Transgenic Animals

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This paper is meant to investigate the application of gene transfer in order to create transgenic animals and the ethical dilemma that arises from it.

CHAPTER 1: PREFACE

1.1 Motivation

The first time we have learned about transgenic animals was at our teacher‘s presentation about genetic engineering and it was quite fascinating to learn about both the wide applications of transgenic animals in various fields of research and pharmacology and the ethical question that such a drastic interference into an animals life and health raises.
With our proceeding research and the interview with a professional we wish to learn more about the techniques of gene transfer as well as gaining a satisfying answer to the ethical dilemma that science is facing.

CHAPTER 2: INTRODUCTION

2.1 What is the context of the chosen topic? Are there any recent events?

Transgenic animals are used for relatively various purposes that can be divided into three major categories of which the first one aims at gaining information on gene functions and regulations as well as human diseases. The second category aims at creating high value products (e.g. with transgenic livestock for the production of medical substances) that can be used for human therapy. The third category aims at the improvement of animal products that are used for consumption by humans. Since over 95% of transgenic animals are rodents of which the most often occurring rodents are mice, this paper focuses mainly on the first and the second category dealing with the usage of transgenic animals in the medical sector.
With regard on the first of the three categories mentioned in the paragraph before, transgenic animals can be seen as important tools for researching on human diseases. They help understanding gene function and therefore gaining knowledge about how prone which people are to suffer from a certain disease, how a disease will develop once a person suffers from it, and to determine what happens if therapy against the disease is started.
Besides that, transgenic animals can be a tool in the production of pharmaceutical products. Especially mice are of great use in that sector. They are genetically modified to produce human antibodies or to produce antibody drugs. Seven out of eleven antibody drugs released between 2006 and 2011 were derived from transgenic mice.
However, not only rodents such as mice are genetically modified but also farm animals whose blood, milk or eggs can be used in drug production when genetically modified. These animals can deliver recombinant (relating to or denoting an organism, cell, or genetic material formed by recombination) proteins that help to treat human diseases. While mice have been used the most successfully as transgenic animals, the development with farm animals has been slow up to the present time. There are currently few biomedical products that are approved for use in the medical sector. One of these is human antithrombin III which is a protein that can be produced with the milk of genetically modified goats. The protein can be used to prevent clots in patients with hereditary antithrombin deficiency, which is a disease resulting in a lack or in a shortage of

antithrombin in the blood. Since the protein is produced with the milk of transgenic goats, these animals can be seen "living pharmaceutical factories" for the production of substances in large amounts that otherwise would be rare. For example, a relatively low number of transgenic goats (about 80) is needed to cover the entire demand for antithrombin of Europe.
A second example for such biomedical products approved for medical use is a recombinant human C12 esterase inhibitor. It is produced in the milk of transgenic rabbits and is used to treat hereditary angioedema, which is a genetic disorder that can cause blood vessels to expand and lead to swellings of the skin.

2.2 Discovery of the Transgenic Animals

There are two components of highest importance for the development of the production of transgenic animals. The first of these two components is the ability to transfer embryos. The second one is the ability to manipulate them. Techniques to conduct these processes were discovered and used between 1891 and the 1940s. The first genetic modification of animals, however, is reported in 1974. The virologist Rudolph Jaenisch and the mouse embryologist Beatrice Mintz demonstrated that it is possible to modify genes in mice by injecting the SV40 (Simian-Virus 40) into mouse embryos in an early stage of development. The modification of the genes became detectable since all of the mice that resulted from that experiment carried the gene in all of their tissues.

2.3 Where and Why Are Transgenic Animals Used?

Experiments like the one described in chapter 2.2 were the foundation for the production of transgenic animals. Since the mid-1980s, transgenic mice became a useful tool to understand the progression of cancer as well as a powerful tool to get insights into experimental drugs.
There are different reasons why mice are particularly useful. One major reason is that their genome is similar to the human genome. Aside from that, the physiologic and behavioral traits of mice can be extrapolated ("translated") to human diseases. Other reasons are high practicality (it is relatively easy to manipulate the cells and embryos of mice) and the comparatively short reproduction cycle of mice.

