Chicken Egg Yolk Antibodies, Production and Application

Polyclonal antibodies, widely used in research and diagnostics, are conventionally isolated from the blood of immunised mammals, especially rabbits. The fact that antibodies can also be detected in the yolk of eggs laid by immunised hens, led to the development of the yolk antibody technology as an alternative method less stressful to animals. Since hens can be kept under nearly natural conditions and antibodies be isolated from the collected eggs, this technology has become an interesting alternative to the blood-taking techniques.
For their work on yolk antibodies, the authors of this book received the FISEA prize (International Foundation for the Substitution of Animal Experimentation, Luxembourg) in 1997.
Protocols on how to keep and immunise hens and on the extraction, isolation and use of antibodies from yolk are described in detail in this manual. These practical instructions are complemented by a short introduction to the hen's humoral immune system and a section on the pros and cons of chicken yolk antibodies compared to those of mammals' serum. Avian antibodies have been shown to be in some applications even more effective than mammalian antibodies, especially when phylogenetically highly conserved antigens have been used for immunisation.

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IgY-Technology

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Preface

As early as 1893 Klemperer published his observation that there must be neutralising proteins (i.e. antibodies) in the yolk of eggs laid by immunised hens. As is often the case in the history of science, this finding was hardly acknowledged and even ignored for a long time. Only later, when animal protection came to be regarded as a serious ethical claim for the scientific community as well, did these results came back to mind. Animals are now perceived as fellow creatures in need of protection, so the extraction of specific antibodies from the eggs of immunised hens was regarded as an attractive alternative to blood-taking methods.

In 1992 – nearly a century after the original discovery – yolk immunoglobulins became the topic of a joint research project. Various research groups which already had experience with yolk antibodies started to work together and were financially supported by the German Ministry for Education and Research. The aim of this project was to find out whether these avian antibodies are as effective as the traditionally used polyclonal antibodies from mammals, especially from rabbits. It could be shown that in the case of phylogenetically highly conserved antigens, avian antibodies are even more effective than mammalian antibodies.

The project went on for six years, during which IgY-technology won increasing attention and acceptance. There are now commercially available cages for keeping hens under acceptable conditions. In addition to laboratory protocols for extracting IgY from yolk, there are also easy-to-use commercial kits for the isolation of immunoglobulins. Also, secondary antibodies coupled to detectable markers are now available. Within a few years, IgY-technology has developed to a recognized alternative which is less stressful to animals than the conventional procedures. In

other words, there is no longer any excuse for not using this well established technology.

In acknowledgement of their work on yolk antibodies, the scientists working together in the joint project received the FISEA prize in1997. FISEA – the International Foundation for the Substitution of Animal Experimentation, Luxemburg – awards this prize to researchers working on new alternative methods that eleminate or reduce the use of animals or are less stressful to animals.

Apart from the animal protection aspect, there are some advantages in using yolk antibodies compared to mammalian. For instance, a single immunised hen can yield an amazing abundance of antibodies, many of which show a different specificity than those a rabbit would produce after the same immunisation. These benefits have already led to scientific results which could not be achieved with mammalian antibodies, neither with polynor monoclonals.

The only obstacle was the lack of a reliable collection of protocols and laboratory instructions for this technology. Researchers or students who wished to apply this technique, were still obliged to search for information from literature in various fields of specialisation. This book now presents all relevant protocols and information needed to use IgY-technology easily and successfully.

The protocols are based on our own experience and on those of our colleagues working in the same field. All procedures have been tested and most have already reached a routine level. They are presented in an easy-to-follow way, supported by many practical hints and notes. Included are detailed instructions on how to keep and immunise hens and protocols for the extraction of antibodies from yolk and for the various applications. The protocols were selected with the aim to cover as broad a spectrum as possible. In some cases, it will of course, still be necessary to refer to special literature. Therefore, a list of the relevant references is given in the appendix.

We do hope that this laboratory manual will enable also the unexperienced scientist and everybody working in a laboratory to use this technology without initial problems.

We are aware that one can never avoid some mistakes finding their way into a text and wish to apologise for these possible errors in advance. We would welcome any suggestions which support the book's aim to disseminate IgY-technology and thus serve the protection of animals.

Berlin, Leipzig
Juli 2000

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Abbreviations

Ab	antibody
ABTS	2,2'-azino-bis(etylbenzthiazoline-6-sulfonic acid)
AGIDT	agargel double immunodiffusion test ("Ouchterlony"-technique)
AI	avidity index
ALP	alkaline phosphatase
BGG	bovine gamma globuline
BSA	bovine serum albumine
CCK	cholecystokinin
CD	cell differenciation
CRP	C-reactive protein
DAB	diaminobenzidine
DMSO	dimethylsulfoxid
DNP	2,4-dinitrophenol
ECVAM	European Centre for the Validation of Alternative Methods
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
FACS	fluorescence activated cell sorter
FCA	Freund's complete adjuvans
FIA	Freund's incomplete adjuvans
FITC	fluorescein isothiocynate
GABA	γ-aminobutyric acid
HPR	horseradish peroxidase
HSA	human serum albumin
i.d.	intradermal
IE	immunoelectrophoresis
IEA	immunoelectrophoretic assay
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M

IgY	immunoglobulin Y
i.m.	intramuscular
i.p.	intraperitoneal
ISCOM	immune stimulating complex
i. v.	intravenous
kDa	kilo Dalton
KLH	keyhole limpet hemocyanine
LPS	lipopolysaccharide
mAb	monoclonal antibody
MAP	multiple antigen peptide
MBP	myelin basic protein
MDP	muramyldipeptide
MHC	major histocompatibility complex
OPD	ortho-phenylenediamine
PAGE	polyacrylamide gelelectrophoresis
PBS	phosphate-buffered saline
PCSL	Pam_3Cys-Ser-$(Lys)_4$ – lipopeptide
PEG	polyethylenglycol
pNPP	p-nitrophenylphosphate
POD	peroxidase
RIA	radio immunoassay
RIE	"rocket" immunoelectrophoresis
rpm	revolutions per minute
RZ	Reinheitszahl
s.c.	subcutaneous
SD	standard deviation
SDS	sodium dodecylsulfate
SoAg	somatic antigen
SPF	specific pathogen free
SRID	single radial immunodiffusion ("Mancini"-technique)
TBS	tris-buffered saline
TMB	tetramethylbenzidin
Tris	Tris-hydroxymethyl-aminomethan
TRITC	tetramethylrhodamine isothiocynate
v/v	volume/(total)-volume
w/v	weight/ (total)-volume

Contents

Short Introduction to Hens' Humoral Immune System

MICHAEL ERHARD and RÜDIGER SCHADE

Introduction

The hens' immune system differs from mammals' in various ways. In this brief introduction we shall try to give a basic idea of the special features of the hens' humoral immune system, especially as regards the structures and functions of antibodies.

Like the immune system of mammals, that of hens is divided into two major components, one of them being non-specific and innate and the other specific and acquired. Naturally between the two there are numerous interactions of critical importance in the event of an immune response. Hens too, for instance, have antigen-presenting cells, and many functional mechanisms are regulated via interleukines. Unfortunately there are few data about hens, since there has been less research internationally into the immune system of hens than into that of rats and mice. But hens do have a feature not shared by mammals, as Klemperer (1893) pointed out: the passive immunity of the offspring, which in mammals reaches the foetus through the placenta or colostrum, has to come through the fluid parts of the egg in ovipars. While the egg is still in the ovary, hens transfer their serum immunoglobulin Y (IgY) into the yolk. As the egg passes down the oviduct, IgM and IgA from oviduct secretion are required with the albumin.

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