of our immune system are likewise largely preserved. For this reason a recent tetanus booster shot does not



CD20 antigen

Fig. 2

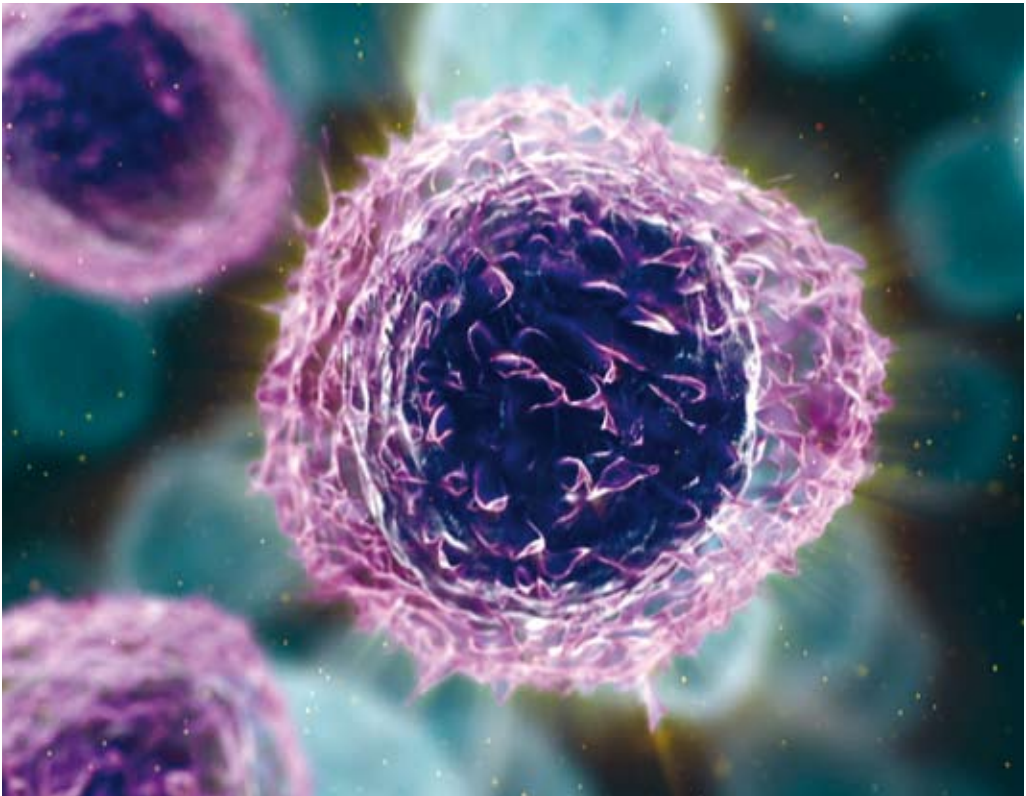
Rituximab selectively targets B cells displaying the cell surface antigen CD20.

need to be repeated, and the individual's lifelong immunity against childhood illnesses is expected to remain intact. The effect is so specific because only B cells produce the CD20 molecule against which rituximab is directed. This was a discovery of basic research that was rapidly exploited in medicine. "Rheumatoid arthritis is a very good example of the close interplay between research and clinical studies", points out Dr Manning. The situation is similar with other components of the immune system that can give rise to autoimmune diseases. A recent example of this approach is tocilizumab, a therapeutic antibody which is directed against the IL-6 receptor and which therefore interferes directly in the inflammatory process at various sites in the body. Over the past few years this substance has emerged as another highly promising candidate for the treatment of rheumatoid arthritis.

**New approaches  
to treatment**

As a result of these developments, patients with rheumatoid arthritis now have a much broader range of therapeutic options than they did only a few years ago. In this context B cells have become an important target for new drugs. Another potential target on which researchers have set their sights is a cytokine known as B lymphocyte stimulator, which B cells need in order to mature and without which they starve.

Other new drugs are directed against T cells, another type of immune cell. Like B cells, T cells play a role in causing and sustaining rheumatoid arthritis. Attempts were therefore made to kill these cells too using therapeutic antibodies; however, this



T cells

Fig. 3

T cells are important players in the immune response.

resulted in dangerous side effects, since T cells are essential for defence against microorganisms and cancer cells, and do not replenish themselves as easily as do B cells. Drugs being developed at present therefore block activation of T cells – for instance by binding to a particular surface molecule of T cells – without killing them.

In addition to immune cells, a variety of signalling substances, known as cytokines, are involved in the inappropriate immune response that occurs in rheumatoid arthritis. These small molecules, which are mostly derived from immune cells, modulate inflammatory processes, including those in joints. Therapeutic antibodies directed against the three most important candidates of this category, namely interleukin-1 (IL-1), interleukin-6 (IL-6), and tumour necrosis factor alpha (TNF-alpha), have been available for some time. More and more of these substances are now attracting the attention of drug researchers, including interleukin-15 and interleukin-17.

Since we now know in considerable detail just how the immune response leads ultimately to rheumatoid joint damage, this suggests other possibilities for new forms of treatment. One of these involves the use of a therapeutic antibody that directly inhibits bone destruction. This binds to a cytokine known as RANK ligand that is secreted by connective tissue cells in joints in order to initiate the processes of bone destruction. Neutralisation of this cytokine by the drug directly prevents joint damage.

