Egg Yolk Antibodies, State of the Art and Future Prospects

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Summary

Immunisation of chickens and extraction of antibodies from egg yolk belongs to the alternative methods since the animals suffering is reduced by non-invasive antibody-sampling. Also, the number of animals needed to produce a certain amount of antibody can be reduced since chickens produce a significant higher antibody quantity than rabbits. Despite its several advantages this technology (IgY-technology) is rather scarcely used. Traditional behaviour as well as limited or no information at all may hamper a broader acceptance at present. However, significant arguments exist in chicken housing, the choice of appropriate IgY-extraction methods and a lack of information regarding the use of IgY-antibodies. This paper intends to give a short introduction in the IgY-technology, to briefly discuss the state of the art and to inform on recent developments and discussions in this field. The suitability of IgY for special fields of application (as a result of the structural differences between IgY and IgG) is emphasised (e.g. assays combining IgG and IgY, immunisation of chickens against highly conserved antigens). In addition, it is stressed that the IgY-technology as an alternative method can particularly integrate requirements of animal protection (reduce, replace, refine), science (characteristics of avian immune system and resulting properties of IgY) and economy (amount of IgY produced from one chicken).

Zusammenfassung: Eidotter-Antikörper, gegenwärtiger Stand und Ausblick


Keywords: IgY-technology, antibodies, egg yolk, IgY, alternative

1 Introduction

During the last decades the biomedical research has been confronted with growing public interest in the welfare of animals used in experimental investigations. This public discussion encouraged a search for appropriate alternatives in animal experimentation suited to reduce, refine or replace suffering of animals or animal experiments at all. That concerns also the production of monoclonal and polyclonal antibodies which are important tools in bio-medical research. Such antibodies are essential components of a variety of diagnostic systems used for quantitative as well as qualitative determination of several substances. Normally, laboratory rodents (frequently rabbits, but also mouse, rats or guinea pigs) or mammals like horse, sheep and goat are used for antibody production. This procedure involves two steps causing physical and psychical stress. The first step is the immunisation itself, whereas the second step consists in the bleeding of animal as a prerequisite for antibody preparation.

For about hundred years (Klemperer, 1893, see fig 1) it is known that specific antibodies of immunised chickens are being concentrated in egg yolk to a similar extent as compared with serum concentration (there are contradictory data in literature, e.g. Larsson et al., 1993, found that the IgY is higher concentrated in the yolk than in the serum). In contrast to the serum immunoglobulins only one class of immunoglobulin is found in the egg yolk (traces of other Ig classes are negligible) which usually is called IgY, but also terms as IgG (because this chicken Ig isotype resembles closely mammalian IgG) or IgA (results from immunological investigations started from a phylogenetical point of view).
are sometimes used. Thus, using chickens for antibody production, the second painful step, the blood collecting, can be replaced by antibody extraction from egg yolk. Therefore, this method belongs to the alternative methods because of an objective reduction of suffering in animals. We call this antibody-production IgY-technology because this procedure includes several aspects like e.g. chicken housing conditions, egg laying behaviour, immunisation protocols, antibody-extraction methods, particular properties of IgY, particular IgY application and molecular IgY structure (see fig. 2).

Although the antibody-production in chickens offers some advantages (see table) it is not as widely used as could be expected. That may be due to several reasons like traditional behaviour, lack of knowledge and experience or incorrect information about the procedures. According to our opinion there are two main arguments which hamper a wider acceptance and application of the egg yolk antibody. These are problems with chicken keeping and problems with antibody extraction. In the following we discuss some of the basic requirements/knowledge to the practice of IgY-technology.

**IgY - Technology**

- housing conditions
- immunisation
- adjuvant
- titre development
- IgY extraction
- IgY-characteristics
- IgY-application

![Figure 2: Summary of the several aspects which are included in the term IgY-technology.](image)

2 **Chicken housing**

Immunisation of chicken with subsequent collection of eggs for antibody production requires appropriate keeping conditions which should meet to a large extend with the natural behaviour of the bird.

Regarding housing adequate proposals have been made:

- Housing in cages in groups of two hens (conventional keeping conditions/SPF-condition).
- Housing in groups on floor (inside/outside).