2.4 Are There Alternative Treatments?

Not only mice can be used for research on diseases and experimental drugs but also sheep, pigs, goats, and rats. Aside from the goat, the usage of these animals is limited due to technical constraints (restrictions).
However, there are other procedures (e.g. in immunology), which replace transgenic animals in vivo research with in vitro experiments, where immune cells are isolated (e.g. in a petri dish) and then cultured.
In addition to that cell lines are used, although they come with many disadvantages. Therefore, the results have to be interpreted with high caution, because they do not always reflect physiology.
Much more precise experiments can be conducted with the application of newer tools like CRISPR/Cas and other genome engineering systems.
For all these processes, no transgenic animals need to be created, however these experiments are mostly used to complement in vivo research and cannot replace the use of transgenic animals in many cases. In most applications however a balanced mix of transgenic animals, human tissues, computer modeling, and genome engineering systems is attempted.

CHAPTER 3: DESCRIPTION OF ENGINEERING TECHNIQUE

3.1 Explanation of the Applied Technique (Explanation of How Transgenic Animals Are Created)

There are currently three main methods of creating a transgenic animal, that are DNA microinjection, embryonic stem cell-mediated gene transfer and retrovirus-mediated gene transfer:

a) DNA microinjection:

Historically the earliest applied technique (Gordon and Ruddle, 1981) is DNA microinjection, where a single gene or a combination of genes is transferred from one specimen to the fertilized ovum of another that may be from another species.
The introduced genes can then influence the expression of some of the specimen's genes, however this is a delicate process that has a lot of randomness involved and cannot produce consistent results. The ovum is then placed into the oviduct of a female that has been prepared to be a recipient by mating with fertilized males.
Even with its low success rate and its unpredictability it is the most widely applied technique mostly due to its technical simplicity and the wide range of species it can be applied to.

b) Embryonic stem cell-mediated gene transfer:

This method relies on the insertion of the desired DNA in a vitro culture of stem cells ("basic" cells that can turn into any type of cell) that are then inserted into an embryo. The result is a chimeric animal (made up of genetically different cells). The method is most commonly applied to provoke a precise desired mutation via homologous recombination and works particularly well with mice.

c) Retrovirus-mediated gene transfer

Here a retrovirus (virus that has single-stranded RNA as its genetic material) is used to introduce the desired genetic material into a carrier's cell that in return enables these to infect new host cells. Granted the retrovirus integrates into a germ cell, this method aims to produce a chimeric animal as well.

All these methods have a fairly low success rate, if they happen to succeed however and the treatment does not lead to an abortion, the specimen are then tested for the transgene and are inbred until their genome is homozygous.

CHAPTER 4: DOCUMENTATION OF THE INTERVIEW

4.1 How We Found Our Interview Partner

During our search for interview partners, we found an article of the University of Basel titled "Efficient genetic modification of immune cells" from April 2018 dealing with a new method developed by Prof. Dr. Lukas Jeker and his Team that "enables genes in living T-cells in mice to be modified quickly and efficiently". Lukas Jeker is

a professor of Experimental Transplantation Immunology and Nephrology at the University of Basel. He is also active in the University Hospital of Basel, working in the department of Transplantation Immunology and Nephrology. We contacted him and sent our questions as a document. The main topic of it were the details of the modification of genes in animals, alternative procedures, and the ethical dilemma raised by the usage of transgenic animals. Both our question document as well as the answers provided by Prof. Dr. Jeker appear in the appendix of this report.
The answers given by Prof. Dr. Jeker will also be the main information source for the discussion in chapter 5.