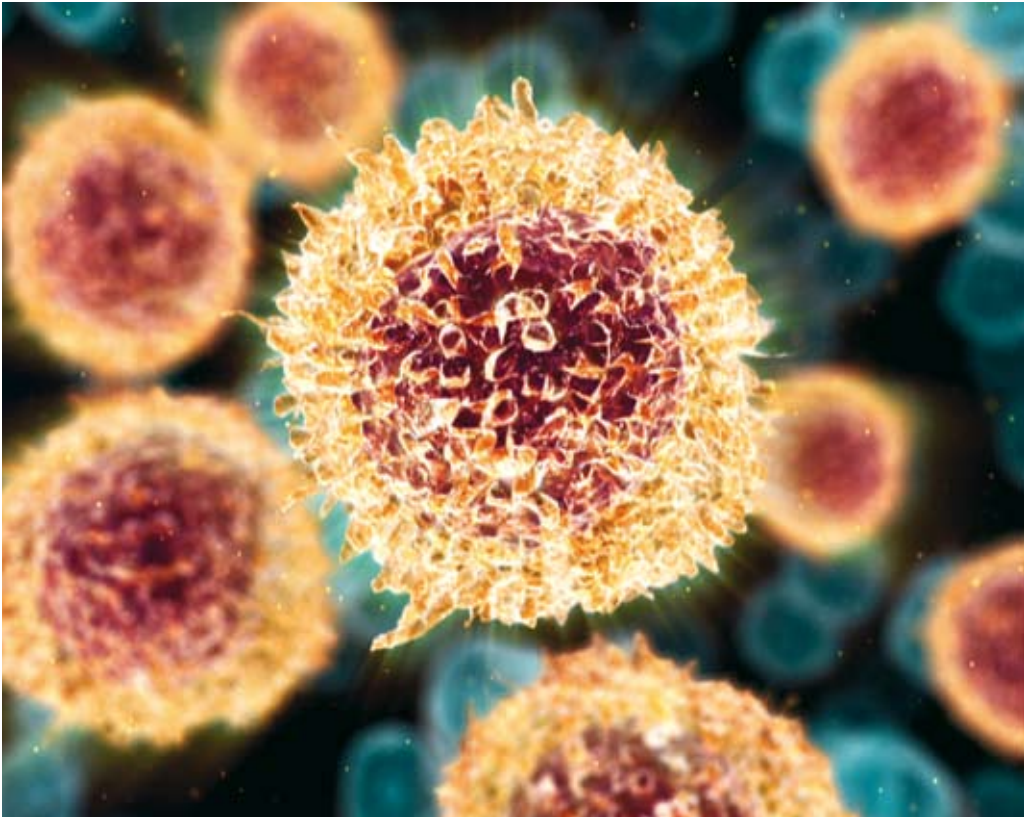
“Ultimately, we don’t know what’s the best site at which to attack”, says Dr Manning. “A certain part of the immune system may be more affected in one patient than in another.” As in a complex machine with countless switches and levers, there are many different sites at which it might be possible to intervene in a malfunctioning immune system. Biopharmaceuticals now provide us for the first time with direct access to individual switches within the system. By contrast, earlier forms of treatment were limited to turning the main power supply on or off, or to turning many switches more or less at random, with the result that they also interfered with the correct functioning of the immune system. According to Dr Manning, “Our challenge is to have available a range of therapies that act at different sites in the immune system. In the long run we want to be able to identify the appropriate component of the immune system in each individual patient so as to be able to start effective treatment as soon as possible.” If this objective is to be achieved, important advances in diagnosis (see Chapter IV) and more new drugs will be required.

## Rituximab and rheumatoid arthritis

“Rituximab is our first step into rheumatoid arthritis, but it is also much more than that”, declares Dr Urs Schleuniger, Business Director Hematology and Autoimmune Diseases, Roche Basel. “Certainly, it has redefined the whole understanding and importance of B cell therapy in many autoimmune diseases.” Back in 1997, when Roche launched rituximab as the first effective biological treatment for non-Hodgkin’s lymphoma, a type of blood cancer in which, among other things, B cells proliferate unchecked, scarcely anyone could have imagined that such a substance could ever play a central role in the treatment of rheumatoid arthritis. “Rituximab is now the gold standard in treatment of non-Hodgkin’s lymphoma; it has dramatically increased both the cure rate in aggressive lymphoma and survival times in indolent lymphoma”, says Dr Schleuniger. “Thus, it has already had a profound impact in another field of medicine, and extensive use has shown it to have a good safety profile.” Although a possible role of B cells in the pathogenesis and progression of rheumatoid arthritis was conjectured several decades ago, the focus of medical research remained for a long time on T cells. Professor Jonathan C. W. Edwards, of University College London, was one of the few researchers who still believed that B cells play a central role in the pathogenesis of autoimmune diseases. “Edwards had a specific hypothesis on the importance of B cells in rheumatoid arthritis, and with rituximab he had the opportunity to test this hypothesis”, recalls Dr Schleuniger. “In a pilot study he showed rituximab to be effective, and that led to a huge surge in interest.”

## Rheumatoid factor as a turning point

The central element of Edwards’s hypothesis was rheumatoid factor, which is found in the blood of many rheumatoid arthritis patients. This is a group of antibodies directed against endogenous antibodies – with the consequence that molecules bind together to form immune complexes. “Rheumatoid factor is thus directed against the body’s own immune system. Along with TNF-alpha, IL-6, and other cytokines produced by stimulated immune cells, this is precisely what drives the inflammatory process”, explains Schleuniger’s colleague Manning. Whereas the prevailing view had long been that T cells were the principal culprits in rheumatoid arthritis, Edwards postulated a central role for autoantibodies – and thus for the altered B cells that suddenly start producing these molecules. Edwards’s idea was that if one were to completely eliminate the active B cells present in an affected patient, the subsequent generation of cells would be free from such “black sheep” B cells. “Most of the T-



**Mature B cells**

**Fig. 4**

B cells play a key role in the targeted treatment of rheumatoid arthritis.

cell directed therapies that were tested clinically in the last 20 years didn't work", recalls Manning. "It is only recently that research results have shown what an important role B cells and cytokines play in inflammatory processes. This was a breakthrough in the treatment of rheumatoid arthritis and other autoimmune diseases."

