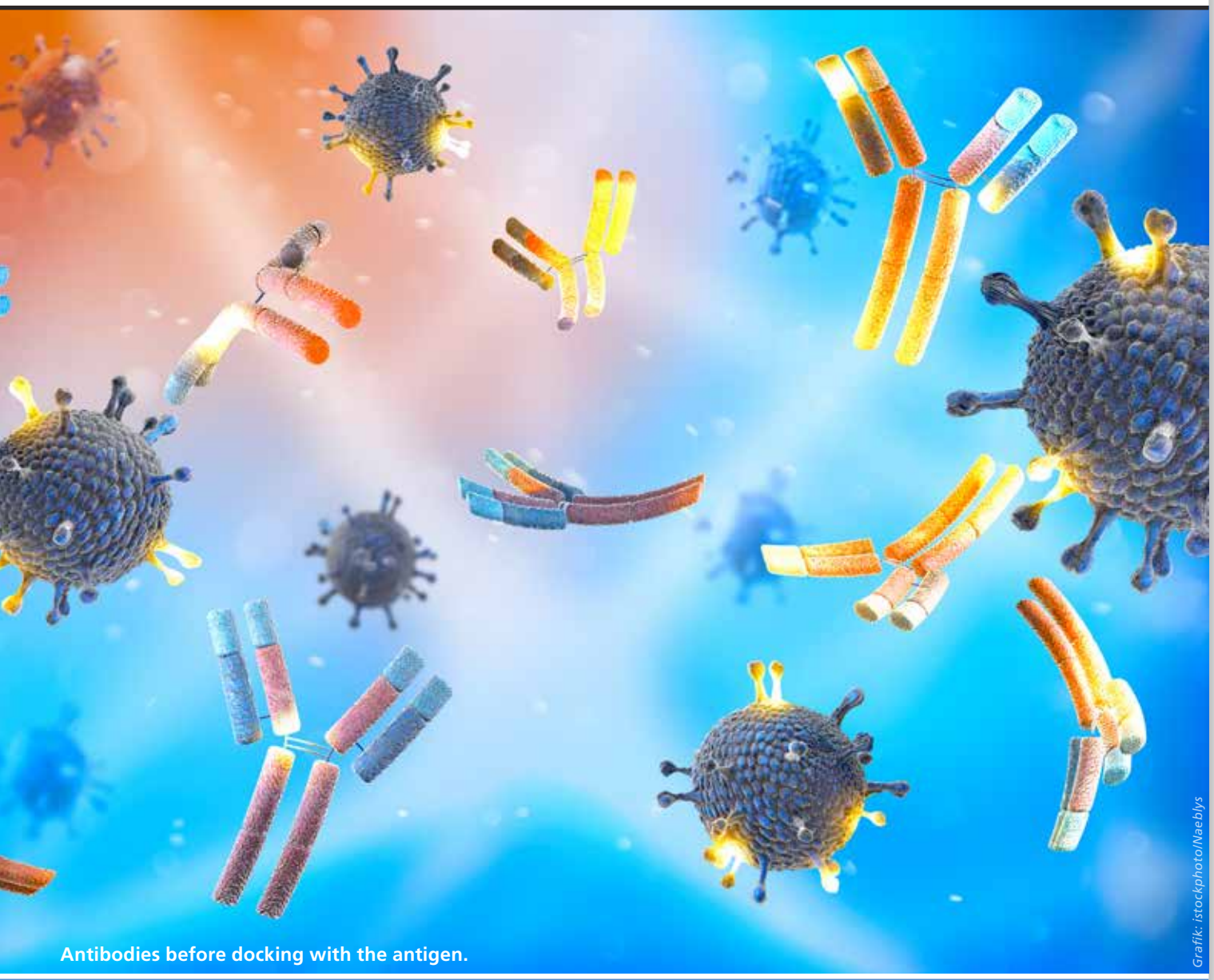
Replacement of the Year 2022



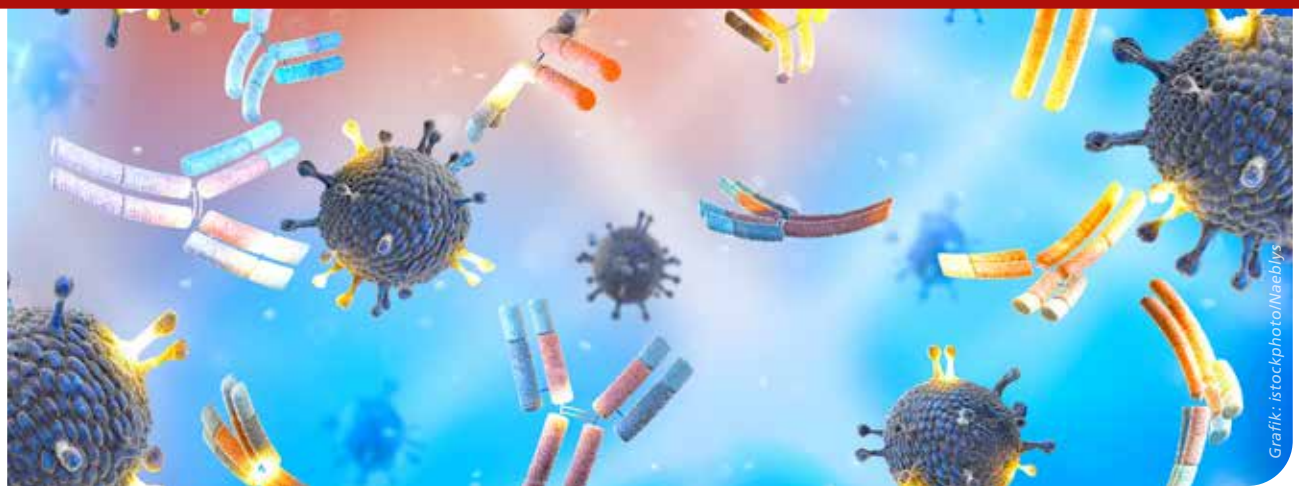
Antibodies before docking with the antigen.

