Xiao-Ying Zhang
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IgY-Technology: Production and Application of Egg Yolk Antibodies

Basic Knowledge for a Successful Practice

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Editors
Xiao-Ying Zhang
College of Biological Science and Engineering
Shaanxi University of Technology
Hanzhong, Shaanxi, China

Ricardo S. Vieira-Pires
Center for Neuroscience and Cell Biology
University of Coimbra
Coimbra, Portugal

Patricia M. Morgan
School of Natural Sciences
National University of Ireland Galway
Galway, Ireland

Rüdiger Schade
Institute of Pharmacology
Charité – Universitätsmedizin Berlin
Berlin, Germany

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Preface

Adopting new scientific methodologies can be a slow process. Researchers gain expertise with a particular model or set of techniques, and it often requires groundbreaking advances to garner support for new ideas. IgY technology has developed quite slowly given its potential, but we consider that now is the right time to provide scientists and practitioners with an overview of IgY and IgY technology research and to underpin the urgent requirement for standardization and shared perspectives of this technology. We have also attempted to provide the theoretical background combined with the practical approaches to support new researchers in the field as well as review the advances to date and set out the challenges ahead for more established researchers.

The book in divided into three parts. Part I describes the biological basis of IgY and IgY technology. IgY is a fascinating molecule important in the evolution of immunoglobulins and the immune system. Birds are phylogenetically distinct from mammals and have several unique features, and given the predominant use of mammalian immunoglobulins in research, we have emphasized the differences between mammalian and avian systems.

Part II of the book covers the core methods employed in IgY technology: not only the established methods such as hen keeping, immunization and antibody monitoring, but also some novel methodological needs, such as IgY delivery and dosage form design. The demand for proteins used as therapeutics is soaring. The methodologies for the production of monoclonal IgY and transgenic hens are discussed.

Part III of the book summarizes and discusses the applications of IgY technology. The real impact of any technology is in its beneficial contributions to society. The potential of IgY in maintaining animal health has in some part been realized, but opportunities still need to be exploited for treatment of human diseases. The ability to generate polyclonal IgY, which does not need expensive equipment, may address critical needs where costs are a factor. It is also instructive to see how basic research combined with innovation and entrepreneurial spirit is driving the development of new products and processes.

Through this monograph, it is our anticipation and hope to create a shared outlook among the new researchers and established practitioners of IgY technology. The global spread of IgY technology is evidenced by our contributors to this book. It

would be advantageous to formally establish an "IgY Society" to link the globally spread researchers, to link academia and industry, and to draw others with different research backgrounds and skills into the pursuit of the many benefits of IgY technology. We hope that we have communicated our enthusiasm for this area of research, and we hope that this book will play some part in expanding the membership of the community of IgY researchers.

Hanzhong, Shaanxi, China — Xiao-Ying Zhang
Coimbra, Portugal — Ricardo S. Vieira-Pires
Galway, Ireland — Patricia M. Morgan
Berlin, Germany — Rüdiger Schade

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On a personal level, one of us (PMM) would like to acknowledge the support and encouragement provided by Gerry Morgan, throughout the planning and execution of this book. It would not have been possible without him. RVP would like to thank his family for their long-term support particularly during the journey of writing this book.

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Dr. Patricia M. Morgan
Dr. Ricardo S. Vieira-Pires

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Contents

Contributors

Hyeong Chul Ahn ADBioTech Co., Chuncheon-si, South Korea

Marina Bok INCUINTA, CICVyA-INTA, Comodoro Rivadavia, Argentina

Catherine D. Caulfield Ovagen Group Ltd., Carrentrila, Ballina, Ireland

Pablo Chacana UEDD INTA-CONICET IPVet, Instituto Nacional de Tecnología Agropecuaria, Comodoro Rivadavia, Argentina

Brindha Chelliappan Department of Microbiology, PSG College of Arts & Science, Bharathiar University, Coimbatore, Tamil Nadu, India

Chinnappan Gandhimathi Sri Ramakrishna Dental College and Hospital, Coimbatore, Tamil Nadu, India

Ankit Choraria PSG College of Arts and Science, Coimbatore, Tamil Nadu, India

Fazle Elahi ADBioTech Co., Chuncheon-si, South Korea

Álvaro Ferreira Júnior School of Veterinary and Zootechnics, Universidade Federal de Goiás, Goiânia, Brazil

Mara G. Freire CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

Shikun Ge College of Biological Science and Engineering, Shaanxi University of Technology, Hanzhong, China

Carol A. Harley i3S - Instituto de Investigação e Inovação em Saúde and IBMC – Instituto do Biologia Molecular e Celular, University of Porto, Porto, Portugal

Anders O. Larsson Department of Medical Sciences, Uppsala University, Uppsala, Sweden

Carlos Leónidas Leiva UEDD INTA-CONICET IPVet, Instituto Nacional de Tecnología Agropecuaria, Argentina

Chiuan Herng Leow Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Pulau Pinang, Malaysia

Ben Li College of Biological Science and Engineering, Shaanxi University of Technology, Hanzhong, China

Antonysamy Michael Department of Microbiology, PSG College of Arts & Science, Bharathiar University, Coimbatore, Tamil Nadu, India

Leonard M. Moran Ovagen Group Ltd., Carrentrila, Ballina, Ireland

Patricia M. Morgan Emerita Lecturer, School of Natural Sciences, National University of Ireland Galway, Galway, Ireland

