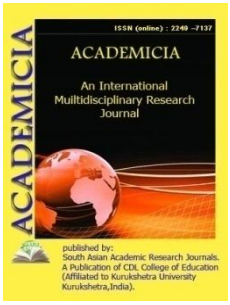
ACADEMICIA
An International Multidisciplinary Research Journal
(Double Blind Refereed & Peer Reviewed Journal)



DOI: 10.5958/2249-7137.2021.02372.7

A REVIEW ON TRANSGENIC ANIMALS PRODUCE HUMAN ANTIBODIES

Milind Pandey*; Ashish Singhai; Rajesh Sharma*****

*Department of Pharmacy, Teerthanker Mahaveer University,
Moradabad, Uttar Pradesh, INDIA
Email id: milind.pharmacy@tmu.ac.in

**Department of Pharmacy, Teerthanker Mahaveer University,
Moradabad, Uttar Pradesh, INDIA

***Department of Pharmacy, Teerthanker Mahaveer University,
Moradabad, Uttar Pradesh, INDIA

ABSTRACT

Laboratory mice offer a convenient supply of monoclonal antibodies with a wide range of affinity or specificity (mAbs). The intrinsic immunogenicity of rodent antibodies has hampered the development of these molecules as medicinal treatments. The use of transgenic mice producing repertoires of human antibody genetic codes has been investigated as a method of producing low immunogenicity mAbs for in vivo treatment. Over a dozen pharmaceutical as well as biotechnology firms have already used this technique to create novel therapeutic mAbs, and there are now at least 33 medicines in clinical testing—including many in pivotal trials—that include variable sections expressed by human sequences from transgenic mice. The preliminary results from these studies provide a peek into the safety and effectiveness concerns that these compounds may face. Nonetheless, real product approval is needed to properly verify this technology as a medication discovery tool, which is the greatest hurdle thus far. It may be feasible to expand this technique beyond rodents in the future by using transgenic farm animals to create and synthesize human sequence polyclonal sera directly.

KEYWORDS: *Antibodies, Biotechnology, Transgenic Animals, Therapeutic Mabs.*

INTRODUCTION

Transgenic animals are commonly used as models in biomedical research in the lab. Genetically engineered animals, mostly mice, account for almost 95% of those utilized. They are essential tools for studying human illness, since they are utilized to better understand gene function in the context of disease susceptibility, progression, and treatment response.MAbs were one of the first medicinal compounds to be authorized by the FDA thanks to contemporary biotechnology. However, it took 8 years for the US Food and Drug Administration to approve the next therapeutic mAb following the launch of muromonab-CD3, a murine mAb targeting CD3 that was authorized in 1986 for treating severe organ transplant rejection (FDA). The immunogenicity of mouse antibodies in healthy patients was one factor contributing to the gap, which could also lead to rapid clearance, reduced efficacy and an elevated chance of infusion reactions, which can range from relatively harmless fevers and rashes to cardiopulmonary and anaphylactic-like adverse events[1]. Biotechnology or pharmaceutical firms have addressed this issue by developing reduced immunogenicity antibody molecules utilizing molecular biology techniques. In vitro, mouse antibodies were reengineered to exchange framework amino acids with human sequences5,6. Novel, laboratory-derived antibodies have also been discovered by screening libraries of human and synthetic immunoglobulin sequences.

The FDA has already authorized 17 therapeutic monoclonal antibodies (mAbs) (Figure 1). Except for three, they've all been modified to be less immunogenic and include at least some human DNA. Human sequencing mAbs may provide a possible answer to the immunogenicity issue that has plagued rodent-derived antibodies. Early efforts to mine genuine human antibody repertoires through cancer or highly contagious patients, however, mainly yielded IgM antibodies with poor affinity or specificity. Although recent advancements have solved many of the technical challenges involved with producing human mAbs directly from human B cells, the human immune system's natural tolerance for human antigens, as well as the fact that human patients cannot be exposed to the same kinds of vaccination schemes used to generate rodent antibodies. Our capacity to reach human B cell–derived antibodies is limited to the wide range of sites that rodent antibodies can access. In this review, they describe the present state of medicines produced from transgenic mice containing human immunoglobulin repertoires, an alternate method for producing low immunogenicity therapeutic mAbs. Because the immunogenicity of rodent antibodies was the main reason for creating transgenic mice platforms, this study focuses on immunogenicity evidence for human sequence antibodies in the clinic. Transgenic technology is frequently addressed in relation to the development of polyclonal antibody–based medicines[2].

