Anna Stevenson

Chemical reactions for making antibodies - what does the future hold?

Antibodies are globular proteins made by b lymphocytes with a shape complementary to one antigen. They are an integral part of the human immune system for fighting disease, and their high specificity to one antigen makes them useful for detecting foreign molecules in our blood. They are used in blood tests to diagnose diseases; to treat immune deficiencies; to protect RhD positive foetuses when the mother is RhD negative; and to cure snake bites.[1] The antibodies used for these processes are currently obtained by either using human antibody donations,[2] or by injecting antigens into an animal and harvesting the monoclonal antibodies in a lab.[3]

Given the limitation of these processes, being able to mass produce synthetic antibodies outside of the body is important for both research into, and treatment of, many diseases. Until recently the only possible methods to create antibodies all had some biological origin. However, there are now exciting developments in antibody production using chemical reactions such as: molecularly imprinting polymers; using polymers which have bonded to carbon nanotubes; and creating synthetic antibody mimics. These developments although in their infancy provide a potentially much better solution for future use in cancer treatments and lab research relative to existing methods.

What are antibodies?

Antibodies consist of 4 polypeptide chains in a Y shape. The antigen binds to the variable regions at the ends of the Y shape (Figure 1a).[4] Antibodies production is triggered in lymphocytes by the invasion of a pathogen. Animals chemically make the antibodies by a condensation reaction between amino acids forming peptide bonds as seen in figure 1b, which form a polypeptide chain. The chain then forms an alpha helix or beta pleated sheet. This then folds to form the 3D tertiary structure of the protein, where disulphide bridges, ionic and hydrogen bonds form holding the protein together. Four polypeptide chains then bond together by disulphide bridges to form an antibody as seen in figure 1a.

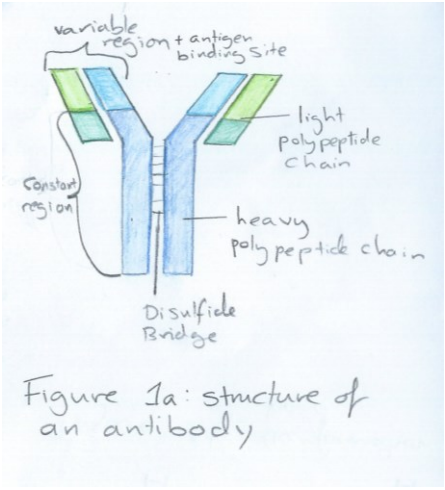


Figure 1a: structure of an antibody

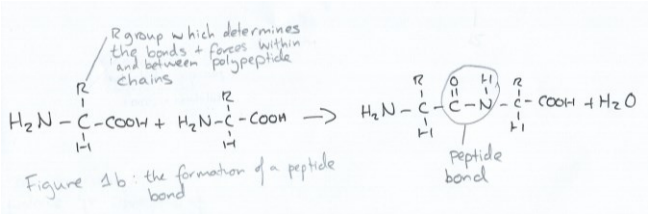


Figure 1b: the formation of a peptide bond

This process gives rise to about 10^{11} unique antibodies, and so it will be very time consuming and expensive to chemically synthesise them all. [5] However, given our bodies can create new antibodies for future strains of diseases, today's research only needs to focus on creating antibodies for today's known diseases.

Monoclonal antibodies (biological synthesis)

Until 1975, antibodies were made by injecting either inactivated toxins from bacteria or snake venom into a horse's blood to trigger an immune response. These antibodies were then extracted and injected into humans. In 1975, Kohler and Milstein created antibodies by injecting antigens into a mouse triggering an immune response to produce antibodies, as described above. They removed the mouse's spleen cells and fused them with myeloma cells, a form of tumour cells,

to form hybridoma cells.[6] These cells were then grown in a lab before separating the b lymphocytes and collecting the antibodies for use (figure 2). This was completed relatively quickly as there were no inhibitions on the growth of the tumour cell. Since then, 20 such monoclonal antibodies have been approved for clinical usage and hundreds more are in clinical trials. This is already having a significant influence on the future treatment of cancers and auto-immune conditions.[7] For example, Herceptin is a drug used to treat breast cancers containing Trastuzumab antibodies which inhibit the HER2 protein from signalling cell growth[8]. In clinical trials, it increased survival rates for breast cancer by 37%.[9] However, not all monoclonal antibodies trials are successful. In 2006, the TGN1412 drug almost caused the death of 6 patients from organ failure due to an overproduction of cytokines. This demonstrates the benefit of developing synthetic antibodies that do not trigger an autoimmune response.[10]