Martin G. Murphy Ovagen Group Ltd., Carrentrila, Ballina, Ireland

Tiago Ôchoa-Pires CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

Greg Papadakos Roslin Technologies, Edinburgh, UK

Viviana Parreño INCUINTA, CICVyA-INTA, CONICET, Comodoro Rivadavia, Argentina

Sentila Rajan Bharathiar University, Coimbatore, Tamil Nadu, India

Marguerita Rosa CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

Jafar Salimian Applied Microbiology Research Center, Chemical Injuries Research Center, Systems Biology and Poisoning Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

Rüdiger Schade Institute of Pharmacology, Charité – Universitätsmedizin Berlin, Berlin, Germany

Meenatchisundaram Subramani Nehru Arts and Science College, Coimbatore, Tamil Nadu, India

Ana Paula M. Tavares CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

Celina G. Vega Centro de Investigación en Ciencias Veterinarias y Agronómicas, INTA, Buenos Aires, Argentina
Bioinnovo; INCUINTA, CICVyA-INTA, CONICET, Comodoro Rivadavia, Argentina

Ricardo S. Vieira-Pires CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

Andrés Wigdorovitz Bioinnovo; INCUINTA, CICVyA-INTA, CONICET, Comodoro Rivadavia, Argentina

Rao Wu College of Biological Science and Engineering, Shaanxi University of Technology, Hanzhong, China

Long Xu CBMA, University of Minho, Portugal; College of Biological Science and Engineering, Shaanxi University of Technology, Hanzhong, China

Xiaoying Zhang College of Biological Science and Engineering, Shaanxi University of Technology, Hanzhong, China
Ontario Veterinary College, University of Guelph, Guelph, ON, Canada
CBMA, University of Minho, Braga, Portugal

Fagang Zhong State Key Laboratory for Sheep Genetic Improvement and Healthy Production, Xinjiang Academy of Agricultural and Reclamation Science, Shihezi, China

Part I

Biological Basis of IgY Technology

Development of IgY Technology: A Historical Perspective

1

Xiaoying Zhang, Ricardo S. Vieira-Pires, and Long Xu

Abstract

IgY Technology is a scientific field classically related to the generation, evaluation and use of avian egg yolk IgY antibodies to develop biomedical and biotechnological solutions and applications. The field is more than 120 years old. However, in the last decades, major advances in research and development areas such as Genetics, Biochemistry, Bioengineering and Bioprocessing, have prompted new approaches to this old technology. In this chapter, a retrospective summary is given on the major milestones, key events and developments of IgY technology. We also bring an overview of the main topics and themes gathered and further developed within this book edition. The discussion on the perspective and future trend analysis of IgY technology is given in Chap. 18.

Keywords

IgY technology · Egg yolk antibody (IgY) · History · Animal welfare · IgY product

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X. Zhang (✉)
College of Biological Science and Engineering, Shaanxi University of Technology, Hanzhong, China

Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

CBMA, University of Minho, Braga, Portugal
e-mail: zhxying@snut.edu.cn; xzhang67@uoguelph.ca; zhang@bio.uminho.pt

R. S. Vieira-Pires
CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal
e-mail: ricardo.pires@biocant.pt

L. Xu
College of Biological Science and Engineering, Shaanxi University of Technology, Hanzhong, China

CBMA, University of Minho, Braga, Portugal

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1.1 Introduction

Hens produce an immunoglobulin class, immunoglobulin Y (IgY) whose natural role is the protection of the chicken and the developing chick in the egg. However, the immunization of hens results in the production of specific IgY against the antigen and the process of immunization and recovery of the IgY from the egg yolk, is termed IgY technology. The highly specific polyclonal antibodies are obtained by using different extraction and purification methods. This technology has developed rapidly in recent years and has good potentials in the field of medicine.

In the past two decades, biomedical research has been challenged by the growing public interest in the welfare of animals used in research; resulting in the search for ways to Refine, Reduce or Replace painful animal experiments. This also applies to the production of polyclonal and monoclonal antibodies in animals. Antibodies are important tools in biomedical research and are essential components of diagnostic methods for quantitative and qualitative determinations of a wide variety of biological molecules. Historically, polyclonal antibodies have been produced in rabbits and other rodents like mice, rats and guinea-pigs or in farm animals such as horses, sheep and goats. The production of polyclonal antibodies involves two invasive procedures, firstly the immunization procedure involving several injections and secondly, harvesting of the antibodies from the blood which involves bleeding the animals on several occasions.

1.2 Development of IgY Technology

The first experiments to demonstrate that a serum product could provide protection against a toxin were performed as early as 1890. The German scholar Emil von Behring and the Japanese scholar Kitasato Shibasaburo injected guinea pigs with heat-treated diphtheria toxin and found that the serum could protect animals from what would be a lethal dose of the live toxin. Emil von Behring was subsequently awarded the first Nobel Prize (1901) in Physiological Medicine for his work on diphtheria. Kitasato was experienced in the culture of the anaerobic tetanus bacilli, and worked on the determination of the toxin-destroying activity in serum; his research and the tetanus antitoxin saved the lives of many soldiers during the First World War (Kantha 1991). Started from the aforementioned antiserum work, antibody engineering and antibody based immune therapy have been well developed and have contributed to important developments in biotechnology and therapy.