**First steps towards remission**

Edwards's pilot study with rituximab was followed by other studies, including most recently a phase III clinical trial known as the REFLEX study. This was carried out in patients who had stopped responding to anti-TNF therapy, currently the most effective form of treatment. "Rituximab showed itself to be very effective and safe in these patients who are in most urgent need

of effective therapy because they no longer respond to other forms of treatment”, says Dr Schleuniger.

And the REFLEX study yielded some other very promising results. According to Dr Schleuniger, “We saw that the disease process slowed down considerably. Both the narrowing of the joint space and the amount of bone erosion were reduced by half. Another advantage is that the treatment consists only of two infusions every six to twelve months. With current forms of treatment patients are treated at intervals of days to weeks.”

Based on the results of this study, rituximab has become the only B cell therapy to be licensed for use in the treatment of rheumatoid arthritis, namely for patients who do not respond to, or fail to tolerate, treatment with TNF antagonists. Over the next few years applications are also to be made for authorisation to use the drug in patients with earlier-stage disease.

#### Also effective against other autoimmune diseases

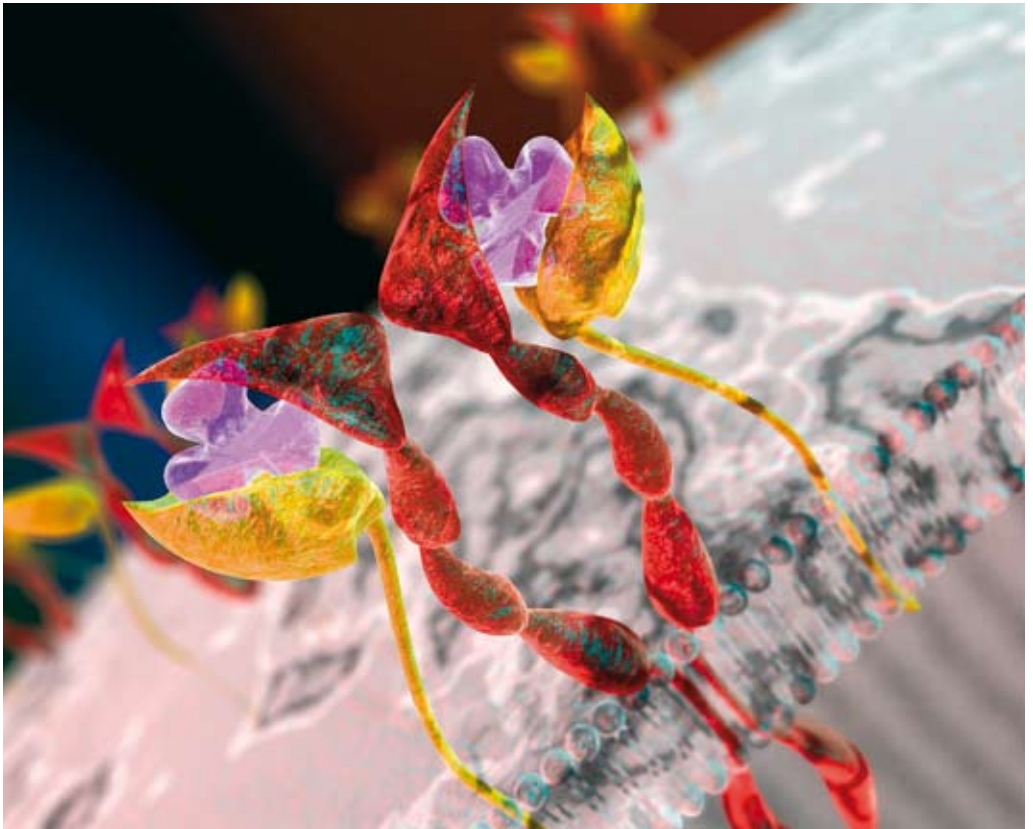
In view of the surprising successes achieved in the treatment of rheumatoid arthritis and the new awareness of the importance of B cells in the pathogenesis of this disease, scientists soon set about extending their investigations to other autoimmune diseases. It was already known from other approaches to treatment that many drugs act simultaneously and in similar fashion against a number of these diseases. For instance, the TNF antagonist infliximab was originally developed for Crohn’s disease but has now been licensed for use in the treatment of rheumatoid arthritis and psoriasis, among other diseases. Furthermore, “For rituximab there are already phase II data on multiple sclerosis”, says Guido Magni, Global Head of Medical Science, Roche Basel. “The indication rheumatoid arthritis is just our starting point.” And his colleague Anthony Manning adds: “Our knowledge of the immune system is already helping us to treat rheumatoid arthritis, but the innovative approaches to treatment that are arising from this will also benefit patients who suffer from other autoimmune diseases.”

Meanwhile, a new drug known as ocrelizumab is undergoing clinical trials. Like its close relative rituximab, this drug is a therapeutic antibody directed against CD20 on the surface of B cells. However, whereas rituximab is a “chimeric” antibody, i.e. an antibody with substantial components from two different species, in this case human and mouse, ocrelizumab is predominantly human in composition. In addition, it activates the immune system in a slightly different way than does rituximab. This has two advantages: firstly, it can be administered more rapidly – so that Steve Robson, for example, could leave his book

at home when attending for his next infusion; and secondly, it is less immunogenic and consequently may be generally better tolerated than rituximab. Urs Schleuniger commented as follows on this new drug's prospects: "A phase III trial on use in rheumatoid arthritis was started early in 2007, and we are going to extend the studies to other autoimmune diseases such as lupus and multiple sclerosis."