4.2 Summary of the Information Provided by Prof. Dr. Jeker

The interview contains eleven questions of which we marked two as optional by putting these questions (No. 9 and 10) in brackets. Despite this, Prof. Dr. Jeker answered each question.
The first four questions focus on the application of transgenic animals. The answers contain a detailed description of what animals Prof. Dr. Jeker and his team use in their work and how these animals are used in their research. Alternative treatments, their advantages and disadvantages are also part of this section of the interview.
The following questions 5 and 6 deal with the detailed advantages and disadvantages of the usage of transgenic animals and also thematize the ethical dilemma regarding their use. This also includes a description of the criteria for the approval of experiments with transgenic animals.
In the following section (questions 7-10) Prof. Dr. Jeker describes how genes of an animal are changed, the difficulties of the procedure, how the success of creating a transgenic animal can be detected, and what happens with animals that are part of unsuccessful experiments.
The last section (question 11) deals with the progress that has been achieved recently using transgenic animals.

CHAPTER 5: DISCUSSION

5.1 What Progress Was Made with the Application of Transgenic Animals and What Future Research Steps Can Be Expected?

According to Prof. Dr. Jeker a lot of the fundamental understanding of mammals had been gained by working with various animals such as mice, rats or zebrafish. He also points out the enhancements in immunology:
"*In immunology it is important to study what happens in the entire organism since the immune system is distributed throughout the body. In contrast to solid organs like the heart, there is no single "immune organ". Genetic ablation of a gene in the entire organism can lead to unwanted side-effects if the gene affects multiple cells or if the gene is important during embryonic development.*"
Thus, the need for living organisms derives from the complexity of the immune system
"*with multiple organs combined with many different specialized cell types and soluble molecules as well as interactions with cell types not considered immune cells*".
Such a complex system cannot be mimicked in a petri dish.
In conclusion, transgenic animals can be considered a powerful tool that has enabled numerous advances of immunology in the past few decades, by allowing much more precise research and by providing the possibility

to create animal models of human diseases. This is further illustrated by the importance of such models during the SARS-CoV-2 pandemic:
"*In a very short time we learned to understand the disease (COVID-19) much better and vaccines and treatments (e.g. neutralizing antibodies) were developed and tested. In addition, cellular therapies are currently being translated to clinical use. Such treatments are based on decades of animal research.*"
Therefore, transgenics are likely to be a major part of the future of immunology.

5.2 Discussion of Ethical Aspects, Advantages and Disadvantages

One of the most important advantages of transgenic animals is the precision they enable in biomedical research. According to Prof. Dr. Jeker, an example for this precision are mice which are the animals exclusively used in his research since the genetic tools for work with mice allow very precise questions and answers. In addition to that, the murine immune system is very well characterized and there are numerous immunologic tools available. Besides, that there are many preclinical mouse models.
The great variety of tools available and the precision they offer make research with transgenic mice very well reproduceable:
"*Some mouse strains exist as inbred strains whereas humans are generally outbred, i.e. genetically diverse*", Jeker explains.
With that it came to "standardization" of genetic backgrounds which leads to a reduction in the variability between experiments, resulting in a better reproducibility. In an outbred situation, a higher number of individuals would be required for statistical reasons which would cause additional effort.
However, work with transgenic mice also inherits disadvantages such as high costs and vast time expenses. Jeker especially highlights the long periods of experiments with transgenic mice:
"*It can take years to generate a new genetic mouse model, then months to years to characterize it and additional years to pursue scientific questions.*"
Furthermore, strict regulations increase the efforts. To conduct experiments, the scientists need to absolve continued special training including theoretical and practical courses to be allowed to work with animals. Aside from that, detailed planning is needed to receive approval. The experiments need to be outlined beforehand and applications are required to be written.
This includes developing procedures that limit the inconveniences for the specimen as much as possible. To reach that goal, strategies like following the so called 3R are taken into account. The 3R stand for "Reduce, replace, refine". "Reduce" means that the number of animals needed and lastly used in an experiment should be reduced to the lowest number possible. "Replace" refers to the ambition to try to use alternative methods whenever possible and "refine" aims at developing experiments that cause a minimum of suffering. Developing experiments that meet these criteria, explaining them in detail, estimating how intense the stress for the animals will be and comparing their suffering with the expected gain of knowledge can end up in writing papers consisting of dozens of pages. Along with the time required to prepare experiments with this level of detail, the time between the submission of the application and its approval can be a period of several months.
In summary it is not possible to ignore that the usage of this method leads to suffering for the affected animals. But to receive approval for an experiment, very detailed planning is needed and the suffering of the animals has to be reduced to a minimum. Combined with the importance of these methods for disease-relevant