Grafik: istockphoto/Naeblys

Phage Display instead of Animals in Antibody Production

Menschen für Tierrechte
Bundesverband der Tierversuchsgegner e. V.

Content



Replacement of the Year 2022:

Phage Display instead of Animals in Antibody Production

Abstract

Antibodies are proteins that are produced by certain cells of the immune system against recognized foreign substances. They are primarily used to defend against pathogens. They can rapidly recognize the structures unique to these pathogens and either mark the antigen for attack by other components of the immune system or neutralize the antigen directly by binding.[1]

These specific properties enable an ever-evolving range of applications. Therefore, antibodies are also produced for research, diagnostic or therapeutic purposes. Enzyme-linked immunosorbent assay (ELISA), fluorescence-activated cell sorting (FACS), Western blot, immunohistochemistry[2] and other immunological methods have become indispensable at today's laboratory work. However, mainly mice and rabbits are still used as live antibody producers.

Every year, nearly one million animals are used in the European Union for the manufacture and production of antibodies,[3] although there are technologies that do not require the use of animals. The procedure is associated with great suffering for the animals.

For years, non-animal methods have been available for the generation and production of antibodies, such as phage display technology. It is extremely versatile and can be used very efficiently to generate an almost infinite range of high-quality antibodies. Such antibodies produced by phage display are already widely used in various fields, including therapeutics.

The JRC's EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) issued a recommendation in 2020 urging end-users and other stakeholders to recognize the scientific validity of animal-free antibodies and to stop using animals for antibody development and production. However, the recommendation has been discussed controversially in the scientific community.

Introduction, Problem

Antibodies are becoming increasingly important in diagnostics and therapy. In so-called individualized medicine (e.g. cancer therapy), they are seen as a great source of hope. Antibody-based drugs are easy to recognize, even for the non-expert, by their name ending in -ab (for "antibody"). However, with the increasing demand for highly specific antibodies, animal welfare issues are also on the rise because antibodies are all too often still produced in animals.

Mice in particular, but also rabbits, are still used as live producers of numerous antibodies. They are not only infected with the foreign body but also administered with effective enhancers to achieve intensification and prolongation of the immune response. The antibody-producing cells are then isolated from the spleen and fused with malignant plasma cells (myeloma cells) to divide them indefinitely. In a particularly painful and distressing follow-up experiment, mice are injected with these constructs into the abdominal cavity, creating excruciating abdominal ascites from which the produced antibodies are extracted.

