SANCTUARY

Definitions

Symbiosis is a term used to describe two different organisms that are in a cooperative relationship which brings benefits to both parties. Our relationship with bacteria is an example of symbiosis, where infection is more the exception than the rule.

Sanctuary is a place of safety and protection from attack or persecution.

Bacille Calmette Guerin (BCG) is a vaccination of a living strain of Mycobacterium tuberculosis (bovine strain) for the prevention of tuberculosis in children and adults. The strain is weakened so that it can be used for immunisation against tuberculosis.

CWDM is a shortened name for cell-wall-deficient mycobacteria. These are mycobacteria that have no cell wall, but instead have an inner membrane and can also produce biofilm for protection. They are difficult to see, but are very common. They are widely considered to be extremely difficult to culture in the laboratory, and for that reason there has been little interest in them

Regional enteritis was an older name for Crohn's disease. As late as 1967, "regional enteritis" was still being reported in medical literature[1], [2].

The Antibiotic Age refers to the period from 1928 until now. The peak was from 1945 until the 1970's when antibiotics were freely used in humans and animals with little regard for antibacterial resistance or utilisation controls. That came later.

Johne's disease is a severe disease of animals caused by Mycobacterium avium ssp paratuberculosis. (MAP), in the bacillary form. MAP in the CWDM form is thought to be a cause of Crohn's disease in humans.

Introduction

Sherlock Holmes would just look at the crime scene, and use deductive reasoning to come up with a satisfying explanation of events leading to a conclusion. (Interestingly, Sir Arthur Conan Doyle modelled his creation, Sherlock Holmes, on a Scottish surgeon, Dr Joseph Bell. Conan Doyle had medical training under Dr Bell and was fascinated by his extraordinary diagnostic skills. Conan Doyle used Bell's diagnostic talents as inspiration for Sherlock Holmes).

These articles are an attempt to try and use deductive reasoning to tie together some of the facts about MAP and Crohn's disease, along with some of our laboratory findings. I hope they also help patients to understand the way researchers try to establish possible causes of Crohn's disease.

One of the first questions the newly diagnosed patient with Crohn's disease is likely to ask is "What causes Crohn's disease?" Depending on who is asked, there are different answers given, all along similar lines. The answer in Google AI is that – ***"The exact cause of Crohn's disease is unknown, but it is believed to be a combination of genetic, immune system, and environmental factors, with smoking being a significant controllable risk factor"***

A cynical microbiologist might point out that this answer covers most known diseases, including "bad habits".

In my world, patients diagnosed as having Crohn's disease are well-informed about probable causes. Some patients have linked their disease onset to a previous bout of food poisoning, after which they struggled to recover completely. Other patients have linked the onset to a holiday in the country, soil contaminated with agricultural waste, antibiotics, vaccinations, or world travel. None of those are far-fetched. If the cause is unknown, the patient will search for clues in their own health history.

Crohn's disease, white cells, inflammation and Mycobacteria

Mycobacteria share some common characteristics. One of these is their ability to lose their cell wall[3]. Losing the cell wall avoids attracting the attention of the immune system, which is always on alert for bacteria. This little-reported strategy is seen when bacteria are under pressure from antibiotics, antibodies, the environment, and the body's immune soldiers, the white cells. Mycobacteria without an outer cell wall are called "cell-wall-deficient mycobacteria" (CWDM). Loss of the cell wall was originally associated with symbiosis between CWDM and single-celled amoebae.

In the environment, the amoeba is a convenient “changing room” for mycobacteria, where they can lose their cell wall and enjoy the protection of the amoeba. The CWDM can also absorb nutrients from the amoebae. This relationship is not just a convenient coincidence, but is the result of millennia of symbiotic adaptation between amoebae and CWDM. Living under the same roof often has benefits for both parties, including a food source, and a shelter from immune responses. The relationship favours those mycobacteria associated with causing infections. *Mycobacterium avium ssp paratuberculosis* (MAP), for example, can survive in amoebae. Amoebae have been called “training grounds for mycobacteria”.[4], [5] Human white cells did not evolve directly from amoebae, but they resemble them, and may share some common ancestor.

The best place for CWDM to hide in warm-blooded animals is inside a white cell. This is called an intracellular lifestyle. White cells clean up infections and target specific bacteria and viruses, depending on the type of white cell. High numbers of white cells can be an indication of infection. In chronic inflammatory diseases, such as tuberculosis and Crohn’s disease, the relationship between mycobacteria and white cells is very complex. Some mycobacteria have evolved to survive in human white cells[6].