research that can lead to a reduction of suffering for patients, the usage of transgenic animals can be seen as justified. The progress in immunology (e.g. in the SARS-CoV-2 pandemic) achieved by these methods can be seen as a proof that a the monetary expenses as well as the time consumption for this kind of research turn out to be worthwhile investments since they offer invaluable advances for public health and therefore for the entire society.

CHAPTER 6: SUMMARY OF THIS PAPER

While writing this paper and during the corresponding research we learned about the genetic modification of animals by different techniques such as DNA microinjection, embryonic stem cell-mediated gene transfer, and retrovirus-mediated gene transfer. We interviewed Prof. Dr. Lukas Jeker to gain further insights into the techniques used to create transgenic animals. Furthermore, the interview enabled us to gain more information about the application process and the approval of experiments in context with the ethical dilemma that transgenic research poses. Further on we used the acquired information to discuss recently achieved progress and ethical aspects in more detail and find satisfying answers.

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APPENDIX

Question for the Interview and Answers:

1. **Which transgenic animals did you work with so far and for what purpose?**
   Mice.
   Purpose: My group is interested in immune regulation with a particular focus on T cells. We aim to understand how T cells are regulated on a molecular level. Specifically, we are studying microRNAs, i.e. small regulatory RNAs that fine-tune expression of other genes posttranscriptionally. We use genetic models such as the Cre/Lox system in order to delete or overexpress genes (e.g. microRNAs) specifically in T cells. These advanced technologies allow us to decipher genetic networks in great detail. We also use transgenic mice that harbor cells that express fluorescent proteins (e.g. GFP) in order to track cells after transplantation.
2. **What animals could have been used aside from those that you used?**
   For animal experiments we exclusively use mice since the available genetic tools allow very precise questions and answers. In addition, the murine immune system is very well characterized, there is a plethora of immunologic tools (e.g. antibodies) and many preclinical mouse models exist.
3. **To what extend does the use of transgenic animals ease the research around immunology?**
   In immunology it is important to study what happens in the entire organism since the immune system is distributed throughout the body. In contrast to solid organs like the heart, there is no single “immune organ”. Genetic ablation of a gene in the entire organism can lead to unwanted side-effects if the gene affects multiple cells or if the gene is important during embryonic development. By deleting a gene exclusively in T cells we get much more precise answers about the gene’s intrinsic role in T cells. In addition, this can reduce the burden on the animal if ablating the gene in all cells would negatively impact the mouse. Transgenic animals are therefore very important for immunologic research.
4. **Are there alternative ways to succeed in research/treatment aside from using these animals?**
   Yes.
   a) **What alternative ways are there?**
      We usually complement our in vivo research with in vitro experiments where immune cells are isolated and then cultured. We also use cell lines but these come with many disadvantages. Results from cell lines have to be interpreted with caution as they often don’t reflect physiology. Newer tools like the CRISPR/Cas or other genome engineering systems allow much more precise genetic studies in human cells. We have rapidly adopted this technique and now almost every single project in the lab employs the CRISPR/Cas system. We also increasingly work with human cells.
   b) **Why did you not use these them in the cases you described before?**
      See 3, for in vivo studies we often need living organisms. The complex immune system with multiple organs (e.g. thymus, bone marrow, spleen, lymph nodes, lymphatic vessels, specialized immune structures like Peyer’s patches, BALT, GALT etc.) combined with many different specialized cell types and soluble molecules as well as interactions with cell types not considered immune cells cannot be mimicked in a plastic dish.

