MONOCLONAL ANTIBODIES

Monoclonal antibodies (mAb or moAb) are monospecific antibodies that are identical because they are produced by one type of immune cell that are all clones of a single parent cell. Given (almost) any substance, it is possible to create monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance. This has become an important tool in biochemistry, molecular biology and medicine. When used as medications, the generic name ends in -mab (see "Nomenclature of monoclonal antibodies").

Discovery

The idea of a "magic bullet" was first proposed by Paul Ehrlich who at the beginning of the 20th century postulated that if a compound could be made that selectively targeted a disease-causing organism, then a toxin for that organism could be delivered along with the agent of selectivity.

In the 1970s the B-cell cancer multiple myeloma was known, and it was understood that these cancerous B-cells all produce a single type of antibody (a paraprotein). This was used to study the structure of antibodies, but it was not yet possible to produce identical antibodies specific to a given antigen.

A process of producing monoclonal antibodies involving human-mouse hybrid cells was described by Jerrold Schwaber in 1973 and remains widely cited among those using human-derived hybridomas but claims to priority have been controversial. A science history paper on the subject gave some credit to Schwaber for inventing a technique that was widely cited, but stopped short of suggesting that he had been cheated The invention is generally accredited to Georges Köhler, César Milstein, and Niels Kaj Jerne in 1975; who shared the Nobel Prize in Physiology or Medicine in 1984 for the discovery. The key idea was to use a line of myeloma cells that had lost their ability to secrete antibodies, come up with a technique to fuse these cells with healthy antibody producing B-cells, and be able to select for the successfully fused cells.

In 1988 Greg Winter and his team pioneered the techniques to humanize monoclonal antibodies, removing the reactions that many monoclonal antibodies caused in some patients.

Production

Hybridoma Cell Production

Monoclonal antibodies are typically made by fusing myeloma cells with the spleen cells from a mouse that has been immunized with the desired antigen. However, recent advances have allowed the use of rabbit B-cells. Polyethylene glycol is used to fuse adjacent plasma membranes, but the success rate is low so a selective medium

is used in which only fused cells can grow. This is because myeloma cells have lost the ability to synthesize hypoxanthine-guanine-phosphoribosyl transferase (HGPRT).

This enzyme enables cells to synthesize purines using an extracellular source of hypoxanthine as a precursor. Ordinarily, the absence of HGPRT is not a problem for the cell because cells have an alternate biochemical pathway that they can use to synthesize purines. However, when cells are exposed to aminopterin (a folic acid analogue), they are unable to use this other, rescue pathway and are now fully dependent on HGPRT for survival. The selective culture medium is called HAT medium because it contains Hypoxanthine, Aminopterin, and Thymidine. This medium is selective for fused (hybridoma) cells because unfused myeloma cells cannot grow because they lack HGPRT. Unfused normal spleen cells cannot grow indefinitely because of their limited life span. However, hybridoma cells are able to grow indefinitely because the spleen cell partner supplies HGPRT and the myeloma partner is immortal because it is a cancer cell. The fused hybrid cells are called hybridomas, and since they are derived from cancer cells, are immortal and can be grown indefinitely.

This mixture of cells is then diluted and clones are grown from single parent cells. The antibodies secreted by the different clones are then tested for their ability to bind to the antigen (for example with a test such as EIA or Antigen Microarray Assay) or immuno-dot blot, and the most productive and stable clone is then grown in culture medium to a high volume. When the hybridoma cells are injected in mice (in the peritoneal cavity, the gut), they produce tumors containing an antibody-rich fluid called ascites fluid.

The medium must be enriched during selection to further favour hybridoma growth. This can be achieved by the use of a layer of feeder fibrocyte cells or supplement medium such as briclone. Production in cell culture is usually preferred as the ascites technique is painful to the animal and if replacement techniques exist, this method is considered unethical.