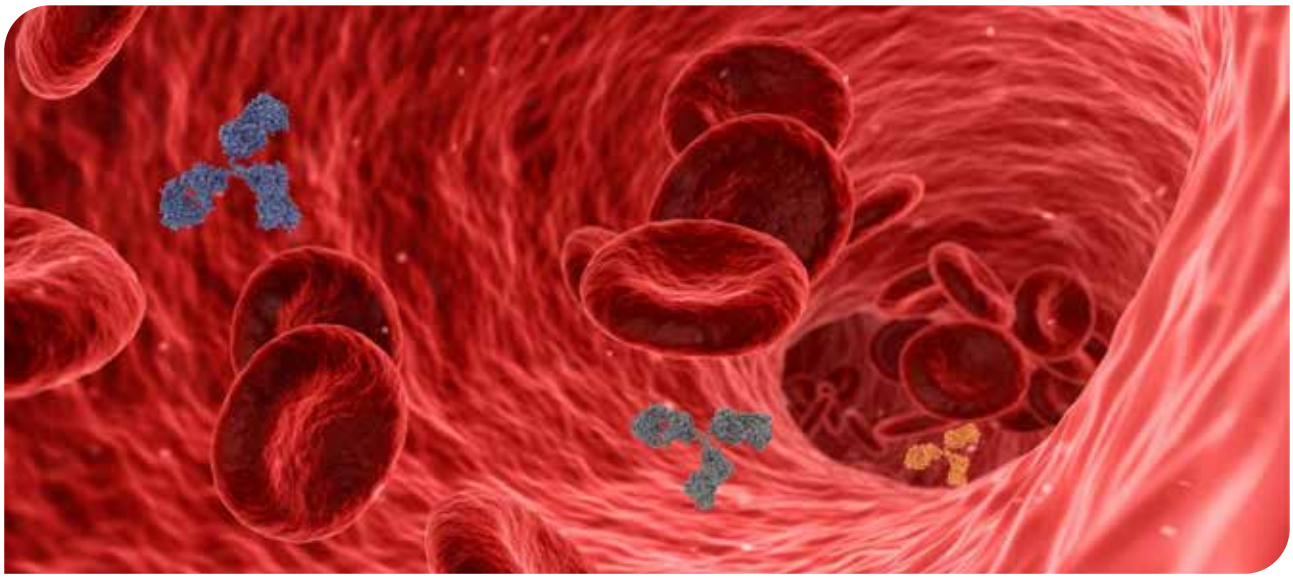
Since the late 1980s, researchers are already recommending to ban highly stressful experiments and using animal-free methods instead. But the animal experiments are still being carried out in the EU. In 2018 for example, almost 55,000 animals – mainly mice are used for this purpose – even though that there are powerful and reliable animal-free methods such as the phage display available in the meantime. In May 2020, the EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) of the Joint Research Center had published a recommendation that end-users and other stakeholders should recognize the scientific validity of animal-free derived antibodies and stop using animals for their development and production. Many animal-derived monoclonal antibodies used in research have been found to cross-react with other molecules, they said. Nearly one-third of the hybridomas randomly selected in one study contained one or more additional productive heavy or light chains. Such nonspecific antibody reagents in research wasted costs, time, and resources; the effects on diagnosis and health management are tremendous, it said.

The European Validation Authority estimates that researchers do not use animal-free antibodies frequently enough because of a tendency for existing methods, a lack of awareness of the current state of science, and scientific misunderstandings. Economic and contractual constraints and limited access to facilities kept them from producing such animal-free antibodies, it said.

Scientists, especially in basic research and immunology, criticized this initiative as too early. Meanwhile, researchers are working on the phage display of the future, developing animal-free techniques not only for monoclonal but also for polyclonal antibodies.

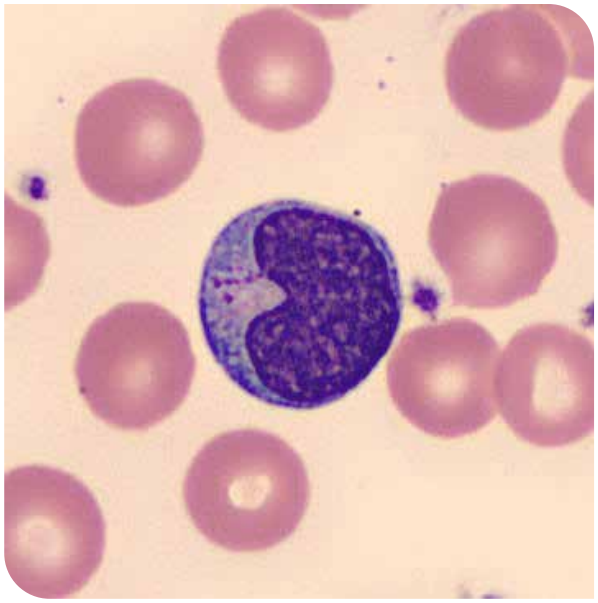
What are antibodies and what are they used for?

Antibodies (also: immunoglobulins) are proteins that are produced by certain cells of the immune system against recognized foreign substances. They are mainly produced for defense against pathogens.[4] The immune system defends the organism against antigens, i.e. foreign materials. For this purpose, it has an innate (non-specific) and an adapted immune system.



Antibodies are transported in a blood vessel to their target site. *Graphics: swiftsciencewriting, Pixabay*

While macrophages, granulocytes, monocytes, mast cells, and natural killer cells, as well as barriers such as the mucous membranes or the body's messengers, belong to the innate immune system, lymphocytes are cells of the adaptive immune system.[5] An intermediate type are the dendritic cells, which can belong to both the innate and the adaptive immune system.[6]



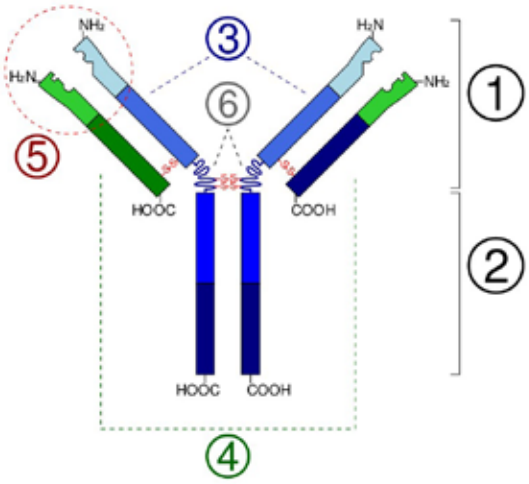
Lymphocytes are highly adaptable to new pathogens and have an excellent "memory" concerning already known pathogens.