**Tocilizumab: modulation of the inflammatory cascade**

At the same time, research is being conducted around the world on other drugs directed against various other molecular targets – and some of these drug candidates are now nearing the goal of marketing authorisation. One such drug is tocilizumab, a ther-



**Tocilizumab in action**

**Fig. 5**

The therapeutic antibody tocilizumab binds to the IL-6 receptor. Via this surface molecule various types of immune cell are recruited to sites of inflammation.

apeutic antibody that is directed against the IL-6 receptor, a molecule found on the surface of various immune cells and in the blood. The cytokine IL-6 is secreted at the start of the inflammatory process; among other actions, it attracts immune cells to the site of inflammation. Urs Schleuniger explains: “Tocilizumab thus inhibits the entire cascade of inflammation that happens as a result of IL-6. It therefore acts not just on the bones and cartilage, but also on the whole body so as to improve anaemia and fatigue, very relevant outcomes for patients.” The product was discovered and developed initially by Roche’s Japanese partner Chugai, which has already completed pilot and phase III studies on use of the drug in the treatment of rheumatoid arthritis and juvenile idiopathic arthritis.

The results of the studies performed to date in Japan have been very promising: “These data were obtained with monotherapy, that must be emphasised, because most studies on rheumatoid arthritis are done in combination with methotrexate”, points out Dr Schleuniger. Particularly impressive has been the impact on symptoms achieved in patients treated with this drug: “These are the best results ever in terms of reduction of signs and symptoms. At the same time, there was a dramatic reduction in joint destruction, with significantly less radiographic evidence of joint destruction after one year.”

#### **Other agents in the pipeline**

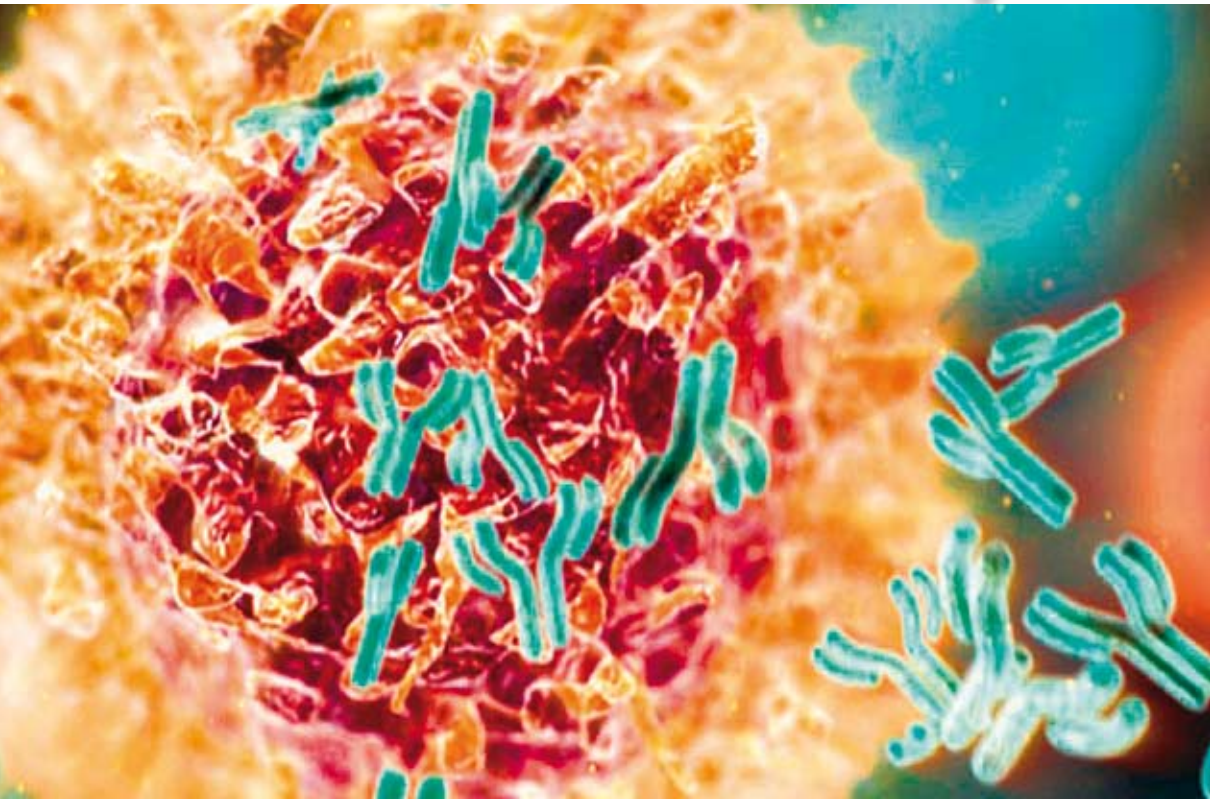
Rituximab, ocrelizumab, tocilizumab – the list of successful and promising therapeutic antibodies for use against rheumatoid arthritis and other autoimmune diseases certainly doesn’t stop there. At the same time, progress is being made with other classes of drug. For example, Roche is presently testing a classical “small molecule” that inhibits p38 kinase, a key enzyme in the inflammatory process. This kinase simultaneously regulates a whole series of cytokines that play a role in inflammation, including TNF-alpha, IL-1, and IL-6, which play a role in the development of rheumatoid arthritis. However, this drug is still some years away from marketing authorisation.

Nevertheless, the wide-ranging efforts being undertaken by researchers worldwide to develop new therapeutic options indicate that patients with autoimmune diseases such as rheumatoid arthritis have cause to hope that in the coming years their diseases can be better controlled – and possibly even cured.

