

Historical science linked to the immune system

The history of vaccination

In the library of St George's, University of London, a large brown and white cowhide is proudly displayed inside a glass case. The hide belonged to Blossom – a rare-breed Gloucester cow who played an important part in the history of medicine.

Blossom's hide was donated to St George's by the family of Edward Jenner, the family doctor who developed vaccination as a method of protecting people from deadly diseases.

Jenner was born in 1749 in a village in Gloucestershire, where his father worked as the local vicar. He started his medical training at the age of 14 when he became the apprentice of a local surgeon, then moved to London to complete his education at St George's. In 1772, he returned to his home town to work as a country doctor.

The speckled monster

Throughout Jenner's life, epidemics of smallpox – nicknamed 'the speckled monster' – were common in Europe. The disease is caused by the variola virus, which can spread through close contact with an infected person or object. About two weeks after the virus infects a person a high fever takes hold, followed by a rash, which appears in the mouth before spreading across the whole body over the course of a day.

Over the next few weeks, the rash develops into pus-filled sores which crust over and form scabs. When the scabs fall off, they leave deep scars behind. An infected person is contagious from the day the rash appears until the last scab falls off, which is usually about three weeks. During the 18th and 19th centuries as many as 98 per cent of children who caught the disease died from it; those who survived suffered from disfiguring scars, and up to a third became blind.

Although there was no way of treating smallpox once the infection had taken hold, 18th-century doctors did attempt to protect individuals from infection. The most common method they used was called variolation. This involved taking material from a fresh human smallpox sore and inserting it under the skin of an uninfected person. The hope was that the person would go on to develop a less serious form of smallpox disease, then once fully recovered would have lasting immunity.

However, many people treated this way developed serious, life-threatening smallpox infections that could then be easily passed on to other people. The crude method of variolation also carried a risk of transferring other blood-borne infections such as syphilis. Although many doctors were concerned about these risks, by the mid-18th century thousands of children were variolated each year – including an eight-year-old Edward Jenner. Luckily, he survived the procedure.

According to an unusual piece of countryside folklore, there was another method of protecting yourself. It was common knowledge among the dairy-farming communities of rural England – such as Jenner's Gloucestershire home – that milkmaids who caught a disease called cowpox never suffered from the far more deadly smallpox. Indeed, the two viruses are closely related, although cowpox causes only a mild illness. Jenner was convinced that the relationship between the two diseases was more than an old wives' tale.

Testing on humans

When a local milkmaid caught cowpox from a dairy cow called Blossom, Jenner decided to carry out a proper test of the theory. He enlisted a young boy called James Phipps as his subject, and rubbed some pus

from one of the milkmaid's cowpox sores into a scratch on the boy's arm. As Jenner expected, the boy became unwell with cowpox, but recovered after a few days.

The next stage in the experiment was to infect the small boy again, this time with matter from a smallpox sore – the risky process of variolation. This time, Phipps did not become unwell. The boy's immune response, developed during his cowpox infection, was also able to defeat the related smallpox virus. Jenner now had his first piece of evidence for a new, safe method of preventing smallpox infection. He wrote up his experiment and submitted it to the Royal Society in London, a group of influential scientists who published new theories and discoveries in their journal.

Jenner's work was the basis for a whole new area of medical science: immunology, the study of the immune system.

The faculty members of the Royal Society were not convinced. Demanding more evidence, they sent Jenner back to rural Gloucestershire to repeat his experiment on several more children. Jenner even infected his 11-month-old son with cowpox. Having bolstered his claims with this new evidence, he submitted his results to the Royal Society again in 1798 – this time they were accepted and published. The word for this now commonplace practice was coined: vaccination, from the Latin word 'vacca' for cow.

Jenner's new technology had the potential to save thousands of lives, especially children's. However, having won over the Royal Society, Jenner now had to convince the general public that such a strange practice could really protect individuals from this universally feared disease.

There was widespread disgust at the idea of transferring matter from a sick animal into a person. Public opinion was slow to change, but in 1840 Britain banned the old practice of variolation and in 1853 – over 50 years after Jenner's results were originally published – vaccination was made compulsory in the UK.

It was another 127 years before smallpox was eradicated all over the world, through widespread vaccination coverage – the first disease for which this had ever been achieved.

Jenner's work was the basis for a whole new area of medical science: immunology, the study of the immune system. Research into vaccines gathered pace throughout the rest of the 19th and 20th centuries with advances in understanding of infectious disease. Today, vaccination is an essential part of modern healthcare, preventing many dangerous diseases around the world.