Microscopic image of a lymphocyte (in blue-violet) in a blood smear, surrounded by erythrocytes and platelets.
*Photo: El*Falaf – Eigenes Werk, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=31217102*

In the beginning, the immune system reacts against surface structures of viruses, bacteria, or other foreign pathogens (antigens) by a certain type of immune cell, e.g. the dendritic cells. Such dendritic cells in the blood flowing through the spleen, find these particles and present them on their surface. As a result, so-called T lymphocytes are activated. T cells recognize the body's cells that have been infected by viruses and kill them.[7] Otherwise, T-lymphocytes can activate B-lymphocytes. The latter proliferate strongly then, transform themselves into plasma cells and create antibodies that bind to the pathogen structure thus preventing it from entering the host cell.[8] Antibodies can also act as a signal to activate other immune cells, such as phagocytes, which take up and digest the pathogen or natural killer cells that kill the pathogen. A good overview is provided by the page antibodies online.de.[9]

Antibodies are Y-shaped and consist of polypeptides composed of amino acids. The amino acids give each antibody its specific ability to bind certain antigens.

The two tips or arms are responsible for binding antigens and are referred to as the variable region, while the stem of the "Y" is referred to as the constant region and is the part that interacts with effector cells – differentiated lymphocytes with specific roles in the immune



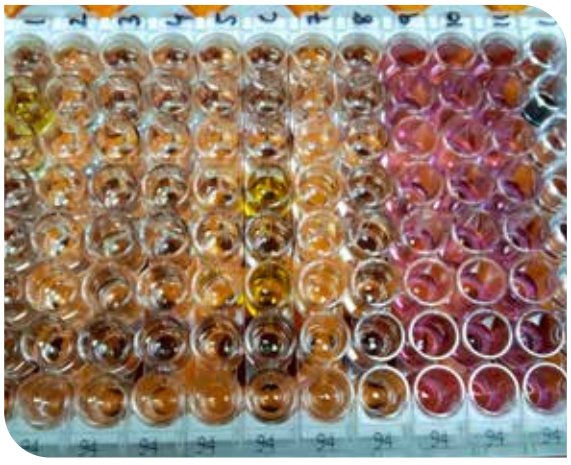
Structure of a typical IgG antibody: 1st Fab section, 2nd Fc section, 3rd heavy chains, 4th light chains, 5th antigen binding site (paratope), 6th hinge region, (*) -S-S disulfide bridge.

Graphik: Y tambe, Wikipedia (CC BY-SA 3.0)

Antibodies in research and diagnostics

Antibodies are also produced for research or diagnostic purposes. They are used for quantification (ELISA), localization in tissue sections, and purification (affinity chromatography) of molecules. Such methods and others are indispensable in today's laboratories.[9] However, animals like rabbits and mice are still used as live producers. An assay widely used in diagnostics is, for example, the ELISA, in which the antigen or an antibody is immobilized on a surface in a microtiter plate. The antigen or antibody is detected here, for example, by a fluorescently labeled or enzyme-conjugated secondary antibody.[12]

A distinction is made between monoclonal antibodies and polyclonal antibodies. Polyclonal antibodies are produced by different B cells of a host – usually an animal such as rabbit or mouse. As a group of antibodies, they target different molecular structures of a pathogen.[13,14] In contrast, monoclonal antibodies, which originate from a single B lymphocyte, bind highly specifically to only one epitope of a pathogen.[15,16]



A typical HBaAG test by Elisa procedure: The color change (red), for example, allows the detection of antigens or antibodies after having undergone a hepatitis B virus infection.

Photo: Md Ariful Islam, iStockphoto.

The monoclonal antibody is highly specific; since monoclonal antibodies detect only single epitopes, they cross-react less frequently with other antigens. However, in the case of a mutation of the antigen (e.g., a virus), they may not bind well or at all. Polyclonal antibodies represent the largest proportion of commercially available antibody reagents and are characterized by the recognition of multiple epitopes. They often have a high binding tendency (overall affinity) to the target antigens, are inexpensive, and can be produced quickly. However, they have the problem of varying in specificity from batch to batch.[17]

Traditional antibody production in rabbits and mice

In 2020, 56,996 rabbits (2019: 75,326) were used for routine production in Germany, which is required by law. Routine production means the production, extraction, storage, or propagation of substances, products, or organisms (according to Section 8a [1] No. 3 of the German Animal Protection Act, Deutsches Tierschutzgesetz).[18] Substances and products meant here are primarily monoclonal and polyclonal antibodies as well as immune sera. Primarily mice are used for this purpose, but also rabbits. The animals are treated according to an already known and proven procedure.[19]

Rabbits are social by nature. They require adequate space to be housed together with one or more social partners and need cage enrichment (hiding places and elevated areas). The cage size must allow at least one hobble, stretched outlying and sitting upright with the ears not touching the cage ceiling.[20]

Photo: anilbolukbas, iStockphoto

When producing monoclonal antibodies, traditionally a mouse or rabbit is first infected with this particular antigen. The activated B lymphocytes are isolated from the spleen and fused with degenerate plasma cells (myeloma cells) that can divide indefinitely.[21] The resulting cell is called a hybridoma.[22,23] Clones with specific and high-affinity antigen recognition are then selected from the hybridomas and the antibodies characterized. The animals are anesthetized, e.g., subcutaneously, and the largest possible blood volume is collected via the anterior vena cava.[24] At the end of the production, the animals are usually euthanized. Rabbit-derived antibodies produced in this manner are favored by some scientists because the animals' antibodies have better antigen recognition than those of mice. They are thought to have more effective mechanisms for generating greater antibody diversity, and their immune systems exhibit optimal antibody affinity, compared to those of mice and other rodents.