Recombinant

The production of recombinant monoclonal antibodies involves technologies, referred to as repertoire cloning or phage display/yeast display. Recombinant antibody engineering involves the use of viruses or yeast to create antibodies, rather than mice. These techniques rely on rapid cloning of immunoglobulin gene segments to create libraries of antibodies with slightly different amino acid sequences from which antibodies with desired specificities can be selected. These techniques can be used to enhance the specificity with which antibodies recognize antigens, their stability in various environmental conditions, their therapeutic efficacy, and their detectability in diagnostic applications. Fermentation chambers have been used to produce these antibodies on a large scale.

Applications

Once monoclonal antibodies for a given substance have been produced, they can be used to detect the presence and quantity of this substance, for instance in a Western blot test (to detect a protein on a membrane) or an immunofluorescence test (to detect a substance in a cell). They are also very useful in immunohistochemistry which detect antigen in fixed tissue sections. Monoclonal antibodies can also be used to purify a substance with techniques called immunoprecipitation and affinity chromatography.

Monoclonal antibodies for cancer treatment

One possible treatment for cancer involves monoclonal antibodies that bind only to cancer cell-specific antigens and induce an immunological response against the target cancer cell. Such mAb could also be modified for delivery of a toxin, radioisotope, cytokine or other active conjugate; it is also possible to design bispecific antibodies that can bind with their Fab regions both to target antigen and to a conjugate or effector cell. In fact, every intact antibody can bind to cell receptors or other proteins with its Fc region.

The Science of HIV & AIDS

The biology and impact of the world's worst pandemic

In the UK the plight of AIDS today gets much less attention from the public and the media than it did back in the 1980's and early 1990's. This often leads to the misconception that AIDS is no longer a problem in this country; in reality, the increasing prevalence of HIV proves that this is simply not true.

Worldwide, accepted definitions, facts and figures on HIV and AIDS include:

Acquired immune deficiency syndrome (AIDS) is a collection of symptoms and infections that result from specific damage to the immune system by the human immunodeficiency virus (HIV).

HIV is transmitted through the direct contact of a mucous membrane, or the bloodstream, with a bodily fluid containing HIV.

The late stage of the condition leaves individuals prone to opportunistic infections and tumours.

Whilst antiretroviral treatments for AIDS and HIV exist to reduce the mortality and morbidity of HIV infection to date there is no known cure; even access to these antiretroviral treatments is not routine in all countries.

Researchers believe that sometime in the 1930's a form of simian immunodeficiency virus jumped to humans who butchered or ate chimpanzee bush meat in the Democratic Republic of Congo. The virus became HIV-1, the most widespread form found today.

The world's first known case of AIDS has been traced to a sample of blood plasma from a man who died in 1959. During the 1970's HIV continued to spread undetected around the world and hence the pandemic began. Today an estimated 40 million people are living with HIV worldwide.

As of January 2006, the Joint United Nations Programme on HIV/AIDS and the World Health Organization estimate that AIDS has killed more than 25 million people since it was first recognised on June 5th, 1981.

The stigma associated with HIV/AIDS is severe and extends to providers and volunteers involved with the care of HIV infected patients. AIDS exerts its toll also on societies, devastating their economies, decimating their labour forces and orphaning children.

Thus, it is one of the most destructive pandemics in recorded history, surpassing even the Black Death. By 2015 it is estimated that 60 million people have died of AIDS.

In 2004 the global spending on AIDS was $6.1b. Estimated global AIDS spending required in 2007 for prevention and care is $20b. Less than 3% of all money spent on AIDS goes towards developing a vaccine for the disease.

In February 2007 a study published in the Lancet showed that male circumcision could dramatically reduce the risk of HIV infection. The randomised control trial showed that circumcised men were 60% less likely to pick up HIV.

