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Mutant Mouse Models in Clinical Research

Pawel Kisielow, Klaus Rajewsky, Harald von Boehmer

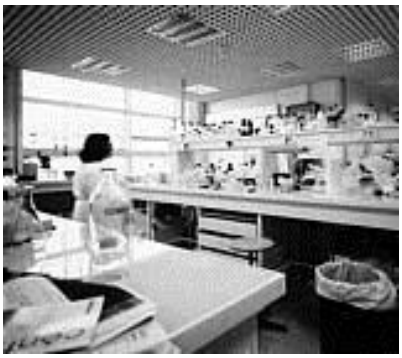
Even if the only effect of a harmless cold is to force the victim to reach for a handkerchief to deal with what is little more than a nuisance, in reality there is a merciless battle to the death taking place within the body, involving highly sophisticated defense mechanisms called up from the biochemical box of tricks. Without the immune system and without this army of defense cells and their helpers, every little infection would represent a threat to a person's life.

Körber Prizewinners: Harald von Boehmer, Pawel Kisielow, Klaus Rajewski (from left)
(Photo: Friedrun Reinhold)

The primary culprits are viruses and bacteria lying in wait to gain a foothold and multiply within the human body. The immune system makes short work of most of these, but not all. For example the AIDS virus which penetrates and hides within the defense cells themselves is so virulent that to date the human body must bow to it, in almost all cases. However, the defense system also has its problems with the body's own mutating cells spreading out of control in the form of a cancerous tumor, as these cells are not intruders, but originate from the body's own tissue. And then again, the immune system can also 'derail' and direct its attacks at the body's own cells. This gives rise to what are termed autoimmune illnesses, such as diabetes mellitus, arthritis or multiple sclerosis – from which an estimated five percent of all people suffer.

Only if medical experts and biologists understand the defense system and are familiar with its every detail they can be in a position to develop treatments to support a weakened immune system, to combat cancer or to deal with autoimmune illnesses. This year's Körber Prizewinners have made significant progress towards this objective. Professor Klaus Rajewsky justifies the laboratory work they have undertaken with genetically modified mice: "The use of mice in immunological research has a long history behind it and for many years now they have been the preferred animal for experimentation. Gene technology came into the picture as well and the technology we have perfected with the mice – targeted mutagenesis – has been a great help in driving our research project forward." The lab mice have to serve the purpose as humans cannot be used in the guinea pig role, while the immune system is so complex and multi-faceted that it cannot be investigated in a test tube or in cell cultures. As the immune system of the mouse operates similarly to its human equivalent, it is possible to observe and investigate illnesses in it, with mutant mice serving as model cases.

In the course of the project, the working groups were frequently required to exchange and have animals involved in the experiments transported elsewhere. When one day – practically overnight – Deutsche Bundesbahn, the German railway company, decided that they would no longer carry live animals, it



In the Paris laboratory Boehmer's staff analyzed tissues and cells, and their products. (Photo: Friedrun Reinhold)

became necessary to identify ad hoc alternatives. "This led to us delivering the mice ourselves, sometimes driving along with them sitting on the back seat of the car," recalls Rajewsky with a grin. Researchers now have three different techniques at their disposal. The oldest, developed in the USA, makes it possible to transfer a foreign gene – what is termed a transgene – into mouse embryos so that when it is born it carries this gene within all of its body cells. However, this kind of transgene is only in evidence in specific cells, as it has to be activated by a switch, on a tissue-specific basis. The second technique, similarly originating from America and fully developed in Cologne, makes it possible to modify genes within the animal's body, and, for example, to switch to them on or off at specific times. The third method, developed within Rajewsky's working group, represents a ground-breaking technique which by crossing-breeding mice of two specific types makes it possible to produce animals in which genes can be activated or deactivated in some cells only, for example defense cells.

Blood cells are removed from a genetically-modified mouse. (Photo: Friedrun Reinhold)

The main protagonists in the drama of an immunity battle are two groups of white blood cells – termed T lymphocytes and B lymphocytes (also known as T and B cells). Also in play are a large number of other cells and an entire host of signaling and auxiliary substances. Both types of lymphocyte can recognize cells or viruses foreign to the body by the characteristics of their surface structures, referred to as antigens. They achieve this through the lock and key principle, as the immune cells have receptors fitting the antigen like a key in a lock and accordingly attach themselves to it specifically. T lymphocytes combat intruders by, for example, latching on to infected cells and killing them off. B lymphocytes emit antibodies into the blood where they attach themselves to the causative organisms, marking them as "enemies" for other defense cells, damaging them or rendering them harmless.