The higher binding affinity of rabbit antibodies compared to mice is thought to provide a better signal-to-background ratio for display in diagnostic applications and research.[25] Nevertheless, a great number of antibodies are generated in mice.

Adjuvants

To enhance the effect of antibody generation and to achieve the desired results more quickly, so-called adjuvants are used. The adjuvant causes an amplification and prolongation of the immune response, e.g. by attracting various immune cells to the antigen and a delayed release through depot formation. By adding a suitable adjuvant, the amount of antigen to be used and/or the number of immunizations can be reduced.[26,27]

Adjuvants are particularly stressful for the animal in both monoclonal and polyclonal antibody production. Often the so-called Freund's adjuvant is added. By causing local tissue irritation and destruction, the adjuvant is classified as relevant to animal welfare. Adjuvants can cause side effects of varying severity depending on the type of antigen, animal species, injection site and volumes, such as granulomas, abscesses, fistulas, ulcers, necrosis, peritonitis, or fatal emboli.[28] A distinction is made between complete and incomplete Freund's adjuvant. In complete adjuvant, the animal's immune system is stimulated by kerosene oil, mannose monooleate as an emulsifier, and destroyed mycobacteria. The incomplete Freund's adjuvant also has all these components except the bacteria. Thus, it is less stimulating.[26] The use of complete Freund's adjuvant is classified as an experiment causing serious impairment of the welfare or general condition of the animals.

Criticism of animal-derived antibodies

There is much criticism of how antibodies are produced in animals, but also of their properties. For example, polyclonal antisera from the blood of immunized animals are only available in limited quantities – once an animal's serum has been used up, the experiments performed with it can never be reproduced. In addition, the immunoglobulins (antibodies) contained are always unknown mixtures, which can lead to a risk of undesirable side reactions.[29] Since an antigen usually has different epitopes, antibodies are also produced against the different epitopes, when an animal is immunized. The proportion of desired antibodies in the total pool of antibodies is at most 10% and is often very much lower.[30]

In addition, unlike animals, humans are unable to produce the sialic acid N-glycolylneuraminic acid (Neu5Gc), which is typically found in antibodies produced by mouse cells and has been linked to immunogenicity in humans.[31] Neu5Gc is also found in pork and beef and has been linked to heart disease and cancer in humans.[32] Manufacturers are therefore encouraged to avoid using materials of animal origin. Monoclonal antibodies derived from hybridoma cells also have drawbacks. In addition to being costly and time-consuming to produce, monoclonal antibodies are often not so monospecific, as the hybridomas may be genetically heterogeneous and thus produce antibodies of varying specificity.[29]

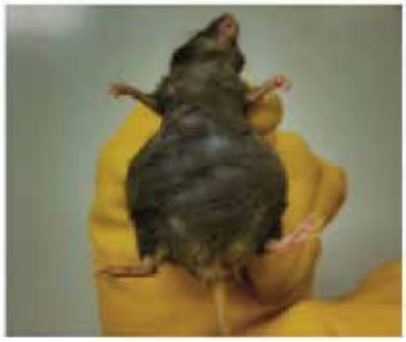
The ascites mouse: in (exceptional) cases allowed despite the high burden

In the ascites method, hybridoma cells are injected into the abdominal cavity of a mouse where they induce abdominal dropsy (ascites) producing tumor.

Between 2015-2018, a total of 176,232 animals were used in the European Union to produce monoclonal antibodies by the mouse ascites method. The EU competent authority recorded an increase of 22% between 2017 and 2018. In Germany itself, more than 7,000 animals were used between 2015 and 2020, mainly mice but also rats and rabbits.

The ascites formation is associated with significant pain and suffering for the animals. The monoclonal antibodies are released by the tumor cells into the peritoneal fluid. To isolate the antibodies, the fluid is collected by a puncture after a certain time. As early as the 80s/90s, scientists campaigned for a ban on this torturous procedure.[33] As early as 1992, scientists were able to produce monoclonal antibodies and hybridomas in vitro from human cells.[34,35,36]

In 2001 and 2003, the legal admissibility of monoclonal antibody production with the ascites mouse was justified in the German Animal Protection Report of the Federal Government. Three so-called "exceptional cases" were repeatedly cited in German and European politics in the following years:
Animal experimentation would be scientifically justified in rare cases when there is an exceptional need for a therapeutic emergency application, there is an existing regulatory approval for a diagnostic or therapeutic in vivo antibody or there are very exceptional circumstances that make it impossible to produce the antibody in vitro.[37] In 2003, the government cited the example of "rescue" of hybridomas when they no longer grow in cell culture or when they are infected.[38] In 2003, the government presented the example of "rescue" of hybridomas, when these no longer grow in cell culture or when they are infected.[38] In this context, the production of recombinant antibodies has been recognized since 2007 as a valid replacement method by the Central Agency for the Registration and Evaluation of Replacement and Complementary Methods to Animal Experiments (ZEBET 299), but is not perceptibly used for various complex reasons.