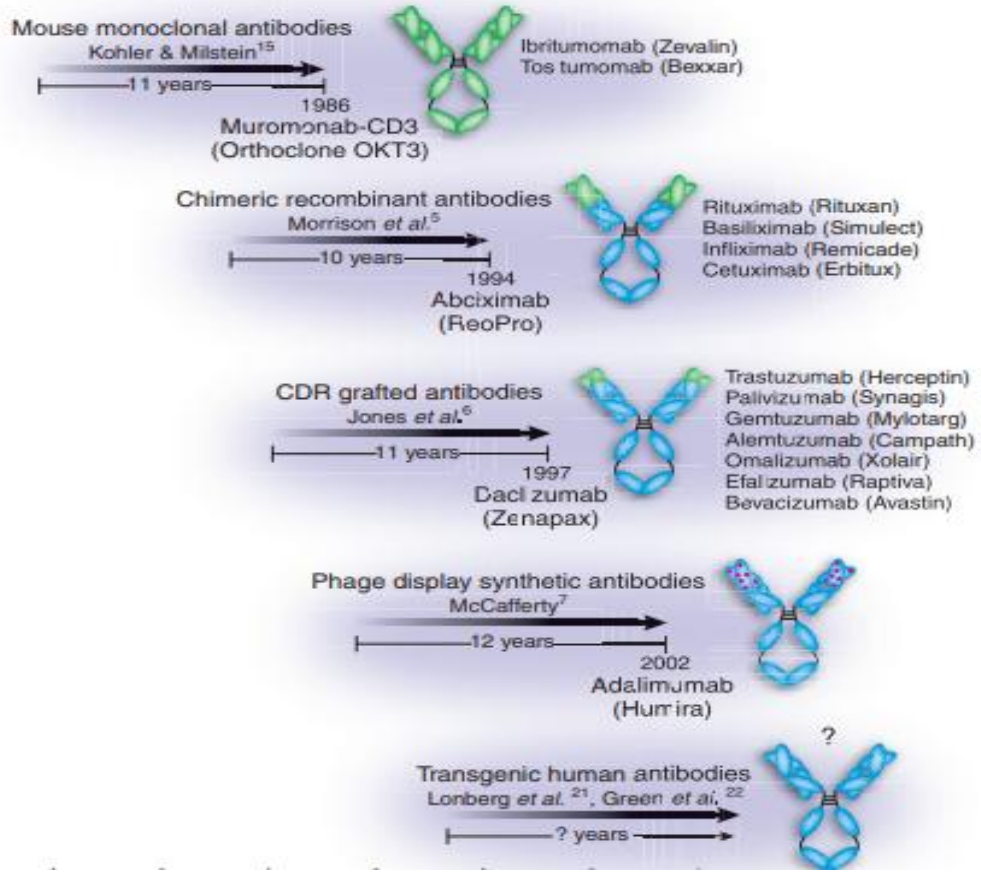


Figure 1:Evolution of therapeutic antibody technology and progress to the clinic[3]**.**

1.1.Mammalian immunoglobulin gene in transgenic mice:

Alt et al.16 proposed that transgenic technology might be used to generate novel human sequence mAbs from un-rearranged, germline-configuration transgenes twenty years ago. Although this was "conceptually absurd," the writers decided that it might "be achieved in the not-too-distant future." The production of a repertoire of human heavy chains, as well as the development of a transgene-encoded immunological response in mice17, were first reported in 1989. The race to create a mouse with diverse human heavy- and light-chain skills and abilities able to make a contribution to a true secondary immune system of high-affinity human mAbs, in the surroundings of disrupted mouse heavy- and light-chain genes, was fueled by this report and the invention of methods for introducing specific modifications into the mouse germ line[4].

The constant region was incorporated in the light-chain transgene. yeast protoplast fusion to deliver minilocus transgenes based on the yeast artificial chromosome (YAC). The heavy chain in this instance comprised 5 VH, all 25 D, as well as all 6 JH gene segments, as well as and constant-region gene segments. This design was VDJ joined and expressed both IgM and IgD antibodies. The light-chain YAC construct contained two functioning V and all five J segments, as well as C. the endogenous -lightchain locus, which contributes just 5% of the B-cell repertoire in normal laboratory mouse strains. A subset of B cells with functional -light-chain expression produces hybrid B-cell receptors and released antibodies with human heavy- as well as mouse-

light chains. Despite the existence of this subpopulation, hybridoma cell lines secreting completely human monoclonal IgM22 or IgG21 mAbs that recognized the target antigens for which the mice had been vaccinated were isolated[5].