The history of HIV / AIDS

The first recognised case of AIDS in the UK was recorded in December 1981, when doctors at Brompton Hospital in London reported the case of a 49 year old homosexual man who had died ten days post referral. He was suffering from a rare infection that almost always occurs in individuals with severely weakened immune

systems. Doctors believed that the condition might be linked to similar cases that had been occurring amongst gay men in the US.

Throughout the 1980's the number of newly diagnosed HIV infections per year in the UK rose steadily. The figure plateaued during the 1990's, averaging about 3,000 cases per year, but then increased dramatically after 1999. By 2005, the annual number of newly diagnosed infections was more than 7,500, and an estimated 63,500 people over 15 years of age were living with HIV in the UK, 20,100 (32%) of whom were believed to be unaware of having been infected. Since the pandemic began there have been 17,161 known UK HIV deaths.

The science bit...

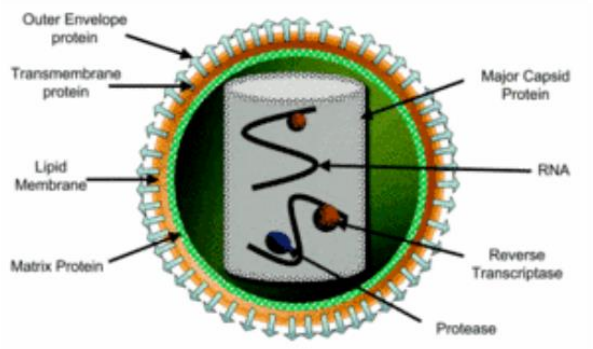
HIV is a retrovirus, meaning that it uses a chemical relative of DNA, called RNA (ribonucleic acid), as its genetic material. It primarily attacks components of the human immune system, including T lymphocytes and other white blood cells that carry "CD4" receptors on their surfaces. HIV enters its target cells by binding to both the CD4 molecule and a chemokine "co-receptor", of which there are two forms, CCR5 and CXCR4. Once inside the cell the virus makes a DNA copy of its genome and then uses an enzyme it carries with it, called integrase, to insert this copy into the cell's own DNA. Either immediately, or after a period of dormancy known as latency, the virus then hijacks the cell and turns it into a virus factory. The newly produced viruses leave the infected cell, destroying it in the process, and move on to invade other CD4+ cells, which are mainly T lymphocytes. These are the cellular linchpins that help to marshall the other components of the body's immune system. As their numbers dwindle the ability of the body to mount an effective immune response to combat other invaders, including bacteria, viruses and fungi, is progressively weakened. This means that HIV kills by slowly destroying the immune system and leaving the infected individual vulnerable to infection by so called "low grade" or opportunistic bugs.

What is the natural history of HIV infection? What are the symptoms?

Most infections with HIV are initially "silent", meaning that a person may not notice that anything is wrong. Then, several weeks after infection, patients often develop a "seroconversion illness", which characteristically includes flu-like symptoms, lymphadenopathy (swollen lymph glands), fevers, loss of appetite and weight, diarrhoea and general lethargy and malaise. During this time infected individuals have very high levels of virus in the bloodstream (10 million viruses per millilitre of blood is not uncommon). The reason for this very high viral load is that the virus is able to replicate (grow) largely unchecked because the immune system has yet to mount an effective suppressive response, including the production of antibodies that can mop up viral particles. As a result the number of CD4+ T cells can fall to very low levels at this time, and the patient is highly infectious.