This is what a mouse with ascites looks like.

From: Guo, T., et al. Oncology Letters 13(3), 2017. DOI:10.3892/ol.2017.5652.
CC BY-NC-ND 4.0

In 2008, Green Party MEP Jens Holm pointed out that the severe pain and stress the mice had to suffer was in no way covered by the EU Animal Experiments Directive in force at the time. He stressed that several in vitro methods were already available for the production of monoclonal antibodies and suggested that the ascites procedure with animals should be banned throughout the Union, especially since, according to his information, the several EU Member States, including Germany, wanted to officially phase out the use of the mouse ascites procedure.[39]

In his response, Environment Commissioner Stavros Dimas indicated in 2009 that the Member States themselves were responsible for enforcing the measures set out in Directive 86/609/EEC. According to the EURL ECVAM Scientific Advisory Committee (ESAC), the in vitro production of monoclonal antibodies is "scientifically acceptable" and "practically available", and therefore, except in rare cases, the production by the mouse ascites method is no longer scientifically necessary. Only a few countries have banned mouse ascites completely, including Switzerland and the Netherlands.[19]

In 2017, 5% of all animal testing in the EU was performed for routine production. Of these, 55% involved the production of blood-based products, but 10% also involved the production of monoclonal antibodies using the ascites method.[40] Other product types, which accounted for 35% of uses, were mostly related to antigen and protein production.

According to ALURES, the European database of non-technical project summaries of EU member states, 54,941 animals were used in routine production for the production of antibodies by the ascites method in 2018, almost all of them were mice.[41] In previous years, rats and rabbits were also used to a lesser extent for this purpose. Between 2015 and 2017, the production of monoclonal antibodies by the mouse ascites method recorded a 66% increase. The severe pain and stress-inducing animal tests were carried out primarily in France, followed by Germany, Spain, Italy, Hungary, and the Czech Republic.[42]

Since the end of the 1980s/beginning of the 1990s, there has been much criticism to end this animal experiment, especially since other methods were already available – with limited success.

Legal basis

According to the regulations of the European Animal Experiments Directive 2010/63/EU, the 3R principles (Replacement, Reduction and Refinement) should be applied in the manufacture and control testing of medicinal products. This also applies to the production of antibodies. This means that animal testing for scientific purposes should only be considered if there are no non-animal alternatives (Recital 12 and Article 47, paragraph 1).[43]

The legal landscape governing the production of monoclonal and polyclonal antibodies is confusing and cannot be fully described here.

The guideline of the World Health Organization (WHO), for example, does not prescribe a procedure and mentions, among other things, the preparation of monoclonal antibodies and related proteins using the ascites procedure with mice. However, the WHO advises against the use of vivo production methods for the manufacture of human therapeutic products, if feasible.[44]

Similarly, the European Pharmacopeia does not prescribe a procedure. It states that, if monoclonal antibodies for use in humans are produced in animals, in addition to the conventional safety assessment, it must also be guaranteed that the antibodies are virus-free, including, for example, spongiform encephalopathy prions.[45] Accordingly, antibodies can be produced in animals or genetically engineered without animals.

After the antibody has been produced, its safety must be ensured, e.g., based on the safety guidelines (Guidelines) of the International Conference on Harmonisation (ICH).[46] Unintended reactions or cytotoxicities of a monoclonal antibody against human tissues are nowadays at least already checked by appropriate immunohistochemical procedures with human tissues. However, this process also involves an antibody that must have been produced according to a specific procedure. Due to the biological activity as well as species and/or tissue specificity of many biotechnologically produced reagents, standard toxicity tests with e.g. rats and dogs are out of the question. However, ICH Guideline S6(R1) considers animal species relevant for monoclonal antibodies where the test material is pharmacologically active due to the expression of the appropriate receptor or epitope (in the case of monoclonal antibodies) and whose tissues have a similar cross-reactivity profile to human tissues. This sounds very much like genetically modified (humanized) mice.

In addition to the safety guidelines, the ICH quality guidelines also play a role (Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products Q6B).[47]

However, many biotechnologically produced medicinal products intended for human use cause allergic immune reactions in animals. Guinea pig anaphylaxis tests are frequently performed. However, induction of antibody formation in animals is not indicative of possible antibody formation in humans. Therefore, such studies are considered little valuable for routine evaluation of this type of product. For monoclonal antibodies and other related antibody products directed against foreign targets (i.e., bacterial, viral targets, etc.), a short-term safety study (see ICH Guideline S6) in an animal species to be justified could be considered; additional toxicity studies, including reproductive toxicity studies, are not appropriate.