Given that each of these two mice strains only retained a portion of the normal human V repertoire, the effective isolation of human mAbs selectively directed against a particular antigen was unexpected. This begs the issue of why mammals seem to have been chosen for their vast germline V repertoires. For the production of different antibody sequences at the six composed regions (CDRs), which allow direct interaction with specific antigens, large germline repertoires may be needed. The expressed antibody repertoire, on the other hand, is a result of three sources of diversity: combinatorial, junctional, and somatic, with the germ line providing just one of them (combinatorial).

The capacity to produce antibodies against a wide range of targets utilizing minilocus transgenes with a small fraction of complete human combinatorial diversity may represent the relative significance of these three sources of variation. Although the germ line fully encodes naïve B-cell CDR1 and CDR2 sequences, junctional variability, which is preserved in minilocus transgenes, generates most of the heavy-chain CDR3 repertoire. CDR3 sequences seem to be crucial for antigen recognition by unmutated B-cell receptors, and they may account for the majority of the main repertoire. Primary repertoire B cells with low affinity for the immunogen may subsequently undergo affinity maturation through T cells, which has been demonstrated to produce high-affinity antibodies from a small V-gene repertoire[6].

1.2.Human mAb immunogenicity in transgenic mice:

A study of the existing clinical data mentioned above allows us to question if the transgenic mice platforms have really addressed the immunogenicity issue that prompted their creation in the first place. Despite the fact that transgenic mouse–derived human mAbs have yet to complete a phase 3 clinical trial, giving data equivalent to that available for authorized medicines, the first findings are promising.

The lack of severe infusion responses and minimal interpatient variability of drug exposure further testify to this molecule's low immunogenicity. Because mAb is likely to be linked not only to the molecule's intrinsic characteristics but also to the patient's immunological state, data from studies including patients with inflammation or autoimmune illnesses may be useful. None of the 85 patients in the zanolimumab psoriasis study, who each got four doses over a month, showed a significant immune response to the human mAb63[7].

Trials using CTLA-4 mAbs have produced some of the most dramatic results from individuals with increased immune responses. Human immunoglobulin–producing transgenic mice may be beneficial over other technologies merely because of inherent variations in the drug discovery processes required by the various systems, in addition to offering a platform for the identification of low immunogenicity therapeutic mAbs. For generating low immunogenicity mAbs in vitro, a procedure similar to that employed for small-molecule drug development is required: lead identification followed by a potentially long period of lead optimization. In transgenic mice, however, the lead optimization step can be skipped entirely but since B-cell development as well as affinity maturation can produce in vivo optimized antibodies. This enables a process in which

each potential candidate is tested in a series of increasingly sophisticated in vitro and in vivo assays before being selected as a lead in essentially the same molecular form as it will be used in humans[8].

Selections for genetically engineered mouse platforms are based on information from preoptimized leads; however, selection decisions for lead optimization–based processes must rely on data from unoptimized leads, that aren't always relevant to the characteristics of the finished optimized compound. Furthermore, because clonal antibody–secreting cell lines are directly generated by the hybridoma fusion methods commonly used to generate drug candidates from transgenic animals, the process is well suited for screening protocols that use a variety of cell-free, in vitro cell-based, and/or in vivo assays.

1.3.Large animals' polyclonal antibodies:

In many respects, therapeutic mAbs on the market now are far superior than the polyclonal serum treatments pioneered by Kitasato, Behring, and Ehrlich over a century ago for the treatment of diphtheria and tetanus. Although MAbs have proved to be a reliable source of well-characterized, low-immunogenicity, and highly effective medicines, polyclonal human serum–derived and even animal serum–derived antibodies still have a role in the clinic. In certain instances, polyclonal antibodies may be preferred to mAbs for passive immunotherapy, much as the natural human immune system prefers polyclonal antibodies to mAbs when reacting to infections. Polyclonal antibodies have several advantages, including increased potency in immune complex formation, utility in combating infectious diseases caused by multiple strains of pathogens or that require neutralization of multiple epitopes for effective treatment, and the ability to neutralize snake and insect venoms with multiple toxic components. Cows, chickens, and rabbits are examples of nonrodent transgenic animals that may be used in the biological manufacture of human polyclonal antibodies. In the scientific literature, some work toward this aim has already been published[9].