## IV.

# Recognising rheumatoid arthritis

*A number of signs and symptoms must be simultaneously present before a doctor can make a definite diagnosis of rheumatoid arthritis. By that time, however, most patients already have some functional impairment and the ideal time for starting treatment may already have passed. This is because the earlier treatment is started, the less the disease can be controlled. Novel molecular diagnostic tests now offer a way out of this dilemma.*



## New paths in diagnosis

Pain, swollen joints, reduced mobility – many patients are already feeling very ill by the time their doctor announces that they have rheumatoid arthritis. And it's not because they waited too long before going to see the doctor: "Sometimes you simply have to wait until the disease progresses to the point at which disease-identifying characteristics appear", explains Prof. Randall Stevens, Group Leader Pharma Development, Roche Medical Sciences, Nutley, USA. "For rheumatoid arthritis and for many other autoimmune diseases, there is simply no conclusive diagnostic test available. Diagnosis is based on signs and symptoms, physical examination, laboratory tests, the patient's history, and – not least – time."

Nevertheless, the first symptoms that lead patients to go to see their doctor are often quite typical. The most common presenting symptom, pain in the finger joints, is one of the principal symptoms of rheumatoid arthritis, but can have many other causes. The standard criteria used nowadays for diagnosing the disease follow the recommendations of the American College of Rheumatology. The early symptoms include joint pain, morning stiffness, and joint swelling. These are followed by deformities and rheumatoid nodules – lumps up to the size of a table tennis ball that develop under the skin at sites subject to mechanical strain, though they may appear almost anywhere in the body, such as the lung and heart. Only when a certain number of findings are present can the diagnosis be regarded as established.

## A suspicion, not a diagnosis

Patients in the early stages of the disease are therefore often faced with the problem that although the doctor can express a suspicion that their symptoms are due to a progressive autoimmune disease, the presence or absence of such a disease cannot be established beyond doubt. Clearly, this is not ideal in terms of treatment of the patient, since as with all chronic diseases, the prospects for successful treatment of rheumatoid arthritis are greater the earlier the disease is recognised and the earlier doctors are able to intervene.

"Time is the key factor", confirms Dr Werner Zolg, Head R&D Marker Discovery and Proteomics at Roche Diagnostics, Penzberg, Germany. "We have to identify the inflammation in the joint as soon as possible, because the next step, joint destruction, is irreversible." This is confirmed by recent studies on early treatment of rheumatoid arthritis. At least for current standard therapies such as methotrexate, these have identified a critical window of opportunity: if treatment is started during this early

period, the prospects for long-term control of symptoms are significantly higher than if treatment is started only at a more advanced stage of the disease.

Even though early experience suggests that treatment based on biopharmaceuticals can considerably extend this window of opportunity, early diagnosis remains crucial for the subsequent evolution of the disease – because if something is not yet damaged, it doesn't need to be repaired. Nevertheless, "It's often years before standard diagnostic procedures based on radiographs can detect joint changes," says Dr. Zolg, "whereas with modern imaging techniques such as magnetic resonance and ultrasound we often see soft-tissue changes after a few months. But our hope is that biomarkers will be able to recognise the disease within weeks, or even days."

### Blood tests instead of radiographs

Biomarkers are proteins that make it possible to distinguish healthy individuals from diseased individuals. This distinction is based on proteomics, a comparison of the protein sets of different individuals. In the case of rheumatoid arthritis, Dr Zolg and his team are looking for proteins that occur only, or to a significantly greater extent, either in healthy or in diseased individuals. The principle is not new: already, patients' blood or other body fluids are tested in laboratories for components that indicate the presence or degree of progression of a particular disease. If, for example, doctors suspect the presence of liver damage, they measure blood levels of liver enzymes, while if they suspect renal failure they measure blood levels of substances such as creatinine and urea.

In the same way, laboratory parameters are already used to help diagnose rheumatoid arthritis. These parameters include erythrocyte sedimentation rate and C-reactive protein, which are generally elevated in the presence of inflammatory processes anywhere in the body. In conjunction with clinical signs and symptoms, therefore, the values of these parameters can provide evidence of acute joint inflammation. On the other hand, these values do not identify the underlying cause of the patient's pain. So far, attempts to identify affected patients early and definitively by means of a simple laboratory test have had only limited success.

As long as 50 years ago it was possible to demonstrate the presence of certain autoantibodies, including rheumatoid factor (see Chapter III), in the blood of patients with rheumatoid arthritis. Rheumatoid factor is an antibody that mistakenly binds to endogenous, rather than foreign, molecules. The peculiar fea-



Methods of detecting rheumatoid arthritis

Fig. 1

Magnetic resonance and ultrasound imaging detect joint damage from rheumatoid arthritis much earlier than conventional x-rays.

ture of this antibody is that it binds to other antibodies and joins these together to form large aggregations known as immune complexes. This process is now known to play a role in rheumatoid arthritis. Nevertheless, the presence of rheumatoid factor does not prove that the disease is present.

For one thing, rheumatoid factor cannot be demonstrated in the blood of all patients with rheumatoid arthritis. Formation of immune complexes thus appears not to be an essential step in the development of the disease. In addition, the level of this parameter is also elevated in many healthy people and in people with various other diseases. It may be that such people are protected from the disease by other factors. Rheumatoid factor is thus burdened with too many uncertainties to provide definite early diagnosis. On the other hand, it is useful as an indicator of prognosis: patients who consistently show very high blood levels generally go on to develop particularly severe rheumatoid arthritis.

**Biomarkers:  
sensitive or specific?**

The search for suitable biomarkers is thus ongoing. “The main focus of our efforts is on substances that can identify the disease earlier than is possible today”, declares Dr Zolg. Indeed, in the past few years many new candidates for this role have been discovered and investigated. However, none of these has passed the most difficult – and ultimately the decisive – hurdle, name-

ly final validation, i.e. testing of the suitability of the biomarker for its intended purpose.

The most important criteria for a good biomarker are sensitivity and specificity. Both these terms describe the extent to which a biomarker is able to distinguish individuals who have a particular disease from those who do not; however, the terms differ in one important respect. A highly sensitive marker reliably identifies affected individuals, but is liable to identify a few healthy individuals incorrectly as suffering from the disease in question. Conversely, a highly specific marker reliably excludes healthy individuals, but fails to identify a few affected individuals.