However, monoclonal antibodies can be generated not only in animals, but also by recombinant DNA technology (rDNA) or, for example, display technology. Scientists are already working on the possibility of producing polyclonal antibodies without using animals.

There is another way: Antibody generation without animals

Recombinant antibodies can be generated without animals from non-immunized (naïve) donor B cells, from fully synthetic universal antibody libraries, or by cloning the antibody DNA from human donor B cells (e.g. from a patient after an infection).

Recombinant antibodies

Recombinant antibodies are produced using genetic engineering methods. For their production, the gene sequences of essential structures of the antibody (so-called heavy and light chains) are isolated and linked to each other via a synthetic connecting protein ("linker"). The genes modified in this way are expressed in Escherichia coli bacteria, yeasts, eukaryotic cells, or even in transgenic plants. This method is also used to produce biopesticides, for example.[48] Recombinant technology makes it possible to produce antibody molecules against theoretically any antigen without having to immunize an animal.[49]

Phage Display

Antibodies not derived from animals can be selected in vitro from large antigen libraries mainly by phage and yeast display, less frequently by ribosome, bacterial or mammalian cell display. Protein synthesis (amplification) is then usually performed in bacteria. This method can be used to produce large quantities of antibodies of consistent quality.[17]

John McCafferty, Sir Gregory Winter and *David Chiswell* are the founders of Cambridge Antibody Technology. *John McCafferty* is one of the inventors of the so-called scFv antibody fragment phage display, which has advanced the discovery of monoclonal antibodies. The method was developed after antibody production in mice had failed.[50,51,52]

In 2018, *Sir Gregory Winter* and *George Smith* received the Nobel Prize in Chemistry for their development. This versatile technique has been strongly modified over the last three decades, leading to the development of various platforms for combinatorial peptide display.

A phage display library contains an extensive repertoire of peptides or proteins, expressed as recombinant molecules on the surface of genetically modified bacteriophages.[53]

Animal-free recombinant antibodies (multiclonal) can even already be used for multiple epitope recognition where animal polyclonal antibodies have traditionally been used.[17] An "immortal" and inexhaustible antibody biobank of recombinant antibodies against any human protein is already technically feasible today – and offers another important advantage besides the complete avoidance of animal testing and the use for therapeutic purposes: no expensive liquid nitrogen is needed for their storage as with hybridomas – the E. coli clones only need a freezer.[54] Therapeutic antibodies against COVID-19 have also been successfully developed.[55,56] Why has the phage display not become more widespread and become a matter of course? The reasons probably lie on the one hand in the historical development of the technology preferentially in the strongly patent-regulated pharmaceutical field as well as – on the other hand – in the enormous diversity of the fields of application – for each one a demonstration example has to be presented to convince colleagues, estimates *Prof. Stefan Dübel*, head of the Department of Biotechnology at the Technical University of Braunschweig. He initiated the "Antibody Factory" of the German National Genome Research Network and is co-founder of several biotech companies, including the animal-free antibody company Abcalis and Yumab, which develops therapeutic antibodies for humans. He has been involved in numerous innovations in human antibody development, phage display, and hyperphage technology.[54,57]

In vitro immunization

In addition to the production of antibody prototypes with bacteria and bacteriophages, such prototypes can also be produced with human immune cells in the petri dish. In the process known as in vitro immunization, human B lymphocytes, for example, are stimulated in vitro to form specific antibodies. A Potsdam research group first activates human antigen-presenting cells (dendritic cells) with the desired antigen and then cultivates them together with antigen-naïve T and B lymphocytes. After several days of cultivation, specific antibodies are found in the culture medium. These are isolated and purified.[58] However, the research group working on this replacement method points out, that further research is needed since natural processes such as affinity maturation must be realized in the organism under in vitro conditions.[59]

Affinity maturation refers to numerous mutations in the genes of antibodies (called somatic hypermutation), which increases the attraction of lymphocytes to the corresponding antigen and makes the immune response more effective. It occurs in mature B lymphocytes within the medulla of lymph nodes.[60]

Critics note that with bacterial expression, lack of binding of a carbohydrate (glycosylation) or improper spatial folding could sometimes prove problematic. Antibodies generated from gene libraries also sometimes show poor storage stability, without the reasons being clearly understood to date.[30]

Example: Using phage display to improve treatment of diphtheria

In a project funded by the PETA International Science Consortium Ltd., scientists from the Institute of Biochemistry, Biotechnology and Bioinformatics at the Technical University of Braunschweig, together with the British National Institute for Biological Standards and Control (NIBSC), have developed human antibodies that are capable of neutralizing the diphtheria toxin.