QUESTIONS FOR DISCUSSION

- Measles, mumps and rubella (MMR) is one of the vaccines routinely given to children in the UK. In 1998, a scientific paper was published that led to some parents refusing to let their children have the vaccination. What happened, and why?
- Smallpox was the first disease to be eradicated through vaccination. Has this been achieved for any other diseases? Which diseases might be eradicated in the future?
- Blossom (and other cows) played an important role in Jenner's experiments, just one example of the many ways that animals have been used in medical research. Find three examples of how animals are used in research today. Do you think this use is justified or not? Why?

The history of germ theory

Today, we understand that infectious diseases like flu, chickenpox and pneumonia are caused by microscopic organisms – bacteria and viruses. Without this knowledge, we might have never developed ways to treat and prevent such infections. However, this understanding – known as the ‘germ theory’ of disease – was a remarkably recent discovery.

People have created theories to explain human disease for millennia: the Greek physician Hippocrates, born in 460 BCE, thought that ‘bad air’ from swampy areas was to blame. In the 19th century, improvements in microscope technology enabled a generation of microbiologists to investigate further the world of previously unseen disease-causing organisms.

Many of these scientists carried out research that contributed towards the formation of the germ theory. However, scientific proof of the theory was the achievement of two European scientists: Louis Pasteur, a Frenchman, and Robert Koch, who was German.

The birth of pasteurisation

Pasteur was a chemist: his early research focused on the study of crystals. But when he took up the post of head of the Science Faculty in Lille in 1854, he was inundated with demands from the local wine industry for him to research the science of fermentation. For the first time, Pasteur discovered that the process was caused by a living organism, which he called ‘ferment’. This work was a turning point in the young chemist’s career – he now began to apply his rigorous experimental methods to biological questions.

By discovering that fermentation was caused by living organisms, Pasteur had raised many issues: what organisms did ‘ferment’ consist of, and where did these organisms come from? Many other scientists at the time believed that such microorganisms appeared out of thin air – the so-called ‘spontaneous generation’ theory.

The alternative opinion was that these microorganisms originated from other similar microscopic beings. Pasteur set out to conduct a series of experiments that would conclusively resolve the debate.

Essential to these tests was an unusual glass flask with a long, thin, bent tube attached to the neck – Pasteur called it a swan-necked flask. Using these flasks, he boiled liquids (therefore killing all of the microorganisms inside) and then left them to cool. The design of the flask allowed the boiled liquid to be in contact with the air, while preventing any dust or dirt from entering.

Pasteur tested many different liquids in this way, including those which usually fermented very easily. He found that none of them fermented after being boiled. He concluded that the processes of fermentation and decay were caused by microorganisms present in the air, and that these microorganisms could be killed by heating.

Pasteur applied this new understanding to the local industries in Lille: most famously, he developed a simple way to prevent wine being contaminated by unwanted microorganisms. This involved heating the wine to 50–60°C, a technique we now know as pasteurisation, after him. It is still used in the production of many foodstuffs.

However, the most dramatic consequences of Pasteur’s discovery were in the biology of disease. Using the experimental techniques he had begun to develop in his earlier work, Pasteur went on to discover several species of bacteria. He also developed ways of making bacteria and viruses less dangerous so that they could be used for vaccination – the technique for preventing disease discovered earlier by English doctor Edward Jenner. (See History of vaccination for more.)

Proving a point

The huge task of matching specific microorganisms to the disease they cause now lay ahead. About ten years after Pasteur's famous fermentation experiments, in a small self-built laboratory in the countryside near Berlin, the German microbiologist Robert Koch was working to establish a new method for approaching this task.

The first disease he chose to study was anthrax, as it was common among farm animals in the area where he lived. The anthrax bacterium had already been discovered, but nobody had proved that this particular bacterium caused the disease seen in animals.

Koch collected anthrax bacteria from farm animals that had died of the disease and used them to infect healthy mice. He also carried out a control experiment, using exactly the same method but substituting the anthrax bacilli with blood from healthy farm animals. The anthrax-infected mice developed the disease and died, but the control mice remained healthy: Koch now had clear evidence that the anthrax bacteria had caused the disease. He then grew pure samples of anthrax bacteria and showed that these could also cause the disease.

As soon as the right method was found, discoveries came as easily as ripe apples from a tree.

These results were published and quickly recognised as having far-reaching consequences for microbiology. Building on this work, he developed new ways to grow pure samples of bacteria and stain them so they were visible under a microscope.