This contradicts the theory that human blood is sterile and bacteria in the bloodstream are swiftly neutralised by white cells. That does not always happen. Some bacteria can survive and adapt to an intracellular lifestyle. When this happens with mycobacteria, the organism adopts a dormant, or latent form. The cell wall is lost and the mycobacteria do not replicate, but drastically reduce their metabolism. This is accompanied by biochemical changes in metabolic products, including the inactivation of some virulence factors and alterations in mycobacterial genes. The inability of the dormant forms to be regrown is common.

The CWDM can sometimes change back to a cell-walled form, particularly in tuberculous disease, where the disease is reactivated in the patient[7]. The intracellular dormant lifestyle within the white cell is also a powerful antimicrobial resistance factor[8]. It makes antibiotic penetration difficult, and dormancy is also a natural resistance factor. Antibiotics require actively replicating bacteria to work well.

Iflammation is the hallmark of Crohn's disease, but exactly what triggers the inflammatory response is a topic of intense debate. White cells are directed by the immune system to sites of inflammation. There are theories for why this occurs in some individuals and not in others.

An important discovery, called "original mycobacterial sin"[9] was reported by researchers in 1970. An Australian researcher, E.W. Abrahams noticed that when Australian children were vaccinated against tuberculosis using the BCG vaccination, (which specifically targets *Mycobacterium tuberculosis*), many of them instead showed strong evidence of immunity to Mycobacterium avium complex (MAC). MAC is the group of mycobacteria that includes MAP.

Instead of being immune to tuberculosis because of BCG vaccination, the children had instead become preferentially immune to MAC. Abrahams, following on from other researchers, called this "original mycobacterial sin." He proposed that the first exposure of the subject to any Mycobacterium species set the immune alarm system to go off for that specific Mycobacterium species, even if the patient was later exposed to another completely different Mycobacterium species. When the children had been vaccinated with TB mycobacteria in the BCG vaccination, they produced antibodies to MAC instead. This meant that they had been exposed to MAC in the environment before getting the TB vaccination. These children lived in Queensland, a State of Australia.

Queensland is now the epicenter for outbreaks of environmentally acquired *Mycobacterium abscessus* and *Mycobacterium ulcerans*; these are significant agents of skin infections and share some characteristics with MAP[10], [11], [12]. Not only that, but *M. ulcerans* and *M. abscessus* emerged as significant agents of human skin infections in the 1960s, around the same time that the rates of patients with Crohn's disease started increasing[13]. Of course, there may be no direct link between the two events, but some researchers have linked those recently emerging mycobacterial skin diseases with the dawn of **"the antibiotic age."**[14] Widespread use of antibiotics was common in the 1960's in both animals and humans and it was only later that the effects of this became apparent. These included infectious transmission because of resistance to antibiotics.

Some researchers in the early 1900's had observed that Johne's disease in animals resembled a disease in humans called regional ileitis[15], [16]. Crohn had published his definitive paper on regional ileitis in 1932, after which regional ileitis was called Crohn's disease. At that time Crohn's disease was a relatively rare disease, and in 1967 was still referred to in some publications as

regional ileitis[1]. It is sobering to realise that the emergence of Crohn's disease happened so recently in time.

Abraham's theory of "original mycobacterial sin" is worth considering in relation to Crohn's disease. Ramon Juste[17] and other researchers[18] noted the carriage of MAP in the blood of normal healthy patients. In Dr Juste's study there were more healthy human carriers of MAP than there were in patients diagnosed with Crohn's disease.
How are we to interpret these reports? These healthy control patients who were possibly carrying MAP are not going to develop Crohn's disease. Leaving aside the very real possibility that we researchers have been using imprecise methods or "barking up the wrong tree", there are several explanations for the observations.

Explanation 1

MAP is unlike *M. tuberculosis*; *M. tuberculosis* always causes tuberculosis. It is an obligate mycobacterial pathogen. If *M. tuberculosis* is isolated from human samples, it must be there as a pathogenic organism. It is superbly adapted (obliged) to cause disease in humans.

Members of MAC, (including MAP), are opportunistic mycobacterial pathogens. If they are found in human culture samples, it does not mean they are dangerous to the human host. They may only be passing through the person, from the environment. Members of MAC need an opportunity to invade, most commonly via an immunocompromised immune system. In other words, some underlying fault in the immune system opens a door to MAC infection.