**The objective:  
more reliable diagnosis**

Developers of diagnostic tests are therefore faced with a problem, since it is equally desirable for a test not to give false negative results, i.e. fail to recognise affected individuals as such, as it is for the test not to give false positive results, i.e. incorrectly classify healthy individuals as being affected. In other words, sensitivity and specificity should both be as high as possible. In general, this can only be achieved by use of a number of different markers.

In order to estimate the value of potential biomarkers, the sensitivity of the substances concerned is measured at a standard specificity of 95%. In other words, investigators determine the concentration of a biomarker at which it incorrectly classifies only five out of every hundred healthy individuals as being affected by the disease in question (substances that fail to achieve this degree of specificity are eliminated forthwith). The sensitivity of the marker at this concentration, i.e. the percentage of affected individuals that it correctly identifies as such, is then determined. This gives a figure for “sensitivity at 95% specificity”. Rheumatoid factor has a value of only 62.1% for this parameter. In other words, this test, which is presently regarded as being of central importance for the diagnosis of rheumatoid arthritis, fails to identify more than a third of affected individuals. This is because, as indicated above, not everybody who suffers from rheumatoid arthritis produces high concentrations of rheumatoid factor.

In order to develop more reliable methods of diagnosis, Roche set up a large-scale research project. Dr Zolig describes the project as follows: “In a study conducted over five years in six European countries, we collected the most extensive possible set of data on almost 1500 patients with rheumatoid-like diseases, including 367 patients with rheumatoid arthritis. In order to be

able to compare results, we tested every available biomarker in every single patient.”

Only one biomarker that is detectable in blood, namely anti-CCP, an autoantibody that was first identified by a group in the Netherlands, scored better in these tests than did rheumatoid factor. “This is the best marker that we could find. It is far superior to rheumatoid factor and is now regarded as the marker of choice for the diagnosis of rheumatoid arthritis”, says Dr Zolg. “We’re now testing other markers in combination with anti-CCP in order to increase the sensitivity and specificity of the test even further.”

### **Outlook: molecular diagnosis**

Earlier and more precise diagnosis is by no means the only advance that scientists such as Dr Zolg hope will result from the use of new biomarkers, either alone or in combination. For example, prospects for further progress in treatment are also closely linked to success or failure in the search for biomarkers. It is hoped, for instance, that biomarkers will also be able to predict which drugs are most appropriate and likely to work in a particular patient and at what dosage their effect will be greatest.

This is because rheumatoid arthritis, like many other diseases, arises as a result of the combined action of many different factors. Differences in genetic predisposition, lifestyle, and environmental conditions result in the disease having different causes, following a different course, and requiring completely different treatment, in different patients.

For example, a proportion of patients fail to respond to treatment with TNF inhibitors (see Chapter III). At present, therefore, these patients are, at least for a certain period, taking drugs that are ineffective in them. In doing so they are exposing themselves to a risk of serious side effects and are wasting valuable time during which their disease progresses and their joints continue to be attacked. If it were possible to identify these patients at an early stage by means of a test, doctors could start them directly on other forms of treatment and thereby slow down the progression of joint damage.

Biomarkers that can predict such differences between individuals are therefore just as sought after as those that permit early diagnosis. Such “stratification markers” classify patients into strata that respond differently to a given treatment. In some cases – such as that of TNF inhibitors – the question to be answered is whether a particular drug should or should not be given. More commonly, however, the question to be answered is



Early detection of rheumatoid arthritis using biomarkers

Fig. 2

Biomarkers will one day help to detect rheumatoid arthritis earlier.

what dose of a drug should be administered: if the patient takes too little, the drug may not work, whereas if he takes too much, the likelihood that he will suffer severe side effects is increased. This is illustrated by the case of Roche's AmpliChip CYP450, a test based on a chip that recognises the major variants of two genes that play a role in the metabolic breakdown of many drugs. By providing important information on how rapidly the patient breaks down drugs, the result of the test enables the doctor to prescribe an appropriate dose of a particular drug at the outset.

### Predicting the course of illness

A third area of application of biomarkers is in predicting the course, i.e. estimating the prognosis, of diseases. For example, the level of rheumatoid factor can, as mentioned above, often provide important information on how rapidly rheumatoid arthritis will progress in a particular patient. This enables the treating doctor to adjust the treatment accordingly. The basis for

a molecular prognosis of rheumatoid arthritis is the knowledge that certain gene variants are associated not only with an increased predisposition to the disease, but also with different courses of the disease. In particular, certain rapidly progressive forms of rheumatoid arthritis appear to be linked to particular gene variants. Roche Molecular Systems is presently working on ways to distinguish between these variants. Once reliable tests to do this become available, such patients will be able to benefit from early, highly intensive therapy.

And that's not all: biomarkers can also play a central role in the monitoring of treatment. In particular, highly effective biopharmaceuticals such as rituximab and tocilizumab make it possible to tailor the rhythm of treatment to the individual patient. To this end, suitable biomarkers can help in the decision as to when and for how long a patient can be taken off treatment without risking further joint damage. This constitutes an im-



Fig. 3

"I wouldn't swap it for winning the lottery", says Steve Robson, describing what rituximab therapy has meant for him.

portant step towards a more normal life for the patient concerned.

“At Roche our Pharma and Diagnostics Divisions are working closely together to make the most of these new opportunities”, says Dr Anthony Manning, Global Head Inflammation, Autoimmunity and Transplantation Research at Roche, Palo Alto, USA. “We want to be able to provide the right drug for the right patient, we want to be able to determine the right dosage so as to achieve the best possible outcome. This is still just a vision, but I believe that in our lifetime we will see this type of transition in therapy.”