However, Koch is best known today for devising a universal method for testing whether a specific bacterium causes a particular disease, known as 'Koch's postulates'. Using this method, he was able to discover the bacterium that causes tuberculosis – a major killer of the 19th century. The Nobel Prize in Physiology or Medicine was awarded to Koch in 1905 in recognition of his contribution to bacteriology and the understanding of disease.

By 1900 the discoveries of Pasteur and Koch, and the work of their fellow scientists, had led to the identification of 21 disease-causing microorganisms in just over two decades. As Koch himself said, "As soon as the right method was found, discoveries came as easily as ripe apples from a tree."

QUESTIONS FOR DISCUSSION

- What are Koch's postulates? Try searching the term online.
- Joseph Lister was a surgeon during the 19th century. He also made important contributions to the understanding of disease. Search online to find out about his work. What experiments did he do, and how did they support the germ theory of disease?
- Before germ theory became widely accepted, people had other theories to explain the causes of disease. Research two of these and write a paragraph about each. The links above and below might be useful ('From miasmas to germs' in particular).

The history of blood types

Blood typing is necessary for safe blood transfusions, where donor blood is given to a patient in need. If the recipient gets blood that's not compatible with theirs, it can cause a transfusion reaction, which can cause serious symptoms or even death.

Lower and animal transfusions

Early blood transfusion experiments frequently involved animals. In 1665, Richard Lower, a member of a prestigious scientists' organisation called the Royal Society, bled a dog "of medium size", then transfused blood from a mastiff into the first dog. He recorded that the recipient dog recovered "with no sign of discomfort or of displeasure".

Your chance of receiving compatible blood without pre-transfusion testing ranges from 7 per cent (if you're type O negative) to 100 per cent (if you have the 'universal' recipient type, AB positive).

Lower's experiment was followed by transfusions from animals into people, but animal and human blood aren't compatible and the recipients survived only if they received a very small amount of blood. Blood transfusions remained unpredictable, and the Royal Society and the French government banned them in 1668.

Later, human transfusion experiments in the 19th century, performed by Dr James Blundell and Samuel Armstrong Lane, among others, showed some promise, but they couldn't make the procedure more reliable.

Early blood transfusions weren't guaranteed to fail. In some cases, the donor and recipient happened to have compatible blood. Your chance of receiving compatible blood without pre-transfusion testing varies according to your blood type, but it ranges from 7 per cent (if you're type O negative) to 100 per cent (if you have the 'universal' recipient type, AB positive).

Landsteiner and blood types

The man who discovered some of the human blood types that we know today was an Austrian, Karl Landsteiner. His work was influenced by an article on blood typing in goats, which was written by Paul Ehrlich and appeared in the 'Berliner klinische Wechenschrift' in 1900.

Landsteiner discovered the common blood types A, B and O (which he referred to as A, B and C) in 1901, and Adriano Sturli and Alfred von Decastello – who were working under Landsteiner – discovered type AB a year later in 1902. Landsteiner was awarded the 1930 Nobel Prize in Physiology or Medicine for his work.

Ottenberg and others

Six years after Landsteiner's discovery, in 1907, an American doctor named Reuben Ottenberg successfully transfused blood between two people at Mount Sinai Hospital in New York. He was the first person to record pre-transfusion testing for blood compatibility in a clinical setting, although he remarked later that the testing "was only brought in incidentally in a footnote", and concluded that he probably "should have made a separate article".

Ottenberg made several notable discoveries over the next 50 years. His work led to the knowledge that people with type O blood are 'universal donors', which means that their blood will be accepted by people with any of the four ABO system blood groups.

Testing blood types made transfusion much safer, and it got steadily more popular. Yet some recipients were still undergoing transfusion reactions, suggesting that an important part of the puzzle was missing. In 1940 this was revealed as Rh factor, which was discovered by Landsteiner and Alexander Weiner in tests

on rhesus monkeys (hence the label 'Rh', for Rhesus). Whether you test positive or not for the Rh D antigen determines whether you have a positive (e.g. A+) or negative (e.g. AB-) blood type.

Five years later, in 1945, Robin Coombs, Arthur Mourant and Robert Race developed the 'antiglobulin test'. It meant that non-agglutination antibodies could be discovered and studied. This quickly increased the number of blood group systems: today there are 35 recognised blood group systems, and there might be more that haven't been found yet.

ABO and Rh are still the most recognised systems. The NHS website, for example, states that there are eight blood types (A+, A-, B+, B-, O+, O-, AB+ and AB-). This isn't strictly true, but it's all that most people will need to know.