Inspired by the antiserum study of the early 1890s, the German medical doctor Felix Klemperer did a classical experiment in 1892 (Fig. 1.1): laying hens were subjected to tetanus-causing bacilli (*Clostridium tetani*) by intraperitoneal injection every 5–15 days, for a total of five times. The egg yolk was first dissolved and mixed with a $NaCl–Na_2SO_4$ solution, and injected into mice at three different concentrations: high, medium and low doses. On the next day, these different groups were infected with 1.5 times the concentration of what would be a lethal dose (LD_{50}) of tetanus. Mice pre-exposed to high doses of the yolk-based solution all survived

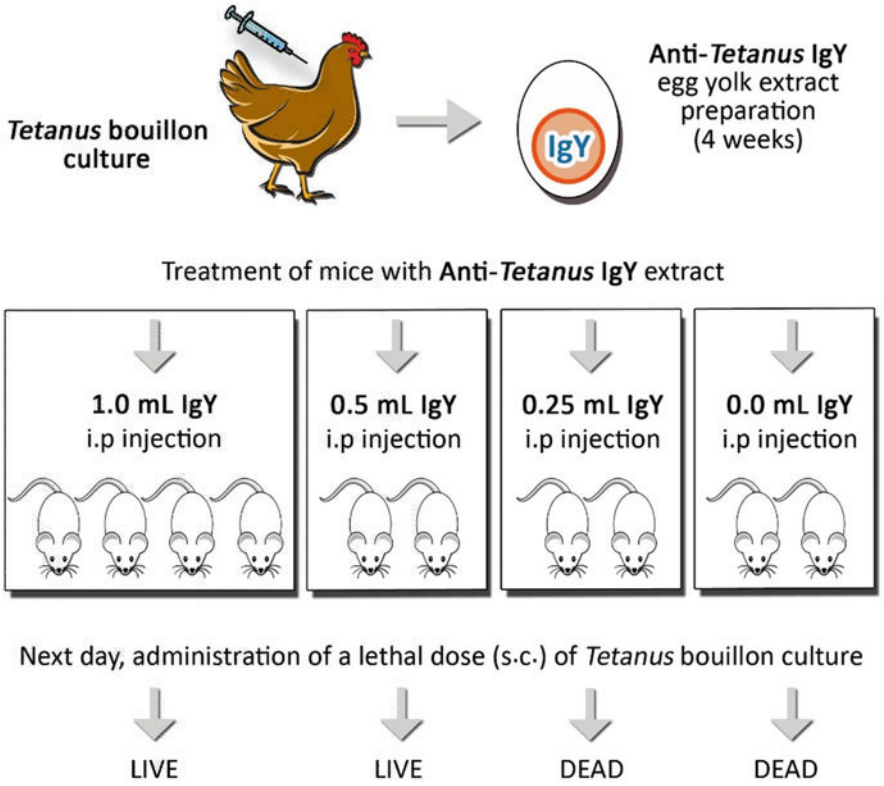

Fig. 1.1 Historical experiment of Dr. Felix Klemperer. The first relevant experiment in the field of IgY antibodies was performed by the German researcher Felix Klemperer in 1893. He showed for the first time that laying hens immunized with tetanus toxin could generate immunity and produce egg yolk extracts with protective characteristics. Adapted from IgYTechnology.com (www.igytechnology.com)

and those exposed to either the low dose or a control group with no pre-treatment died. This study proved for the first-time that immunized laying hens can produce specific antibodies with neutralizing ability, although Klemperer used the term "immunity" ("Immunität" in German) to refer to the "antibody". He claimed "extremely high immunity" obtained from the immunized hen egg yolk (Klemperer 1893). This discovery of a relatively easy way to obtain the specific antibody from laying hens represented a fundamental breakthrough in medical science, especially when considering that it was only 2 years after the discovery of the first mammalian antiserum therapy of Behring. Unfortunately, Klemperer's work and observations were very much undervalued, and its revival only happened after several decades (Fig. 1.2).

The chicken egg yolk antibody was called IgG for many decades since this immunoglobulin isotype exhibits properties quite similar to mammalian IgG (Chap. 5). The molecule was first termed "IgY" by Gerrie A. Leslie and L. W.

XV.

Aus dem Laboratorium der medicinischen Klinik zu Strassburg i. E.

Ueber natürliche Immunität und ihre Verwerthung für die Immunisirungstherapie.

Von

Dr. Felix Klemperer,
Assistent der Klinik.

In einer vor Jahresfrist mitgetheilten Versuchsreihe [1]) konnte ich zeigen, dass das Blutserum der von Natur gegen Mäusesepticämiebacillen und Friedländer'sche Bacterien refractären Kaninchen anderen Thieren gegen die Infection mit diesen Bacterienarten nicht Schutz zu verleihen vermag; dass aber, wenn man den Kaninchen steigende Mengen der Mäusesepticämie- resp. Pneumoniebacillen injicirt, ihr Serum die vorher vermisste immunisirende Fähigkeit gewinnt. Es liess sich dieser Vorgang dahin deuten, dass in beiden Fällen die von Natur vorhandene, relativ schwache Immunität durch die Injection der Bacterien zu höheren Immunitätsgraden gesteigert wurde.

Die Frage lag nahe, ob dieser Vorgang eine allgemeinere Gültigkeit besitze und ob die Möglichkeit, die natürliche Immunität zu steigern, wie wir dies beim Kaninchen gegenüber den genannten beiden Bacterienarten gefunden hatten, auch in anderen Fällen von natürlicher Immunität bestände.