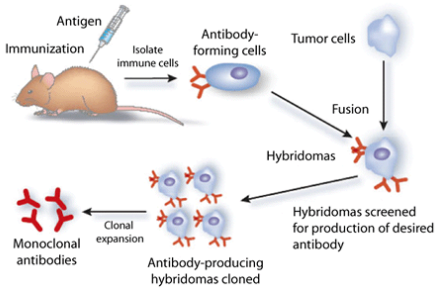


Figure 2: Producing monoclonal antibodies

This method can be used to create large amounts of antibodies in fermenters with thousands of litres of tissue culture for hybridoma cells. A drawback is that to create an antibody for every human antigen would require at least a few hundred thousand mice and would have major ethical problems due to the pain it causes the mice. It may also require many time-consuming steps to purify and alter the antibodies to prevent rejection by the body.

An alternative method is to add genes from antibodies into bacteriophage genomes to create recombinant DNA. This method halves the time scale from 4 months for monoclonal antibody production to 2 months, but is expensive and can present false positives when phages binding to antigens they are not specific to.[7] Monoclonal antibodies can also be made from parts of two different antibodies bonded together to creates a bispecific antibody with two different target regions. It can simultaneously bind to killer T cells and tumour cells to treat cancer. There are currently 35 of these antibodies in clinical trials, and the patents associated with 6 of these bispecific antibodies have been sold for a cumulative value of US$3.5 billion, which shows the potential companies think these antibodies have for future use in medical treatments.[11]

Molecularly imprinted polymers (chemical synthesis)

Scientists have started developing methods to create antibodies from non-living sources. Molecular imprinting involves shaping monomers with amino or carboxylic acid groups around a template molecule, which is similar in shape to the antigen and so acts like a mould. A cross-linking agent is added so that the monomers can undergo polymerisation to form a fixed 3D structure. The binding chemicals are held firmly in place preventing the shape changing when the template is removed. When the solvent molecules are removed, they create space to allow the target molecules to enter and bind. The polymers can be used to make highly specific detectors that can be used for biosensors.

In 2008, Kenneth Shea with a team at the University of California used this method to manufacture antibody nanoparticles from plastic. They first looked for monomers that had a high affinity for melittin, a toxin in bee venom.[12] They shaped and polymerised monomers around a melittin molecule to form a longer chain. The venom was then dissolved away to leave a cavity with a complementary shape to melittin (figure 3). Finally, the antibodies were purified before being injected into mice. They injected two groups of mice with a lethal dose of bee venom, but only gave antibodies to one group. This increased the survival of the mice receiving the antibodies from 0% to 60%, showing that the antibodies could recognise and bind to the toxin over the other antigens in the mice's blood. These antibodies were then destroyed by the liver but did no harm to the mice.

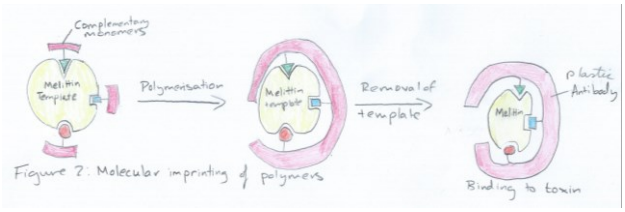


Figure 3: Molecularly imprinting polymers

Molecular imprinting is cheaper than other synthetic methods, takes only 1-2 hours[13], and causes less ethical conflicts.[14] Philipp Holliger said that the limitations with this method were due to the antibodies being unable to communicate with the immune system and so even though they can perform some functions, they do not give future immunity against the toxin.[15]

In 2016, Shea developed an antivenom treatment by reacting polymers with different chemical functions to produce nanoparticles. These were incubated with PLA_2 molecules, a snake toxin, and those with the best specificity selected. In the blood, the toxins bind with the PLA_2 molecules due to the high specificity. The nanoparticles act in a similar way to the antibodies used in antivenoms, and with further development, will be able to bind to the other principle proteins in snake venom to provide a cheaper treatment for snake bites.[16]

Carbon nanotubes (chemical synthesis)