c) **Would there be any advantages in using these alternative techniques?**
We use a balanced mix of mouse, human tissues, primary cells, cell lines, computer modeling, recombinant DNA and protein technology etc. We pick the best tool for each question.

d) **Would the experiment/study/research have been impossible to conduct if transgenic animals could not have been used?**
Clearly there are experiments that would be impossible without transgenic animals.

5. **What are the advantages and disadvantages of using animals for such purposes?**
See above:
Advantages: defined genetic models, the immune system is too complex to be modeled in a dish or in silico. Reproducibility: some mouse strains exist as inbred strains wheras humans are generally outbred, i.e. genetically diverse. The “standardization” of a genetic background dramatically reduces the variability between experiments. For statistical reasons the number of individuals generally needs to be higher in an outbred situation.
Disadvantages: costs are very high, experiments can be very long. It can take years to generate a new genetic mouse model, then months to years to characterize it and additional years to pursue scientific questions. In addition, regulation is very strict, experimenters need special training (theoretical and practical courses, continued training) to be allowed to work with animals and to perform experiments on animals. Experiments need to be outlined beforehand, then an application needs to be written, often dozens of pages where the experiments are explained in detail, where the stress on the animals is to be estimated and where the expected gain of knowledge has to be weighed to the the suffering of the animals. This needs to be justified. From submission to approval it can take many months.
6. **How do you view the ethical dilemma that the use of transgenic animals poses?**
See 5, we work in a disease-relevant area and as a physician I have seen first-hand what certain diseases can mean for patients. I am therefore convinced that the experiments we perform are justified. However, I am aware that I am causing suffering for some of our mice and that is a dilemma indeed. As explained above we try to limit the stress and suffering to a minimum, among other measures by following the 3R: Reduce, replace, refine. Ie.. we try to use alternative methods where possible, we try to reduce the number of animals and to refine procedures to reduce the suffering as much as possible.
7. **Could you describe the method of creating transgenic animals that you applied most in more detail?**
We use embryonic stem (ES) cells that are then genetically manipulated to engineer the desired modifications into the genome. After carefully selection of correctly engineered cells (in a dish) those cells can be implanted into mouse embryos and implanted into female mice. Pups born from these mothers will be chimeric, i.e. contain some cells that are genetically altered and others that are unmanipulated. By breeding them one can, with some luck, obtain mice that contain the genetic manipulation in all cells of the body.
8. **What are the most sensitive parts of this process? What can go wrong?**
Culturing of the ES cells can be challenging since when they start to differentiate they lose their ability to develop into an entire mouse. Also, the genetic alterations need to be carefully monitored before implanting the ES cells to mice.

9. **(How do you know if the procedure of creating a transgenic animal was successful?)**
   This depends on what the goal was. If e.g. a gene was supposed to be inactivated one can look for the genetic alteration by analyzing the DNA, or by measuring if the gene is still transcribed into mRNA and/or translated into protein.
10. **(What happens with the animals if the procedure was not successful?)**
    Animals would get euthanized.
11. **What progress can we expect from the application of transgenic animals in research? What problems may we expect to be solved with this technique?**
    Much of the fundamental understanding of mammals came from working with various animals (e.g. mice, rats, zebrafish). The ability to genetically alter mice added much precision and dramatically increased our understanding of biology. It also allowed to create animal models of human diseases. During the SARS-CoV2 pandemic it became clear how powerful animal models are. In a very short time we learned to understand the disease (COVID-19) much better and vaccines and treatments (e.g. neutralizing antibodies) were developed and tested. In addition, cellular therapies are currently being translated to clinical use. Such treatments are based on decades of animal research.