Die Beantwortung dieser Frage schien mir nicht nur für das Verständniss der angeborenen Immunität von Bedeutung zu sein, sondern mehr noch für den weiteren experimentellen Ausbau der sogenannten Serumtherapie. Die Untersuchungen der letzten Jahre (Ehrlich) haben gezeigt, und Behring [2]) hat es jüngst noch mit besonderer Schärfe ausgesprochen, dass die Vorbereitung eines Thieres zur Lieferung eines Serums von starker immunisirender Fähigkeit zwei Acte umfasst: Einmal muss das Thier überhaupt erst immunisirt,

1) Berliner Klinische Wochenschrift 1892. Nr. 13.
2) Die Blutserumtherapie I. S. 40 u. ff. (Leipzig, Georg Thieme. 1892.)

Fig. 1.2 First IgY publication by Felix Klemperer in 1893

Clem in 1969 "based upon physical-chemical and antigenic characteristics", in order to better distinguish the yolk antibody which showed "insufficient correlation with any of the known human immunoglobulins" (Leslie and Clem 1969).

Since the middle of the twentieth century, as animal ethics and welfare has gained more attention in academia, the research results of Klemperer have gained more prominence and this was advanced by the animal welfare research of Russel and Burch (Russell and Burch 1959).

Since the 1980s, IgY has been widely used owing to the availability of standard commercial reagents such as IgY-specific purification kits (Chap. 11) and tagged secondary antibodies against IgY.

The term "IgY technology" was first used in 1995 and defined as "IgY production and application" in 1996 and recognized by the European Centre for the Validation of Alternative Method (ECVAM), as an international standardized technology, recommended to replace the use of mammalian IgG for animal welfare purpose (Schade et al. 1996) (Fig. 1.3).

In 2001, the world's first IgY laboratory manual was published in Germany, which standardized the laboratory practices of IgY technology (Schade et al. 2001).

In 2004–2007, an interdisciplinary study of the comprehensive utilization of eggs was launched through a Cooperative Organization Science and Technology action (COST 923) in European Union framework, with the aim to improve the comprehensive utilization of egg ingredients with new functions and usage, and to encourage the non-food applications of eggs. The biomedical use of IgY became the focus of the action plan, as emphasized in the project summary publications (Huopalahti et al. 2007).

In 2011, an IgY monograph in Chinese was published.

An analysis of the publications on avian IgY antibodies and IgY Technology performed on NCBI database (time window 1950–2020) with different search terms, namely "*IgY Technology*", "*IgY Antibodies*" and "*IgY*", shows a prominent increase of the numbers of published works since the 1990s. The publications cover a broad range of research fields including immunology, zoology, ornithology and poultry science, veterinary medicine and animal husbandry science, food engineering, medical sciences (e.g., Gastroenterology, Stomatology), demonstrating the breadth of application of IgY Technology research and development (Fig. 1.4).

1.3 Application of IgY Products at a Glance

IgY has the advantage of stable chemical properties, high yield, and low cost for its production. Hens are phylogenetically distant from mammals and thus can be used to raise antibodies against conserved mammalian proteins. Its passive immunity function can be used to study how to resist viral and bacterial diseases. Therefore, it has the potential of developing as a functional food and drugs for human use, and as a feed additive and veterinary drug to animals. For example, one of the research focuses of IgY technology has been treating gastrointestinal diseases and diarrhoea with specific IgY, which have been used more intensively in veterinary medicine, but also in human health. These will be further developed in Chaps. 15 and 16.

IgY-based products in the pipeline involve specific antibodies for the treatment of several pathogens such as *Helicobacter pylori*, *Streptococcus mutans*, *Candida*