Artificial chromosomes containing the complete human germline heavy-chain and -light-chain loci were implanted into transgenic calves. Human heavy- and light-chain antibody transcripts were rearranged properly in the calves. To produce homozygous heavy-chain knockout mutant calves, the same group utilized successive gene targeting in fibroblast cells along with nuclear transfer cloning. Combining these methods with light-chain knockouts may result in a novel transgenic platform for generating human-sequence polyclonal antibodies from animals. The capacity to hyperimmunized the animals against particular pathogens or human disease–associated proteins, as well as better lot uniformity and decreased danger of human pathogen contamination, are all possible benefits of this approach[10].

2. DISCUSSION

Mice have also been genetically engineered to generate human antibodies spontaneously, which may be used as treatments. Between 2006 to 2011, seven of the eleven monoclonal antibody medicines authorized by the FDA were generated from transgenic mice. The use of transgenic farm animals to generate vast amounts of complicated human proteins for the therapy of human illness is also being investigated. Currently, therapeutic proteins are generated in mammalian cell-based reactors, but this method is costly. The cost of constructing a new cell-based

manufacturing plant for one therapeutic protein, for example, was projected to be above $500 million in 2008.

Monoclonal antibodies with a broad range of affinity or specificity are readily available from laboratory mice (mAbs). Rodent antibodies have been hindered in their development as medical therapies due to their inherent immunogenicity. The use of transgenic mice expressing human antibody gene repertoires as a means of generating low immunogenicity mAbs for in vivo therapy has been explored. Over a dozen pharmaceutical and biotechnology companies have previously utilized this method to develop new therapeutic mAbs, as well as at least 33 medications are now in clinical studies, with several in pivotal trials, that contain variable portions produced by human sequences from transgenic mice. Nonetheless, in pivotal trials, each of these mAb-based therapies will need to demonstrate meaningful patient benefit. It's possible that one of these chemicals will be the first novel biologic produced in a transgenic mouse if it proves to be helpful to humans. This method has the potential to generate large transgenic farm animals that may be used to make therapeutic monoclonal antibodies antibody in the future.

3. CONCLUSION

High-affinity humans sequence mAbs against a broad range of potential therapeutic targets have been generated using transgenic mice that produce human antibody repertoires. The first results from clinical trials of 33 transgenic-derived human mAbs show a variety of medicines that are both active and well tolerated. Nonetheless, each of these mAb-based medicines will need to show real patient benefit in pivotal studies. If one of these compounds proves to be beneficial to patients, it may be the first new biologic developed in a transgenic mouse. This technique has the potential to create huge transgenic farm animal that can be utilized directly to produce therapeutic human-sequence polyclonal antibodies in the future.

REFERENCES

1. S. B. Manesh, R. O. Samani, and S. B. Manesh, “Ethical issues of transplanting organs from transgenic animals into human beings,” *Cell J.*, 2014.

2. G. Chala, “A Review on: Antibody Engineering for Development of Therapeutic Antibodies,” *Int. J. Immunol.*, 2015, doi: 10.11648/j.iji.20150303.11.

3. N. Lonberg, “Human antibodies from transgenic animals,” *Nature Biotechnology*. 2005, doi: 10.1038/nbt1135.

4. P. Mehta, A. Sharma, and R. Kaushik, “Transgenesis in farm animals-A review,” *Agric. Rev.*, 2017, doi: 10.18805/ag.v38i02.7944.

5. R. Chang, X. Liu, S. Li, and X. J. Li, “Transgenic animal models for study of the pathogenesis of huntington’s disease and therapy,” *Drug Design, Development and Therapy*. 2015, doi: 10.2147/DDDT.S58470.

6. K. E. Harris *et al.*, “Sequence-based discovery demonstrates that fixed light chain human transgenic rats produce a diverse repertoire of antigen-specific antibodies,” *Front. Immunol.*, 2018, doi: 10.3389/fimmu.2018.00889.

7. M. V. Shepelev, S. V. Kalinichenko, A. V. Deykin, and I. V. Korobko, “Production of

recombinant proteins in the milk of transgenic animals: Current state and prospects," *Acta Naturae*. 2018, doi: 10.32607/20758251-2018-10-3-40-47.

8. L. M. Houdebine, "Production of pharmaceutical proteins by transgenic animals," *Rev. Sci. Tech.*, 2018, doi: 10.20506/rst.37.1.2746.

9. L. M. Houdebine, "Production of pharmaceutical proteins by transgenic animals," *Comp. Immunol. Microbiol. Infect. Dis.*, 2009, doi: 10.1016/j.cimid.2007.11.005.

10. M. Brüggemann *et al.*, "Human Antibody Production in Transgenic Animals," *Archivum Immunologiae et Therapiae Experimentalis*. 2015, doi: 10.1007/s00005-014-0322-x.