## Glossary



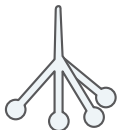
Antibody



Intact/processed antigen



B cell



Complement

**ACR criteria** a set of criteria recommended by the American College of Rheumatology (ACR) for the classification and diagnosis of rheumatoid arthritis; they include morning stiffness, symmetrical joint involvement, rheumatoid nodules, and radiographic changes.

**ACR70** a seventy percent improvement in symptoms in patients with rheumatoid arthritis based on ACR criteria.

**Antagonist** in the medical sense, a substance that counteracts certain cells or endogenous substances.

**Antibody** a soluble receptor produced by B cells that circulates in blood and tissues and binds to a specific antigenic determinant (epitope).

**Anti-CCP** an autoantibody directed against cyclic citrullinated peptides (CCPs). CCPs can often be demonstrated in the blood in the early stages of rheumatoid arthritis and are therefore used as a diagnostic biomarker.

**Antigen** a (generally foreign) protein that stimulates the production of antibodies.

**Antigen receptor** a receptor present on the surface of B or T cells that recognises a specific surface feature (epitope) of an antigen.

**Arthritis** painful inflammation of joints; the most common form is rheumatoid arthritis.

**Autoantibody** an antibody directed against endogenous structures.

**Autoimmune disease** a disease in which the immune system attacks and damages endogenous tissue.

**B cells** lymphocytes that mature in the bone marrow and are specialised for producing a specific antibody.

**B lymphocyte stimulator** a protein that B cells need in order to survive and undergo maturation.

**Biomarker** a biomolecule (mostly a protein) used in medical investigation of patients. Biomarkers can be used, for example, to confirm a diagnosis, predict the course of a disease, help determine a person's individual risk of developing a particular disease, or indicate which drug is suitable for a particular patient at what dosage. Ideally, biomarkers should be measurable in blood.

**Biopharmaceuticals (also biologics)** drugs, mostly therapeutic antibodies, produced by biotechnological means.

**CD20** a molecule present on the surface of B cells but not present either in B precursor cells or in mature plasma cells; it is therefore specific for the mature B cell population and is used as a target for drugs (e.g. rituximab) intended to eliminate B cells.

**Chemokines** signalling substances that chemically attract cells.

**Chemotherapeutic agents** generic term for low-molecular-weight drugs that block the metabolism of bacterial or tumour cells; chemotherapeutic agents that interfere with the replication of immune cells are used to treat rheumatoid arthritis.

**Chimeric antibody** a genetically engineered antibody that contains components derived from different organisms – mostly a combination of mouse variable regions and human constant regions.

**Complement** a group of about 20 proteins that control the inflammatory reaction, activate phagocytic cells, and attack microbial cells.

**COX2 inhibitors** anti-inflammatory drugs that block cyclooxygenase-2, an enzyme that helps intensify and maintain inflammatory processes.

**C-reactive protein** a complement protein formed in the liver; as its blood level is increased in inflammation, a high value can suggest the presence of rheumatoid arthritis.



Dendritic cell

- Crohn's disease** a relapsing autoimmune disease characterised by inflammation of the intestinal wall and digestive disturbance.
- Cytokines** signalling substances of the immune system that immune cells secrete in order to activate or inhibit other cells.
- Dendritic cells** phagocytic (devouring) cells with long processes (dendrites) present in bodily tissues that constantly ingest endogenous and foreign material, migrate into regional lymph nodes, and present the ingested material to the lymphocytes present there.
- Disease-modifying antirheumatic drugs (DMARDs)** orally administered drugs for the treatment of rheumatoid arthritis, e.g. methotrexate; by definition, DMARDs interfere with the disease process and are not merely directed against symptoms.
- Double-blind study** a method of evaluating drugs in which neither the patient nor the doctor or study director knows whether the test drug or a placebo (dummy drug) is being administered.
- Effector cells** mature B or T cells that have proliferated greatly after antigen contact and are now performing their immunological function, B cells having become antibody-producing plasma cells and T cells having become cytotoxic T cells or regulatory T-helper cells.
- Epitope** part of an antigen that binds to a soluble antibody or cell-bound antigen receptor.
- Erythrocyte sedimentation rate** a laboratory parameter that measures the rate of sedimentation of red blood cells in anticoagulated blood; as it is increased in inflammation, a high value can suggest the presence of rheumatoid arthritis.
- Fibroblasts** connective tissue cells that produce and maintain structures such as tendons and joint capsules.
- Glucocorticoids** steroid hormones produced in the adrenal cortex in response to stress; among other medicinal applications, they are used to prevent acute reactions to infusions.
- Gold salts** inorganic substances used as disease-modifying antirheumatic drugs in rheumatoid arthritis; administered by intramuscular injection.
- Gold standard** the procedure recognised as being the best or most reliable method of treating or diagnosing a disease.
- Humanised antibody** a therapeutic antibody that is identical to human antibodies.
- Immune complex** reaction product of a variety of antibodies and antigens that may also contain complement.
- Immunological tolerance** absence of an immune reaction to endogenous structures.
- Indication** a disease for the treatment of which a certain therapeutic intervention or drug is suitable.
- Interleukins (II)** signalling substances secreted by leukocytes that act locally to regulate the immune response by influencing other cells. The most important interleukins for the pathogenesis of rheumatoid arthritis are II-1 and II-6.
- Leukocytes** generic term for white blood cells; these include cells of the specific immune system such as B and T cells and also nonspecific immune cells such as macrophages and neutrophils.
- Lupus** see systemic lupus erythematosus.
- Lymphocytes** cells of the specific immune system that are present especially in lymphatic tissue; classified as B or T lymphocytes, or simply as B or T cells.



Macrophage



Mast cell



Neutrophilic granulocyte



Natural killer cell



Plasma cell

**Macrophages** long-lived phagocytic cells that ingest and digest foreign material.

**Magnetic resonance imaging** an imaging technique that provides high-resolution images of the interior of the body.

**Mast cells** cells present in the vicinity of blood vessels of nearly all tissues that secrete inflammatory substances, especially histamine, when required.