Human recombinant antibodies that can neutralize the diphtheria toxin were generated without the immunization of animals using the antibody phage display of the TU Braunschweig. A combination of the antibodies that best neutralize the toxin is a promising candidate for

further clinical development to replace diphtheria antitoxin (DAT) from horse serum in the future. The results were published in the journal Scientific Reports and commented on in an article in the journal Science.[61]

European validation authority recommends animal-free antibodies

In May 2020, the EU Reference Laboratory for Alternatives to Animal Testing of the Joint Research Center (EURL ECVAM) had published a recommendation recommending end-users and other stakeholders recognize the scientific validity of animal-free antibodies and stop using animals for the development and production of antibodies. The recommendation was based on the opinion of the Scientific Advisory Committee (ESAC) of EURL ECVAM.[17]

It has been criticized that many animal-derived monoclonal antibodies used in research cross-react with other molecules. Nearly one-third of the hybridomas randomly selected in a study contained one or more additional productive heavy or light chains (see graph on page 6) that compromised specificity or sensitivity (Affinity). Non-specific antibody reagents in research wasted costs, time, and resources, and the negative effects on diagnosis and health management were tremendous, they said. The costs of producing antibodies from an antibody library are comparable to those of the production of monoclonal antibodies by immunization in animals, but there is a time advantage (several months versus a few weeks using a recombinant library), they said.

Currently, animal-free antibodies would not be used more frequently because of a tendency to use existing methods, a lack of awareness of the current scientific status, scientific misunderstandings, economic and contractual constraints, and limited access to facilities that produce such molecules.

Therefore, government agencies, funding agencies, and scientific publishers were recommended to advocate animal-free antibody production and use. EU governments should also provide subventions to antibody producers or customers who require customized antibody production.

Pharmaceutical industry: transition period of more than 10 years necessary – hybridome replaceable

The European Animal Research Association (EARA) and the European Federation of Pharmaceutical Industries and Associations (EFPIA) appreciate the forward-looking approach of EURL ECVAM, but consider its recommendation to be premature, especially in the area of vaccines and therapeutics. EARA/EFPIA is rather convinced that the existence of multiple methods for antibody generation – in vivo or in vitro – complement but do not replace each other.
The rationale: there would be technological, scientific, and regulatory issues that would first need to be addressed so that the methods can fully replace animals in all areas, where antibodies are used. Any switch from animal-generated antibodies to non-animal antibodies should only take place once it has been proven that the technology is mature enough. On the one hand, the current technology and infrastructure are not suitable for large scale. On the other hand, the biomedical sector must be able to ensure that the technologies for producing animal-free antibodies have reached the necessary development and maturity degree to guarantee antibodies of consistent quality and quantity, which is currently not yet the case. Post-translational modification is an enzyme-dependent process in which an endogenous protein that has just been formed is altered by attaching a molecule. This can change the properties of the protein. It is one of the reasons why immunologists favor animal testing. They argue that such modification is difficult to accomplish in vitro.

They also argue that immunization of rabbits is still the best choice for producing high-affinity antibodies against haptens. (Haptens are molecules or ions that are not capable of eliciting an immune response on their own, but only when they bind to an endogenous carrier protein).

For research purposes, the majority of antibodies would be derived from animals. Their elimination would mean a shortage of available research tools in Europe and worldwide. EFPIA and EARA, therefore supported investment in expanding the supply of such tools, including, for example, the recombinant production of antibodies that are currently still derived from hybridomas. EFPIA and EAZA also advocate a science-based transition period that exceeds 10 years.[62]

Outlook

Engagement to end animal testing for the production of monoclonal antibodies has been in place since the 1990s. Development has progressed steadily over the decades. However, it is clear from the discussions that there is still a lot of persuasion work to be done to achieve acceptance of the new process.

Not so long ago, the commercial use of the phage display was limited to a few selected biopharmaceutical companies that owned the intellectual property rights of the process. Thus, the rights to most of the monoclonal antibodies approved or in clinical trials that were derived from phage display libraries also belonged to commercial companies. However, most of the important patents for phage display technology in Europe and the United States have now expired. Therefore, the expiration of patents should encourage academic and biotechnology start-ups to develop their libraries to develop more antibodies for clinical use.[63]

The importance of phage display is not ignorable

The use of the phage display in synthetic biology, immunotherapy, diagnostics, development of bioassays and biosensors, drug discovery, and others demonstrates its reliability and reproducibility. For example, the phage display has also provided deep insight into the protein interactions involved in the pathogenesis of SARS-CoV-2 and has driven the search for potential antibodies for other human and animal coronaviruses as well. High-affinity antibodies or small molecule therapeutics against viral proteins of SARS-CoV have already been identified.[64] The recent breakthrough in antibody drugs is largely due to the contribution of phage display technology. In addition to the antibodies mentioned above, bi-specific antibodies that recognize two antigens in a single antibody format are currently being commercialized.[65] Phage display also plays an important role in cancer immunotherapy, in which peptides derived based on of this process mimic cancer antigens (mimotopes) therefore enabling effective antibody therapy against cancer.[66] Due to the great potential, technological boundaries of the technology are continuously being pushed.[64]

Concerning the positive recommendation of EARA and EFPIA, the production of antibodies with hybridomas should be replaced as a first step. In a second step, the still missing performance of the phage display could be extended by special support of the development and thus the animal experiment could be replaced step by step in the next years.

Why should it not be mandatory to work with the phage display in areas of application that are already feasible today?

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