In 2013, a team at MIT coated nanotubes with amphiphilic polymers to find potential nanosensors. These specially designed polymers have hydrophobic regions that hook into the nanotube whilst the hydrophilic regions coil around the tube forming a corona that recognises a specific target molecule based on the distance between each of the loops, figure 4. This technique is known as Corona Phase Molecular Recognition. The amount of target molecule present can be detected under laser light as it alters the fluorescence due to the interactions between the polymer and the nanotube. The polymers do not break down inside tissues and so are much longer lasting. In 2013, they had been developed for a form of oestrogen, Vitamin B_2 and L-thyroxine, but the team is currently developing sites for other molecules.[17] Three years later, and after further research, the team at MIT created a method to detect fibrinogen and insulin. They added 20 polymers including DNA and RNA to carbon nanotubes, and selected the one carbon nanotube that could bind to fibrinogen. When the polymer was saturated with fibrinogen the fluorescence of the nanotubes decreased by over 80%, meaning it holds great potential for usage as a sensor to monitor patients on blood thinners.[18]

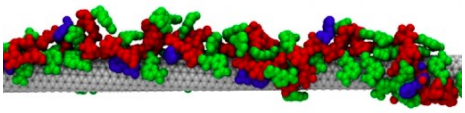


Figure 4: Nanotubes with amphiphilic polymers

The creation of a binding site for fibrinogen demonstrates the progress of this process to detect larger and more complex proteins, showing that the number and complexity of proteins

they will be able to bind to will only increase in the future. For example, there is currently research into detecting proteins associated with cancer and troponin which is released by dying heart cells.

The major drawback of this process is that scientists are unable to work out if there will be any attraction whilst making the antibodies. For example, 19 of the 20 nanotubes the MIT team created have shown no specificity. Another issue is that whilst this is a fantastic method for creating antibodies to detect proteins, they are unable to act as part of the immune system.[17]

Synthetic antibody mimics (chemical synthesis)

Synthetic antibody mimics (SyAMs) were created by a team at Yale University, and are 1/20th the size of antibodies with two binding ends separated by peptides. The team first used a molecule containing an azide group (N_3^-) as a substrate in a cycloaddition reaction to create a carboxylic acid. This was then added in four steps to a linking domain to form an antibody (figure 5a). However, this did not significantly increase phagocytosis of the effector cells. In the second attempt, an azide was reacted with a propargylamine in a cycloaddition reaction in the presence of microwaves to form an amine. An acyl group was added to this with an acid, before undergoing several steps to form two simpler molecules, so that it could be used in another cycloaddition reaction to form a carboxylic acid. This was converted into an antibody (Figure 5b), but although it induced some phagocytosis, it was unable to cause cellular phagocytosis.

Figure 5a. SyAM attempt 3

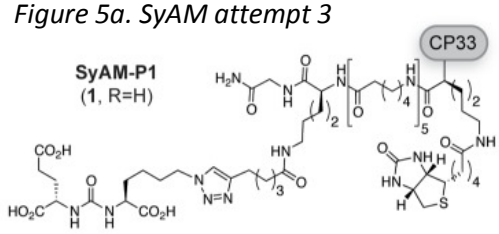


Figure 5b: SyAM attempt 2

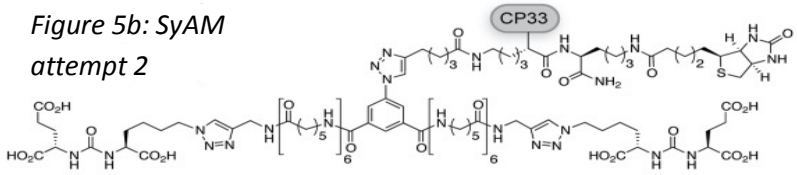
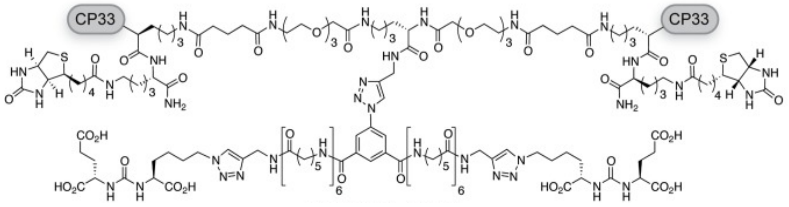


Figure 5c: SyAM attempt 3



In the third attempt, the team added together a diamine with an azide in a cycloaddition reaction in the presence of microwaves. An acyl group was then added to the ester to create a synthetic antibody mimic[19] (Figure 5c).