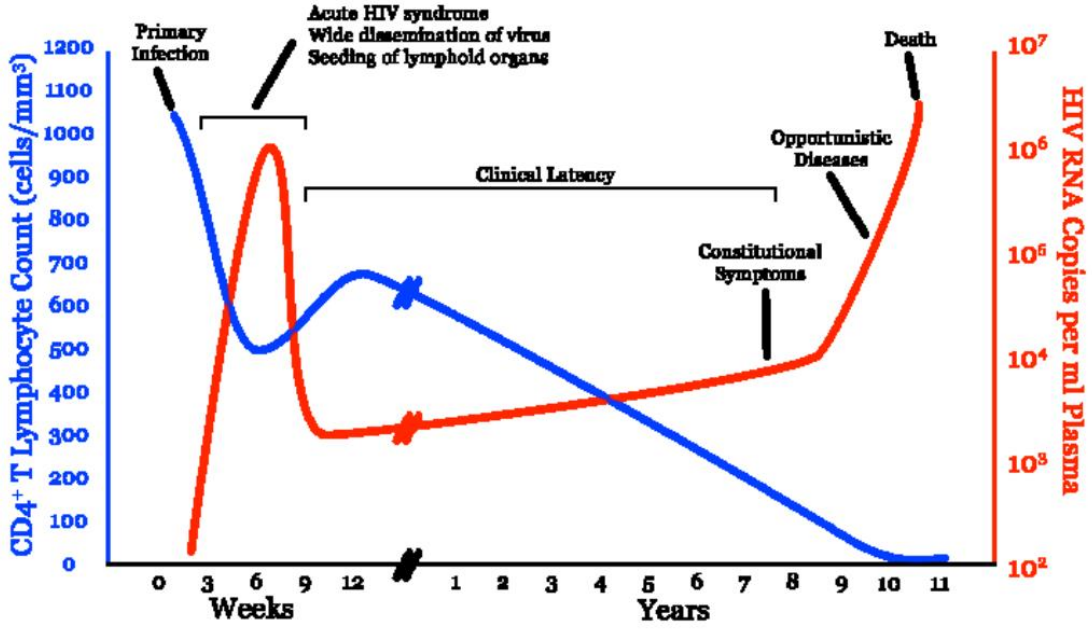
But then the immune system kicks in, and the virus largely retreats, hiding within lymphoid tissues and replicating only very slowly. The levels of virus in the blood

stream become much lower, the patient is less infectious and feels well. Untreated, an infected individual usually remains "healthy" like this for 5 to 15 years.



Structure of the human immunodeficiency virus (HIV) viral particle.

However, the body's immune system only has a limited ability to control HIV. The virus makes mistakes when it copies its genetic code. Roughly once in every 10,000 genetic letters that are copied the virus introduces the wrong genetic base. Since the genome contains about 9000 bases in total, almost every genome copied will contain an error. The result is that these genetic mistakes alter the appearance of the virus and so make it harder for the immune system to recognise and keep up, because it is trying to hit a moving target. Eventually, through this progressive shape-shifting, the virus takes on a form that the immune system cannot respond to, and at this point the pace of the infection begins to accelerate and the number of CD4+ T cells begins to fall.



count falls below a critical threshold (400 per microlitre of blood) the body is no longer able to defend itself. At this point an HIV-infected individual is said to have AIDS, and patients usually begin to develop opportunistic infections caused by organisms that would not normally affect healthy people. These include mycobaterial infections (caused by bacteria related to tuberculosis), the lung infection PCP (pneumocystis carinii pneumonia), oral and genital thrush, complications of CMV (cytomegalovirus), chronic diarrhoea and weight loss, toxoplasmosis, meningitis, dementia, and polyomavirus (JC virus), which is associated with a disease of the brain's white matter known as PML (progressive multifocal leucoencephalopathy). At this point patients are often prescribed prophylactic drugs to help ward off some of these infections including co-trimoxazole, which can slow down the progression of PCP.

Without treatment the median survival time after developing AIDS is only about 9 months. However, the rate of clinical disease progression varies widely between individuals from 2 weeks to 20 years. Many factors affect this rate of progression, including age, quality of health care and the presence of co-existing infections. An individual's genetic make-up also plays an important role because it's now becoming clear that some people are resistant to certain strains of HIV and although they become infected they do not seem to develop AIDS, or they do so only extremely slowly. There are even people who seem to be totally immune to infection with the virus. They carry a mutated cell surface marker called CCR5-delta-32, which prevents HIV from locking onto and invading their cells. Scientists hope that understanding what makes these people able to resist the virus may hold the key to future therapies to block infection amongst susceptible individuals.