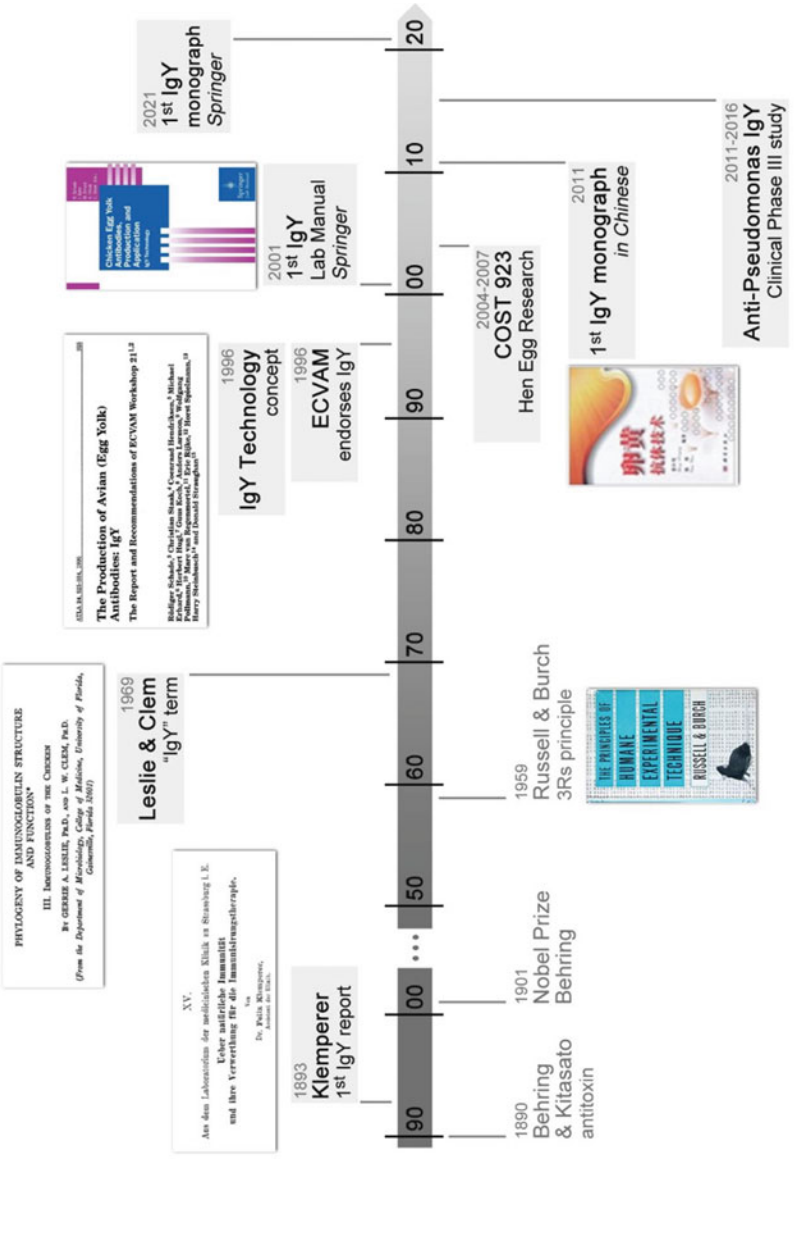

Fig. 1.3 Historical timeline of IgY technology

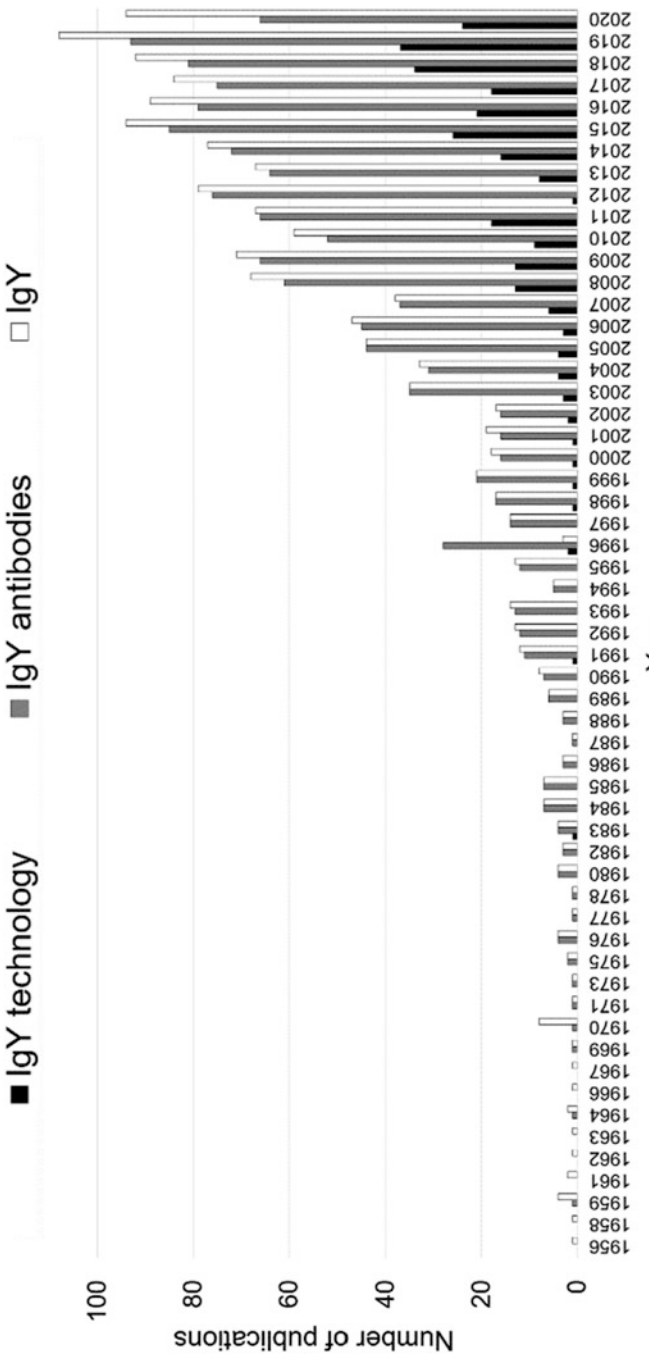

Fig. 1.4 Evolution of publications on avian IgY antibodies. A search of publications on avian IgY antibodies and IgY Technology was performed on NCBI database with different search terms on a time window from 1850 to 2020. The search terms were: "IgY Technology", "IgY Antibodies" and "IgY" and the total number of publications for each term were 268, 1366, and 1491, respectively (December 07, 2020)

albicans, *Porphyromonas gingivalis*, *Salmonella*, *Escherichia coli* and *Clostridium difficile*. IgY products for the treatment of clostridial enteric disease are under a phase II trial for the short-term evaluation of its efficacy and dosage. Moreover, the use of IgY in periodontitis, gingivitis, dental caries, gastric ulcer, dysbiosis, toxins, emerging viral diseases, nutritional diseases, chronic diseases and neoplasms have been presented in numerous patents (Leiva et al. 2020).