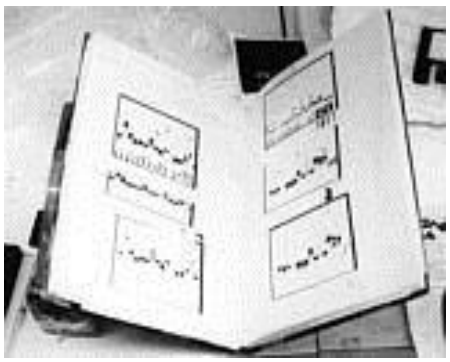
Immunologists have long puzzled over how the wide variety of defense cells are produced and how it is that these cells actually 'know' which structures belong to the body itself and which are foreign. What has become clear in the meantime is that the division of non-specialized precursor cells within the body gives rise to millions of different lymphocytes with a variety of antibodies created by a process of genetic recombination. Most lymphocytes with structures directed against the body's own tissue are immediately eliminated, as von Boehmer and Kisielow were able to prove with the help of transgenic mice. In this way the immune system 'learns' to tolerate the body's own cells. Left over is a large variety of millions of cells which do not damage their own body and which are equipped to deal with all conceivable structures foreign to the body. With this arsenal of genetic technology weapons the immunologists have been able to produce mice with a greater number of T lymphocytes with receptors

directed against quite specific antigens or cell structures, to allow the researchers to accurately trace the progression and reactions of these cells.

Genetically modified mice produce, for example, cytokines which may possibly play a crucial role in the development of diabetes.
(Photo: Friedrun Reinhold)

Harald von Boehmer's Paris working group has concentrated its work on diabetes in the transgenic animal model. In this autoimmune illness, the derailed immune system attacks what is known as the beta cells in the pancreas. The main function of beta cells in the healthy body is to produce insulin and in this way to regulate the level of blood sugar. In people with youth-onset diabetes, it is exactly these cells which are damaged and rendered incapable of controlling the sugar level. As yet it is not known which surface structure on the beta cells triggers the misdirected immune reaction and is attacked by the T lymphocytes. Within this context, von Boehmer and his team introduced a foreign gene into the mouse, with this gene generating a specific surface structure on the beta cells. At the same time they inserted other transgenes which ensure that some T cells feature a receptor directed specifically against that particular surface structure. In this way it is possible to artificially emulate the attacks carried out by the T cells on the tissue of the pancreas using the genetically-modified mice – the mouse thus having become the model for the autoimmune illness of diabetes. When this kind of young animal reaches the age of three weeks, it is can already be observed to be suffering.

"These T cells with the transgenic receptor can be identified at various stages in the mouse described," explains von Boehmer. "This allows us at a very early stage to identify and track what is happening with them. For example, how they start to divide, penetrate into the pancreas and attack the beta cells."



The laboratory log collates the results of the DNA analysis.
(Photo: Friedrun Reinhold)

All that we know as yet is that in the first instance the T cell attack on the pancreas gives rise to local inflammation, with the beta cells subsequently dying off. However, what specifically kills the cells remains a puzzle. It is clearly not the T cells themselves, however it may be substances emitted from them, known as cytokines. "By a specifically-targeted process of switching off what are termed candidate genes suspected to contain important information for triggering cell death, it is possible to identify in the mice the particular gene and substance responsible for the death of the beta cells," states von Boehmer. This is made possible by the methods developed by Rajewsky's team for switching off specific genes. And once the cell killer has been identified, then it can be switched off where possible – for example by means of antibodies or other inhibitors acting against the corresponding cytokine. The immunologists have also created similar 'mouse models' for the autoimmune illnesses of multiple sclerosis and arthritis. While the mice could provide no help for AIDS research as the illness does not appear in them, they still provide the researchers with help in the field

of cancer. Although with the autoimmune illnesses the problem was an exaggerated reaction of the immune system to the body's own cells, exactly the opposite applies for cancer: The 'defense police' do not react strongly enough to the tumor cells running wild, allowing them to proliferate instead of eliminating them. The working group led by Professor Pawel Kisielow in Wroclaw specializes in this group of illnesses. His team breeds special lines of tumor cells to find out whether the mice provide at least a weak immune response to them, and how this defense can be reinforced.

Kisielow first met von Boehmer at a conference in Warsaw in 1980. "We found we shared an interest in T cells," recalls von Boehmer. "As he made an outstanding impression and at that time the conditions for research in Poland were catastrophic, I invited him to Basel where we cooperated for a long time." Rajewsky – who was primarily interested in B cells – also got to know von Boehmer at conferences at which in the first instance the scientists became embroiled in lively discussions. It was only later that they embarked on the experimental cooperation which in 1994 gave rise to an initial joint publication. The intervening years have seen the development of steady, fruitful cooperation among the three teams, holding out promise for the future. This cooperation may well contribute towards coming to grips with the immune system and obtaining a better understanding of illnesses such as diabetes, leukemia or cancer, or perhaps even one day being able to cure them.

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Contact
Körber Foundation
Körber Prize
Kehrwieder 12
D-20457 Hamburg
Phone +49 40 · 80 81 92 -181
E-Mail koerberprize@koerber-stiftung.de