Dr Nadya Markova, a microbiologist from Sofia, looked at the question of how long BCG organisms remained alive in humans after they had been vaccinated. Bulgarian children were routinely immunised with the BCG strain of bovine tuberculosis at birth. The research group demonstrated that the CWDM[19] remained viable within the vaccinated subject for decades, and in some cases crossed the placenta from the BCG-vaccinated mother to her newborn infant[20]. The form of the BCG organism found by Dr Markova and her colleagues, was that of a CWDM. These findings provide evidence that CWDM can be present in healthy individuals.

Explanation 2

As you have probably guessed, the second explanation for the persistence of mycobacteria in nearly all humans, is Abraham's theory of "original mycobacterial sin." The first contact with mycobacteria

sets up an immune system alert in the subject, to warn the body that mycobacteria are present and to keep a watch-out for them (immune stimulation). That first contact with an environmental mycobacterium may result in the long-term carriage of viable CWDM from the contact strain. Markova's research on CWDM in children showed that carriage of living CWDM may persist for decades.

Opportunistic Mycobacterial Infection

Our work has shown that CWDM carriage in healthy humans, **in very low numbers**, is common. In patients diagnosed with Crohn's disease, this also happens. However, the numbers of CWDM present when the patient is in an active phase of the disease, are very high. This can be expected if the subject is an acute phase of an infection.

There are always low numbers of bacteria in transit through the human body. This is quite normal and has been demonstrated by other researchers. They may come from the mouth, through dental disease, or occur as small leaks in the digestive system. Tiny cuts and abrasions will also result in leakage of bacteria into the bloodstream.

However, if there are high numbers of bacteria present in the bloodstream, then a symptomatic patient may require urgent medical intervention. Carriage of CWDM via the white cells in low numbers appears to be common in both healthy subjects and patients with some inflammatory diseases. Why is this happening?

Explanation 1

There are numerous explanations put forward for CWDM presence in blood samples. Apart from blaming the sterile technique of the researchers, the main explanation I have heard is that CWDM are artificial, or that they do not exist. Given more recent research on the blood microbiome, it is now accepted that carriage of bacteria in the bloodstream has been satisfactorily proven by molecular biologists and fills unknown functions.

Explanation 2

Abrahams and others were right.

We carry CWDM within some of our white cells as a long term reminder to our immune system to watch out for mycobacteria. Humans have had millennia of exposure to mycobacteria, in particular

tuberculosis. The evidence for this lies in Egyptian mummies, where tuberculosis infection can be demonstrated by examination of ancient DNA, but also in the immune system of many healthy humans. The innate immune system is the part of the immune system that is inherited from parents and is active at birth. Part of this genetic birthday gift from Mum and Dad includes white cells that can detect and react to the appearance of mycobacteria in lungs and bloodstream. It doesn't have to be tuberculosis.

Of course, E.W. Abrahams observations have led to various responses from other researchers[21], [22], but the possibility of white cells carrying live mycobacteria round the bloodstream has not been one of them. The theory would however help to explain the detection of MAP DNA in healthy controls in low numbers.

SUMMARY

In this article I've presented some odd facts about mycobacteria in general, and MAP in particular. Crohn's disease (regional ileitis) was rare until the 1960's when it became more common. MAP was first linked to Johne's disease in animals in 1913, the same year that a connection was made with regional ileitis, a relatively rare disease of humans. In 1932 regional ileitis was renamed as Crohn's disease, but was still uncommon in the 1960's.

The 1960's was at the height of the antibiotic age, when the long-term consequences of human and animal use were largely unknown and antibiotics were seen as miracle drugs. It was also the period when environmental non-tuberculous mycobacteria were recognised as emerging opportunistic pathogens. The last 20 years have seen climbing global rates of NTM infections, as well as Crohn's disease. Because of the pathogenic role of Mycobacterium tuberculosis in humans and animals, most humans have immune systems that are primed to recognise the risk posed by M tuberculosis complex.

CWDM are closely dependent on humans through intracellular uptake by macrophages. This symbiotic lifestyle may be beneficial to the human host, as protection against tuberculosis. There is abundant evidence that mycobacteria can be present in low numbers in healthy human subjects. 40 years of research into Crohn's disease has also indicated that MAP is able to exist in that state, and is now widespread in the population.

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