As shown by the number of attempts to create these molecules, the process is very complex, and so further development is likely to be time consuming and difficult. The benefit of SyAMs is that they are too small size to be recognised by the immune system, and so cannot be rejected. In current research, they can simultaneously attach to immune and prostate cancer cells to bring them together so an immune cell can destroy the cancer cells, as shown in figure 6. They are currently being developed for other cancers and HIV.[20]

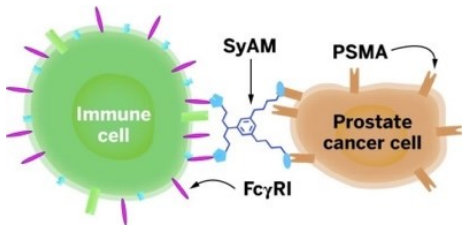


Figure 6: SyAM binding to immune cell and prostate cancer cell

Financial implications

Although currently there is a lot of progress into chemically making antibodies, the development of more synthetic antibodies will be largely determined by funding provided by governments and pharmaceutical companies. For example, there is little research into the development of different types of antibodies to combat snake venoms, as there is little profit due to their high manufacturing costs relative to the patients' ability to pay in typically poorer countries where snake bites are more prevalent[21]. The future of research into chemical antibodies will therefore be driven by demand for expensive synthetic antibodies. Given the unreliability of results from synthetic antibody research, monoclonal antibodies are currently an attractive alternative solution.

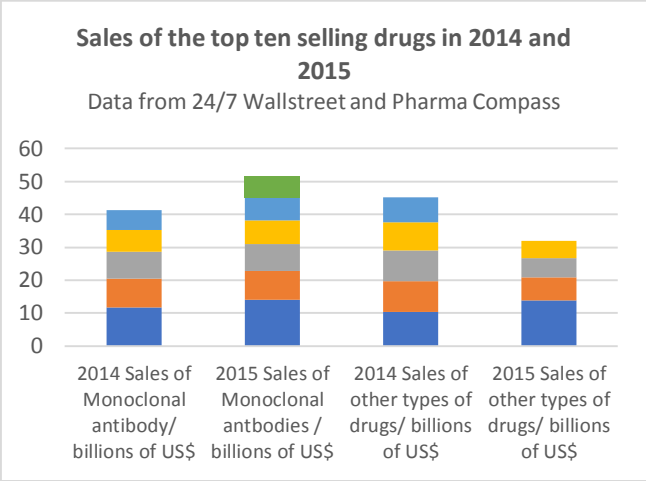


Figure 7

Monoclonal antibodies receive a lot of funding due to the impact they can have on cancer cells. For example, Kymab research group has been given a £81 million grant to undertake research into monoclonal antibodies.[22] Adalimumab, which is a type of monoclonal antibody, was the largest selling drug in 2015 with US$14 billion in sales. As seen in figure 7, there has been a large growth in sales of monoclonal antibodies, and in 2015, six of the top ten selling drugs contain monoclonal antibodies. The high profits from these drugs means there is a risk that research funds are primarily directed to the currently cheaper and more effective monoclonal antibody solutions rather than the riskier option of research into the longer-term development of more effective synthetic antibodies.

Conclusions

For the foreseeable future, synthetic antibodies are unlikely to be able to replicate the production of antibodies by the immune system, but there are lots of exciting developments in the field of antibody production. If current progress continues, it may be possible to mass produce antibody-like compounds with chemical reactions. These developments could revolutionise the treatment for a whole range of treatments from venomous snake bites to curing many cancers.

In the short-term monoclonal antibodies appear to have the most potential for treating diseases given that many are already clinically approved[22], and some scientists think that within the next generation, there will be 100-200 licensed for use[9]. These provide a commercially viable route to treating cancer where there are a limited number of available treatments, but involve the inhumane treatment of many animals to generate industrial quantities of antibodies.

Longer term, synthetic antibody production holds great promise, with the development of melittin antibodies by polymerisation showing that it is possible to create antibodies from an abiotic source which work in a very similar way to those created by the immune system. However, it suffers from a high degree of complexity and failure in development on top of high production costs relative to monoclonal antibody production. This means that even though this production

method may be a superior process in the longer-term, the most cost-effective method currently is mass producing monoclonal antibodies in large fermenters and so will remain the major source of antibodies for now. However, hopefully in the future synthetically made antibodies will be able to be used alongside monoclonal antibodies to provide a cheaper and more ethical treatment of diseases.

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