Traditionally, IgY technology refers to the generation of polyclonal IgY. However, since the end of the twentieth century, monoclonal IgY, monoclonal antibody fragment, particularly IgY-scFv can be also generated by technology convergence (Chap. 13). Also avian transgenesis and the production of biopharmaceuticals in egg white are relevant and will be briefly touched on in Chap. 14. This converges with the overall concept of using chickens as bioreactors for production of high-value proteins including biopharmaceuticals.

IgY technology was rapidly adopted in developing countries and regions such as South America, India, as well as Japan, Germany and other developed countries. The poultry industry is very well developed in China and the technology has broad application prospects there also. IgY technology research and application is broadly applied to veterinary medicine and functional food development (Chaps. 15 and 16). Many countries pay more attention to product exploitation and commercialization (Chap. 17), but the application to medicine requires further research.

1.4 Application of the IgY Technology Driven by Legal Regulations in Favour of Animal Protection

The implementation of the EU Directive 86/609/EEC (European Union 1986) for the protection of animals used for experiments and other purposes has stimulated efforts to increasingly apply alternatives to animal experimentation. Consequently, hens rather than rabbits may be immunized, and in more general terms scientists may even be asked to produce antibodies without using animals. In the longer run, the application of molecular genetics may allow the in vitro production of antibodies and as this stage is reached, classical polyclonal and monoclonal antibodies produced in animals will no longer be required. There is a strong debate on this issue, since many researchers still question the potential of fully synthetic libraries for in vitro antibody generation and selection; there are intrinsic features arising from natural evolution of immune repertoires (e.g., certain bias for preferred antibody structural arrangements) that can only be obtained from animal sources. Therefore, the immunological properties of antibodies produced by gene-technology will first have to be validated. The first production of monoclonal antibodies was welcomed with high expectations (Köhler and Milstein 1975), all of which were not met when the new technology was applied. In fact, polyclonal antibodies are still in use; and due to their immunological properties, they are quite often superior to monoclonal antibodies. Based on the reasons explained we are convinced that under the present legal environment the immunization of hens should be encouraged. Consequently,

the scope of argumentation put forward by scientists who are not convinced of the use IgY technology would be reduced.

1.5 Advantages and Limitations Driving IgY Technology

A common misperception when claiming the main advantages and limitations driving IgY Technology results from the fact that biological and technical aspects are normally brought together in a random manner. Indeed, these are highly correlated hence the difficulty in distinguishing them. Nevertheless, we try to summarize the different dimensions of using avian hosts and avian IgY antibodies, hoping to clearly distinguish each of the features in separately and show how they are compounded to leverage the potential or emphasize the limitations for a given outcome.

Overall, there are five main dimensions grounded on which avian IgY antibody-based technologies have evolved as promising alternatives to conventional research, diagnostic and therapeutic approaches (Fig. 1.5). The five main dimensions are as follow: (1) Animal ethics and welfare—the method is non-invasive as the antibody is collected from the egg yolk not blood, greatly reducing animal harm and distress. (2) Egg crude source—the avian IgY antibody naturally accumulates in high amounts in the yolk of eggs (–100 mg of IgY per egg). As hens lay on average one egg per day, a continuous collection and processing of eggs results in a scalable, cost-effective and highly sustainable way of antibody production. (3) Phylogenetic distance of hosts—birds diverged from mammals more than 300 million years ago, while mammals hold a 98 million years old common ancestor (Chap. 4); this results in lower homology and thus higher immunogenicity between the two groups. It is possible to obtain antibodies against highly conserved mammalian proteins or against proteins that usually evade the immune system in mammals. (4) Genetic organization of avian immune repertoires—birds hold unique antibody repertoires, resulting from diversification based on gene conversion (Chap. 3); this organization allows simplified in vitro molecular cloning of e.g., phage-display libraries (Smith 1985), and similar antibody engineering possibilities as for mammalian antibodies. (5) Molecular structure of IgY—the avian IgY antibody molecule (M.W. 180 kDa) holds unique molecular features in comparison with its mammalian IgG counterpart (M.W. 150 kDa), namely no binding to mammalian Fc-receptor, mammalian rheumatoid factor or mammalian complement proteins (Chaps. 5 and 6). This favours its use in human therapeutics as secondary immunoreactions are less prone to occur.

The limitations to the use of IgY antibodies and related technologies arise naturally from one or several of the inherent features described above. Hence for example a therapeutic IgY, will have an intrinsic variability due to the use of multiple birds, or simply the collection of hyperimmune eggs at different time points with the same birds; changing the birds in the flock will directly compromise the consistency and the reproducibility of the final IgY product, leading to titre and reactivities that are not entirely predictable (Chap. 10). If such features need to be