QUESTIONS FOR DISCUSSION

- Some people refuse to donate or receive blood, even in life-or-death situations. Give examples of people who fit into these categories and explain their reasons.
- Imagine you're a doctor treating an unconscious Jehovah's Witness in the Accident and Emergency department. Your patient has lost so much blood that she can't survive without a transfusion, but you know that this is against her beliefs. Would you go ahead with the transfusion without her consent? Why?
- Investigate why it's not possible to transfuse animal blood into humans. If it were possible, do you think people would accept blood from a pig or dog? Why?

The history of antibodies

Antibodies, also known as immunoglobulins, are proteins in the blood that are created by B cells in response to proteins called antigens, which the body recognises as 'non-self'. Understanding antibodies is useful because it means that we can develop blood tests to diagnose illnesses.

Imagine you're a doctor and a patient comes to see you with a fever, muscle aches, a sore throat and a general sense of lethargy. These symptoms might suggest that your patient's been infected with *Mycoplasma pneumoniae*, which should be treated with antibiotics as quickly as possible – or they might indicate influenza, which should never be treated with antibiotics because it's caused by a virus.

One way to tell the difference between the two, or any other pair of illnesses, is to look at your patient's blood to see what type of antibodies they are producing. This should reveal which antigens are in their system.

Antibodies also play an important part in the development of several diseases. They can render infectious organisms harmless by attaching to their antigens.

Von Behring, Ehrlich and poison-resistant mice

The term Antikörper ('antibodies') was introduced in Paul Ehrlich's 'Experimental Studies on Immunity' in 1891. But his work was pre-dated by Emil von Behring's investigations into "serum therapy" to treat diphtheria and tetanus in 1890.

Von Behring's work was based on Emile Roux's discovery of the diphtheria toxin and was hugely influential at the time: in a speech in 1899, New York's leading public health official, Hermann Biggs, said that "among the remarkable developments in medicine during the last ten years none has been more important in its practical value, nor more revolutionary in its effect on therapeutic possibilities and conceptions than the

discoveries in serum-therapy". In 1901 von Behring received the first Nobel Prize in Physiology or Medicine for the antitoxin he developed.

Ultimately, though, von Behring's serum therapy was only useful against a small number of diseases. Despite his important role in reducing diphtheria, which is still extremely rare in most countries today, his work was eventually overshadowed by the introduction of antibiotics and the work of Robert Koch and Paul Ehrlich.

Ehrlich was fascinated by toxicity and immunity. Independent of von Behring's work, Ehrlich – a great fan of Sherlock Holmes and other detective novels, and a meticulous researcher – began to conduct rigorous experiments using mice.

At first, he fed the mice cocaine; later, he started testing their resistance to different amounts of ricin, one of the world's deadliest toxins. The amount of ricin that it takes to kill a laboratory mouse is extremely small, but Ehrlich gradually increased the doses for individual mice until they could resist doses that would have been lethal if they had been administered straight away.

Even more impressively, Ehrlich eventually produced mice that could survive doses of ricin that were hundreds of times stronger than the lethal dose for mice that hadn't been exposed, making it clear that something in the immune system of the mice was developing resistance to the toxin. He also began to explore whether the immunity was specific, finding that mice that could withstand large amounts of ricin were just as susceptible to abrin (another potent toxin) as mice that hadn't been exposed to either and that abrin exposure had no effect on ricin resistance.

The underlying concept was similar to smallpox vaccination, introduced by Edward Jenner in 1798. What separated Ehrlich's work from Jenner's was his insights into the mechanisms of immunity, particularly the side-chain theory that he proposed in 1900. The theory suggested that side chains within the cells react with antigens and bind them – fitting like a lock and key – to create antibodies, which then travel around the body in the blood.

Edelman and Porter

After the introduction of Ehrlich's side-chain theory, researchers continued to work on antibodies but made few significant discoveries over the next 50 years. "Up to the year 1959," reported the Karolinska Institute in 1972, "our knowledge of [antibodies'] nature and mode of function was very vague and incomplete, in spite of a century of research."

In 1959, though, Gerald Edelman and Rodney Robert Porter revealed their independent discoveries about the chemical structure of antibodies, which led to them jointly receiving the 1972 Nobel Prize in Physiology or Medicine.

Edelman got into immunology in an unusual way: "as a result of boredom". He was serving in the army in Paris when he read a book about the body's reaction to antigens and realised it didn't explain exactly how antibodies function. That's when Edelman made a decisive prediction about his return to America: "When I go back and do research, I'm going to work out how antibodies work."

Both Edelman's work and Porter's focused on breaking antibodies down into smaller parts so they could be handled and studied more easily, which had important implications for diagnostics and therapy.