Treatment

Whilst the number of people living with HIV is rising each year, the number of HIV infections that progress to AIDS has dropped dramatically since 1996. This is primarily the result of anti-retroviral therapies, which are available to slow the progress of the virus. These target essential components of the viral replication cycle and include reverse transciptase (RT) inhibitors, which interfere with the way the virus makes a complementary "cDNA" copy of its RNA genome, and protease inhibitors, which prevent the virus from cutting up the raw materials it needs to form new viral particles.

There are two types of drugs that block RT; these are known as nucleoside and non-nucleoside RT inhibitors. The nucleoside RT inhibitors are structurally very similar to normal DNA bases, but they lack a critical chemical group required to enable a DNA chain to grow. So when the viral RT inserts one of these altered bases into the copy that it's making of its genetic code, it can't finish the job because it cannot add the next genetic letter. An example of this type of agent is the drug AZT or zidovudine (azidothymidine). The non-nucleoside RT inhibitors, which include

drugs like efavirenz and nevirapine, work slightly differently. They target the RT enzyme itself and bind to it, distorting its shape so that it cannot work properly. This stops the virus from replicating.

Protease inhibitors (PIs) only emerged more recently. They work by blocking the action of a protein-cutting enyzme carried by HIV, which is critical to the virus being able to assemble new infectious particles. If this enzyme is prevented from doing its job the virus cannot escape from the infected cell. An example of the PIs includes saquinavir, which is famous for being one of the first drugs produced by building a computer model of the shape of the viral enzyme and then designing a drug specifically to block it.

There are also agents known as fusion inhibitors, which are a newer type of drug that work by stopping HIV from binding with the CD4 receptors that it uses to enter cells. One being evaluated at the moment is called efurvatide.

Doctors have also recently been testing a new agent called raltegravir, which is an "integrase inhibitor". This prevents the virus from inserting a copy of its genetic material into the host cell genome. In a recent trial published in the Lancet doctors randomly allocated 179 patients with end-stage HIV / AIDS to receive either the active drug or a placebo. After 6 months the patients receiving raltegravir showed a 98% drop in the levels of virus in the bloodstream, compared with only 45% in the placebo group. The next step will be to test raltegravir in combination with other HAART regimen drugs in healthier patients who are not approaching the end-stages of their disease. It may make a considerable difference to the rate of disease progression.

So there are lots of drugs with which we can now combat HIV; but there's a problem. Because the virus frequently makes mistakes when it copies its genetic material it rapidly develops forms of the virus that are resistant to the action of these drugs. To slow down the rate at which this happens, rather than use them singly, a cocktail of drugs is used, often one from each of the three classes (nucleoside RT inhibitors, non-nucleoside RT inhibitors and protease inhibitors). This is known as HAART or Highly Active Antiretroviral Therapy (HAART) and it has dramatically reduced the evolution of viral resistance and prolonged the time during which an HIV-infected individual remains healthy and symptom free. However, it's worth emphasising that, whilst drugs help to control the spread of HIV to uninfected cells, unfortunately there is no treatment available at present that can eradicate HIV once integrated into a host.

Side effects...
This means that individuals using HAART have to take medication every day for the rest of their lives, and this often causes severe side effects. When individuals first start treatment they may suffer headaches, hypertension or general malaise (feeling unwell), although these usually improve or disappear with time. Other side effects can include diarrhoea, nausea, fatigue, anaemia, lipodystrophy, skin problems,

neuropathy, mitochondrial toxicity, dyslipidaemia and bone problems. Whilst most people who take anti-HIV medications have some side effects it must not be assumed that everyone gets every side effect that has ever been written down.