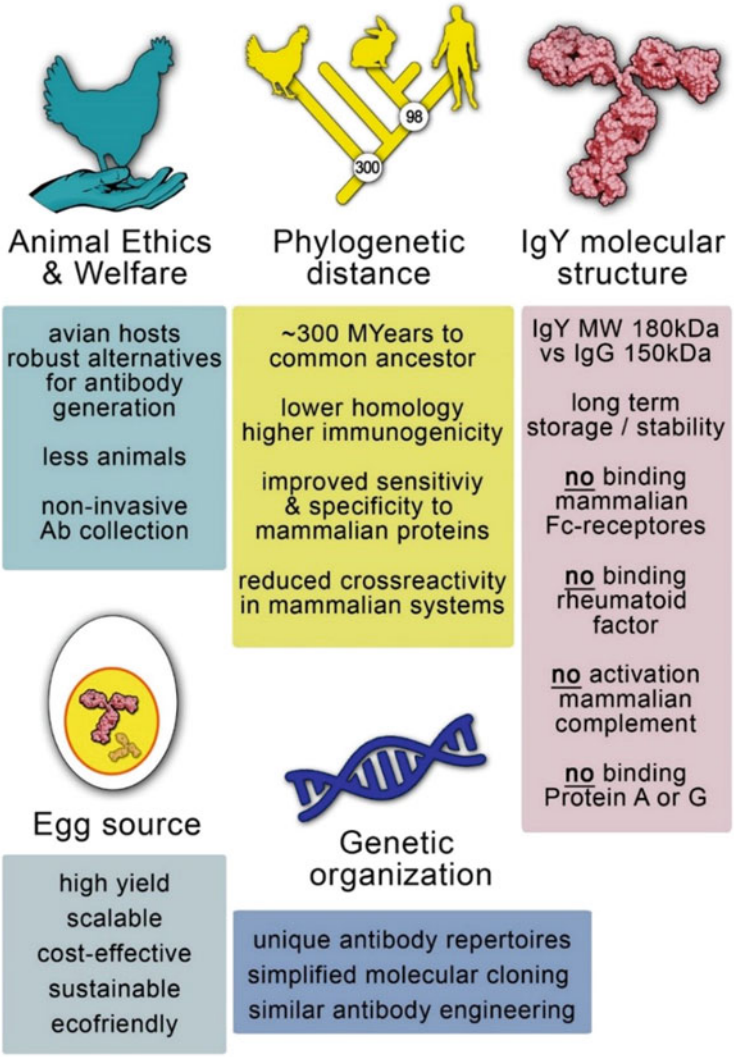

Fig. 1.5 Overall dimensions explored with IgY Technology. Animal ethics and welfare, phylogenetic distance of hosts, antibody crude source, molecular structure of IgY and genetic organization of avian immune repertoires, are the main dimensions grounded on which avian IgY antibody-based technologies have been evolving and growing as promising alternatives to conventional research, diagnostic and therapeutic approaches. Courtesy of IgYTechnology.com (www.igytechnology.com)

tightly controlled, as for e.g., human therapies, one as to account for such technical limitations inherent to any antiserum-like therapies derived from natural sources.

As mentioned above, the IgY molecule, namely its Fc domain (responsible for Fc-receptor binding and signalling functions) does not retain efficient binding

capabilities to Protein A, G or other known microbial-binding proteins used to purify mammalian immunoglobulins. This has been a major limitation in the field for many decades and has driven development and optimization of novel IgY purification methods and approaches. IgY purification is particularly critical for large scale production of functional and therapeutic IgY. Having a simplified method for IgY purification even if only for purification of polyclonal mixtures, would definitely be a major breakthrough and driver for the field (Chap. 11).

The exploitation of avian antibody repertories towards development of novel monoclonal antibodies, faced an initial developmental stage where it was very much undervalued and even neglected. Interestingly, intrinsic technical limitations in translating the hybridoma technology to birds (Nishinaka et al. 1989), namely the absence of an efficient avian tumour cell line, made the approach lag behind in comparison to mammals. Even after the consolidation of phage-display technologies, as birds namely chickens, had become unpopular for monoclonal antibody development, it took some decades for birds to regain attention due to the potential of their distant phylogeny. Currently both phage-display and B-cell sorting technologies enable full exploitation of avian antibody repertories (Chap. 13). On the other hand, the intrinsic avian origin of the selected monoclonal antibodies typically demands further molecular engineering to obtain chimeric molecules with a structural background common to mammalian immunoglobulins.

Implications of all the features, advantages and limitations described above, will be further discussed and detailed in different chapters of the book and will hopefully become clear to the reader.

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The Domestic Hen

2

Shikun Ge, Álvaro Ferreira Júnior, Xiaoying Zhang, and Patricia M. Morgan

Abstract

The egg-laying hen is one of the most common domestic animals. Birds are phylogenetically distinct from mammals and have several unique features: morphological differences such as the presence of air sacs, the medullary bone and cloacal chamber; distinct lymphoid tissues (bursa of Fabricius); and the presence of specific class of immunoglobulin (Ig) termed IgY in egg yolk. Genetic selection of particular traits for laying hens has improved their performance. Currently there are a large number of breeds of hen with different genotypes used for commercial and research purposes. The development of the oocyte and subsequent egg laying is hormonally controlled and formation of the egg as it

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S. Ge
College of Biological Science and Engineering, Shaanxi University of Technology, Hanzhong, China

CMBA, University of Minho, Braga, Portugal

Á. Ferreira Júnior
Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, Goiânia, Brazil
e-mail: alvaro.ferreira@ufg.br

X. Zhang
College of Biological Science and Engineering, Shaanxi University of Technology, Hanzhong, China

Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

CMBA, University of Minho, Braga, Portugal
e-mail: zhxying@snut.edu.cn; xzhang67@uoguelph.ca; zhang@bio.uminho.pt

P. M. Morgan (✉)
Emerita Lecturer, School of Natural Sciences, National University of Ireland Galway, Galway, Ireland
e-mail: pat.morgan@nuigalway.ie

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passes through the oviduct has several defined stages. Hormonal factors affecting the initiation of egg laying and the components of the egg are detailed.