Porter used the enzyme papain to split antibody molecules into three parts, and Edelman focused on breaking the disulphide bridges holding the molecule together. Their work revealed that antibodies consist of two pairs of chains – two short 'light' chains and two 'heavy' chains, which are about twice the length of the light chains – held together in a 'Y' shape by disulphide bridges.

The work paved the way for the development of monoclonal antibody therapy, which is used today to treat cancer and autoimmune diseases.

The history of the major histocompatibility complex

Many major scientific discoveries are the result of work by many researchers, often separated by both geography and time. The discovery of the major histocompatibility complex (MHC) and its vital role in the vertebrate immune system is no exception.

Ancient history

As early as 430 BCE, the Athenian philosopher and historian Thucydides noted that a person who had survived infection with the plague could tend to others infected by the disease without contracting the illness a second time.

In the 19th century, several scientists built upon this knowledge of an immune system that could adapt based on prior exposure to a disease. Louis Pasteur (see 'The history of vaccination') developed the first vaccine to use a live attenuated (weakened) strain of the pathogen, and Paul Ehrlich (see 'The history of antibodies') proposed a theory to explain how antibodies react and interact with antigens, earning him a Nobel Prize. But still, there were vital elements of the immune system picture that were missing.

These gaps were particularly noticeable in the field of organ transplantation. The large number of casualties from World Wars I and II, many of whom sustained life-changing injuries, drove research into how tissue or organs could be transplanted from one individual to another. Successful examples of transplanting material from one part of an individual's body to another part, such as moving skin from the thigh to the nose, date back centuries. However, attempts to transplant tissue and organs between different individuals had largely ended in failure.

Snell, Gorer, Dausset and the MHC

The work of George Snell, an American mouse geneticist, took science one step closer to solving the problem of transplant failure. Earlier research by Clarence Little had shown that tumour tissue transplanted between genetically distinct mice was rejected. This is because the tissues of the donor and recipient were incompatible.

Working with British geneticist Peter Gorer and a large number of selectively bred mouse strains, Snell worked out that a group of closely related genes, which they named the major histocompatibility complex (MHC), was the cause of the rejection. The prefix 'histo-' is used in medicine to mean tissue – it comes from the Greek *histos*, which means 'loom' or 'web'.

Inspired by Snell's work in mice, the French immunologist Jean Dausset looked for, and found, the MHC in humans. Located on human chromosome 6, the proteins encoded by the genes he found present foreign antigens on the surface of cells, which stimulate an immune response. He called the set of genes the human leukocyte antigen (HLA) – they are the most extensively studied of all the vertebrate MHC genes.

Benacerraf and immunological memory

Baruj Benacerraf, a Venezuelan-born American immunologist, demonstrated that genetic factors determine an individual's immune response. He discovered the immune response genes that were later found to code for a type of MHC molecules known as class II. These are involved in the presentation of bacterial proteins.

Class II MHC molecules also play a role in the process that stimulates the production of memory B cells. These cells help our immune system to remember past infections and respond stronger and faster if

we encounter the same pathogen again. (For a reminder on how the immune system works, see our colour poster.)

The combined work of Snell, Dausset and Benacerraf led to them being awarded the 1980 Nobel Prize in Physiology or Medicine for their independent discoveries of “genetically determined structures on the cell surface that regulate immunological reactions”.

Their work, as well as further discoveries by other scientists in the 1950s, made widespread organ transplantation possible. Donors and recipients could be matched to see if they were compatible, thereby dramatically decreasing the risk of rejection following transplantation.

Zinkernagel and Doherty

In contrast to the independent work of Snell, Dausset and Benacerraf, Rolf Zinkernagel, a Swiss immunologist, and Australian Peter Doherty were awarded the 1996 Nobel Prize for their collaborative discoveries concerning the specificity of the cell-mediated immune defence.

Although the vital role of the MHC in the immune response had been determined, the exact mechanism by which it worked was still not clear. Zinkernagel and Doherty found that in order to recognise and kill virus-infected cells, immune cells called T cells need to be presented with an MHC molecule as well as parts of the virus (called the antigen).

They realised that the MHC has a role in enabling the immune system to distinguish between ‘self’ and ‘non-self’. This discovery also led to the distinction between antibody-mediated (humoral) and cell-mediated immunity.

The importance of the MHC beyond transplants is now well appreciated. Scientists have shown that failure to accurately recognise ‘self’ can lead to a range of autoimmune disorders, including rheumatoid arthritis, multiple sclerosis and type 1 diabetes. The MHC has also been implicated in mate recognition in mice, fish and birds, and potentially in humans too.