Another problem with combating HIV is that a number of different strains of the virus can arise due to differences in selection pressures as the virus encounters different individuals, different drugs and different routes of spread. This can result in resistance to multiple anti-retrovirals and frequently occurs through a process called recombination. It occurs because each HIV virion carries two complete RNA genomic strands, meaning that homologous recombination can occur when a cell is coinfected with two different but related strains. The two strains may then exchange genetic material, including drug resistance traits. The process of recombination also therefore poses theoretical problems for the development of a safe vaccine against HIV.

The situation is also made worse by the fact that increasing numbers of patients are found to be carrying resistant forms of the virus at diagnosis, even before any drug therapy has been administered. Indeed, in 2004 an estimated 9% of new HIV diagnoses were found to be drug resistant strains, presumably acquired from individuals who had already received treatment. If patients then acquire additional strains of the virus with different resistance profiles the process of recombination can yield multiply-resistant viruses. In a case described recently in the Lancet this resulted in an individual producing a strain of the virus that was resistant to every available anti-retroviral agent. The patient in question also progressed to AIDS and died within six months of becoming infected.

What is next?

Billions of pounds are spent every year worldwide on caring for and treating individuals with HIV/AIDS and on resources to prevent further spreading of the virus. However, ultimately it is a cure that is required to combat this pandemic. A vaccine to prevent HIV infection, as an alternative method to current therapies, may still be many years away. Not only might such a vaccine have to prime antibodies to attack HIV (the way most vaccines work) but it might also need to increase T cell production. Vaccine trials have been undertaken in South Africa, Kenya, the USA and Thailand, though most have yet to yield promising results. Controversial vaccines made from the blood of HIV carriers have been tested in Nigeria and Thailand. Other developing avenues for treatment of HIV positive individuals include gene therapy, targeted radiation therapy and nanotube technology to block the invasion of HIV into target cells. In April 2007 researchers even identified a component naturally present in human blood (a protein derived from alpha-1-antitrypsin) that can block HIV entry into cells.

In the absence of a vaccine researchers have turned to other approaches to try to combat the virus. A promising discovery, confirmed earlier in 2007, was that male circumcision can dramatically reduce, by 60%, the chances of acquiring HIV. The first clues that circumcision might be beneficial in halting the spread of HIV came

after researchers noticed much lower prevalences of HIV infection amongst communities in which males were routinely circumcised. This hypothesis was tested recently in a series of randomised control trials in which HIV-negative volunteers seeking the procedure were randomly assigned either to undergo circumcision immediately or to wait for a period of time first. The patients were then followed up with regular HIV tests. The trial had to be stopped prematurely on ethical grounds when a large excess of HIV cases were found in the group of individuals asked to wait before undergoing the procedure. Scientists think that the foreskin represents a significant portal of entry for the virus because it is relatively enriched in cell types targeted by HIV, it provides an environment in which the virus can persist for an extended period thus maximising the risk of infection, and the mucosa of the foreskin can develop tiny fissures during intercourse and these facilitate viral entry and infection.

In response to these findings, the WHO / UNAIDS have recommended that it should be considered as an effective preventative measure. According to Kevin de Cock, director of the World Health Organisation's AIDS department, "This is an extraordinary development...Circumcision is the most potent intervention in HIV prevention that has been described". And according to Marie-Louise Newell of the University of KwaZulu-Natal in South Africa and Till Barnighausen of the Harvard School of Public Health "if all of the 2.5 million men in KwaZulu-Natal province had been circumcised, 37,000 new infections could have been prevented in 2007".

But none of these strategies can be effective without education, particularly about safe sex. One in ten girls aged 16-19 in the UK is infected with chlamydia, which can only have been acquired through unsafe sex. This is clear evidence that large numbers of young people are placing themselves at direct risk of HIV, probably because they are from a generation who never saw the "grim reaper" television adverts of the 1980s when AIDS first hit the headlines. HIV is a very real threat and still very much a life sentence. Unless people can be made aware of this then the problem will only continue to get worse.

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