Keywords

Hen · Avian immune system · Reproductive system · Egg

2.1 Introduction

Birds exhibit unique biological features including the ability to fly, the presence of feathers, the ability to maintain a relatively uniform body temperature, migration in response to food sources and climate, and laying eggs. There are almost 10,000 species of birds (Prinzinger et al. 1991), with diverse living spaces, and diverse body structures, such as beak, foot and wing shapes. Avian physiology has two distinct branches; namely the physiology of wild birds (e.g., flight, migration and seasonal breeding) and the physiology of poultry (domesticated birds consisting of predominantly hens but also includes domestic turkeys, quail, ducks, geese, and the more primitive ratites such as ostriches which are discussed in Chap. 9.

2.2 Genetic Selection for Domestic Breeds of Chicken

Within domestic breeds of chicken there are enormous differences in both their genetic makeup and phenotypes (Tixier-Boichard et al. 2012). The differences in modern breeds (*Gallus gallus domesticus*) are probably as a result of their ancestral development from geographically separated subspecies of red jungle fowl (*Gallus gallus gallus*; *Gallus gallus spadiceus*, *Gallus gallus jaboullei*; *Gallus gallus murgha*) and a second species, grey jungle fowl *Gallus sonneratii* (Rubin et al. 2010; Tixier-Boichard et al. 2012) and cross breeding during several rounds of domestication. Whole-genome sequencing shows that several loci have been subjected to strong selection during domestication of chickens and specialization into meat producing (broiler) and egg producing (layer) chickens which are the subject of this chapter (Preisinger 2018; Qanbari et al. 2019). The genetic selection of laying hens resulted in reduced body weight, a shift to earlier sexual maturity, an increase in the quantity and quality of egg production, and decreases in the feed necessary to produce the same number of eggs (Preisinger 2018). Recent genetic trends in egg output, show an increase from 313 to 325 eggs per year from 2010 to 2015 and selection for this trait is likely to result in a further increase of two to three eggs per year (Preisinger 2018). Laying hens still retain some of the biological characteristics of birds, such as the habit of foraging and constant movement, acute hearing, acute daytime vision, neurotic characteristics, utilizing a wide range of food by eating gravel to grind it (Thiruvenkadan et al. 2010); traits which need to be considered in keeping hens (Chap. 8).

2.3 Breeds of Chicken

It is important to note at this juncture that genetic differences exist between different breeds of *Gallus gallus domesticus*: these result in different immunological adaptations as determined in whole blood (Bílková et al. 2017); nest use patterns (Villanueva et al. 2017); excitability and preference for enrichment elements (Kozak et al. 2019); timing of egg laying (Tůmová et al. 2017) and egg laying capacity (Mack et al. 2013; Table 2.1). The most common commercial laying hens include Romain Brown Shell Layer, Hyland Brown Shell Layer, Fresh Brown Shell Layer, Hay Sykes Brown Shell Layer, Elsa Brown Layer, Golden Brown Shell Layer, White Leghorns, Rhode Island Red, Golden Comet, Ameraucana, Barred Plymouth Rock, Golden Laced Wyandottes and New Hampshire Red (Agritech 2009; Ruppenthal 2012). Other breeds of chicken utilized in research in addition to the more commonly used White Leghorn and Rhode Island Red are the following: Anraja, Gramapriya, BlackRock, KalingaBrown (Agrawal et al. 2016); Araucana, Booted bantam, Czech, Minorca and Rosecomb bantam (Bílková et al. 2017); Greenlegged Partridge and Polbar (Kozak et al. 2019); Hy-Line Brown, Bovans Brown, DeKalb White, and Hy-Line W36 (Ali et al. 2016); Silkie and Dongxiang blue-shell (Sun et al. 2013); Erlang Mountainous (Liu et al. 2015); Bovans Sperwer, Isa Sussex, Moravia Barred and Moravia BSL (Tůmová et al. 2017).

Layers produce the most eggs in the first year, with 15%–20% decrease in egg productivity and 15% increase in mortality in the second year (Parkhurst and Mountney 1988). Peak production now approaches the biological limit of one egg per day, but in early production (at sexual maturity) and late production (persistency) genetic variation is still high (Thiruvenkadan et al. 2010).

Apart from breed difference, egg production is closely related to the physical fitness, including physical function, cognitive ability and living habits of hens. Hens with high annual egg production tend to live longer (Villanueva et al. 2017; Dudde et al. 2018). Many regions have rich varieties of laying hens' resources. Compared with the major commercial hens in the world, these hen breeds have received less attention, and they are marginalized by the extensive cultivation of commercial hens (Yang et al. 1996). However, such local layer resources are conducive to the preservation of biodiversity the genome and physiology of the hen and may be important resources for IgY technology.

2.4 Physiology of Chickens

The general physiological parameters of domestic chickens will now be discussed.

2.4.1 Body Temperature

Many birds undergo long distance migration and can adapt to extreme environments (e.g., hot, cold, and desert areas). Despite this, most birds maintain a relatively