**Memory cells** long-lived B or T cells which after antigen contact do not differentiate into effector cells, but instead produce new immune cells as required; they mediate immunological memory.

**Methotrexate (MTX)** a drug originally used in cancer therapy that inhibits cellular metabolism; also used as a DMARD in rheumatoid arthritis.

**Multiple sclerosis** a relapsing autoimmune disease of the nervous system characterised by destruction of myelin-containing nerve sheaths.

**Natural killer cells** immune cells that can recognise and destroy virus-infected and tumour cells.

**Neutrophilic granulocytes** short-lived phagocytic cells which, when necessary, migrate into infected tissue and ingest microorganisms; they can also secrete cytotoxic substances; principal component of pus.

**Non-Hodgkin's lymphoma (NHL)** malignant tumours of lymphatic tissue that cause swelling of the lymph nodes. Whereas the cells of Hodgkin's lymphoma have a characteristic appearance under the microscope, NHL is a generic term for a heterogeneous group of tumours mostly derived from B cells.

**Non-steroidal anti-inflammatory drugs (NSAIDs)** anti-inflammatory drugs not derived from steroid hormones; they include aspirin and phenylbutazone.

**Ocrelizumab** a humanised therapeutic antibody directed against CD20 that acts in the same way as rituximab, but has improved pharmacological properties; a B cell antagonist.

**Osteoarthritis** degenerative joint disease, mostly trauma- or age-related.

**Osteoclasts** specialised cells that break down bone and cartilage; they arise from macrophage precursors under the influence of the cytokine RANK ligand (RANKL) and are overactive in rheumatoid joints.

**p38 kinase** an enzyme found in the interior of cells that controls the production and secretion of cytokines that play a central role in the inflammatory reaction, including IL-6 and TNF-alpha; target for a new generation of anti-inflammatory drugs.

**Pattern recognition receptor (PRR)** a cell-bound or soluble receptor that recognises microorganisms on the basis of common structures (e.g. bacterial cell wall, viral DNA).

**Phase II study** second stage of clinical drug testing prior to marketing authorisation, in which clinical efficacy is tested in a group of about 100 to 200 selected subjects.

**Phase III study** third stage of clinical drug testing, usually involving participation of well over a thousand subjects drawn directly from hospitals or medical practices.

**Plasma cells** mature B cells that produce massive amounts of antibodies.

**Prognosis** predicted course of a disease.

**RANK ligand (RANKL)** binding partner of the receptor activator of nuclear factor kappa B (RANK); formed in rheumatoid joints by T cells and connective tissue cells; stimulates formation of osteoclasts and thereby promotes breakdown of bone and cartilage.

**REFLEX study** a phase III study entitled “Randomised evaluation of long-term efficacy of rituximab in rheumatoid arthritis (REFLEX)” that demonstrated the effectiveness of rituximab in TNF-refractory subjects.

**Rheumatism** popular term for painful joint diseases.

**Rheumatoid arthritis** a chronic progressive autoimmune disease characterised by gradual destruction of joints.

**Rheumatoid factor** an autoantibody that can bind other antibodies and thereby form immune complexes in the absence of antigen; commonly, but not always, detectable in the blood of patients with rheumatoid arthritis.

**Rheumatoid nodules** firm, displaceable lumps up to the size of a table tennis ball that form under the skin, especially at sites subject to mechanical strain (e.g. forearm, back of the hand); a diagnostic sign of rheumatoid arthritis.

**Rituximab** a chimeric therapeutic antibody that binds specifically to a particular surface protein (CD20) of mature B cells and thereby eliminates these cells; used to treat non-Hodgkin’s lymphoma and rheumatoid arthritis, among other diseases.

**Safety profile** experience on the toxicity and adverse effects of a drug acquired during drug testing and clinical use.

**Sensitivity** the ability of a diagnostic test to correctly identify individuals with a particular disease; defined as the percentage of affected individuals correctly identified as such; cf. specificity.

**Specificity** the ability of a diagnostic test to correctly identify individuals not affected by a particular disease; defined as the percentage of unaffected individuals correctly identified as such; cf. sensitivity.

**Stratification marker** a biomarker that can predict how a particular patient will respond to treatment.

**Synovial fluid** “joint oil” – a colourless lubricating fluid that fills the joint space.

**Synovial membrane** a membrane normally only a few cells thick that lines all the internal surfaces of the joint space other than the articular cartilage; it produces synovial fluid. In rheumatoid joints it is generally greatly thickened due to an influx of inflammatory cells.

**Systemic lupus erythematosus** an autoimmune disease with a multitude of signs and symptoms including characteristic purplish skin patches (erythema), joint damage, and kidney damage; caused by autoantibodies directed against structures of the cell nucleus and deposition of immune complexes throughout the body.

**T cells** lymphocytes that mature in the thymus; as T-helper cells they coordinate the immune response, and as cytotoxic T cells they kill infected cells with which they are in direct contact.

**Therapeutic antibodies** genetically engineered drugs (biopharmaceuticals) modelled on human antibodies; they bind specifically to a particular therapeutic target, e.g. certain cytokines or cell surface molecules.

**Tocilizumab** a humanised therapeutic antibody used for the treatment of rheumatoid arthritis; it blocks the IL-6 receptor, a molecule found on the surface of various immune cells that plays a role in the inflammatory response.

**Tumour necrosis factor alpha (TNF-alpha)** a cytokine produced by macrophages and other immune cells that plays a role in the inflammatory response and tissue damage in rheumatoid arthritis.

**Validation** in relation to biomarkers, testing of the suitability of a drug candidate for clinical use, e.g. in terms of predictive value, technical feasibility, or cost-effectiveness.



T cell