Ad 1 There are commercial cages (128x65x80 cm for two chickens) available which are recommended by the Swiss Veterinary Administration (Schweiz-

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**ARCHIV**

**FUR**

**EXPERIMENTELLE PATHOLOGIE**

**UND**

**PHARMAKOLOGIE**

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**Figure 1: The original title of Klemperer's paper, a basic work in the history of IgY antibodies.**

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Table 1: Characteristics of avian IgY in comparison with mammalian IgG

<table>
<thead>
<tr>
<th>Ab-sampling</th>
<th>IgG</th>
<th>IgY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>invasive</td>
<td>non-invasive</td>
</tr>
<tr>
<td>Ab-amount</td>
<td>200 mg IgG/bleeding</td>
<td>50-100 mg IgY/egg</td>
</tr>
<tr>
<td></td>
<td>(40 ml blood)</td>
<td>(5-7 eggs/week)</td>
</tr>
<tr>
<td>Ab-amount/monthly</td>
<td>200 mg IgG</td>
<td>ca. 1.500 mg IgY</td>
</tr>
<tr>
<td>Amount of specific Ab</td>
<td>ca. 5 %</td>
<td>2-10 %</td>
</tr>
<tr>
<td>Protein A/G binding</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Interference with mammalian IgG</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Interference with rheumatoid factor</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Activation of mammalian complement</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>

The data are summarised according to a review published in ATLA (Schade et al., 1991).

Lelystad, The Netherlands there are differences between several chicken lines with respect to the amount of antibody-synthesis. This may be due to different breeding aims: high egg and meat production instead of antibody synthesis. For example, some inbred chicken lines have been shown to be low-responders to proteins like Salmonella pullorum bacterin, human serum albumin and synthetic peptides in contrast to the lines of the B14, B17 and B21 haplotype which are good responders against proteins.

4 IgY-extraction

There are several methods for IgY-extraction (including commercial extraction kits) from egg yolk as discussed in more detail by Dr. Schwarzkopf. One of the most frequently used extraction procedures is the precipitation of protein by ammoniumsulfate, dextran- sulfate or polyethylene glycol. Additionally, separation by chromatographic procedures based on several ion exchange materials are also used. In this context, the main problem is rather the great number of extraction methods than a lack of them. That may discourage potential users of IgY-technology because they find it difficult to select the one method suitable for their specific requirements. In addition, the choice of a certain extraction procedure may also be influenced by the antibody-application planned and the laboratory equipment available. Thus, at present it is difficult to give general recommendations for or against certain extraction procedures. To overcome this situation, a comparative study including criteria like antibody-yield, antibody-purity, antibody-activity, costs and time has been planned during an ECVAM workshop on “Avian antibody”, held in Berlin in March 1996.

4 Immunisation protocol, adjuvant, titre-development

It is common practice to use the i.m. route for immunisation of chickens with the musculus pectoralis as application site. But s.c. injection is also recommended and sometimes may give better results than i.m. immunisation as was discussed at the ECVAM-workshop mentioned above. Booster-injections are performed frequently following a time interval of at least 4-6 weeks. The first immunisation (and also 2–3 booster injections) should be performed before the chickens start to lay eggs. In this way disturbance of egg laying activity can be avoided.

The tissue damaging effects of FCA in comparison with alternative adjuvants have been discussed in detail by Dr. Schmidt (page 30). The crucial point of this topic is the high efficiency of FCA concerning antibody-titre development when compared with alternative adjuvants. In addition, it is difficult to generalise such results since the titre development depends on both the adjuvant and the antigen used. Thus, many different combinations are possible and consequently, many different results can be recorded. However, recommendations can be given based on criteria of animal welfare. Compared to FCA, the cost/efficiency ratio of alternative adjuvants is frequently rather unprofitable.

Further criticism stems from the fact that the antibody-titre development in chickens can not be easily compared with that of rabbits. The study of Dr. Behn (page 18) showed for the first time, that typical titre development as reported for chickens (Bar-Joseph and Malkinson, 1980) may depend on the amount of antigen injected and the use of adjuvant. Otherwise, the titre development strongly resembles titre curves as described for immunised mammals.
5 Advantages and disadvantages of IgY-technology

Aside from the aspect of animal welfare as well as the remarkable amount of antibodies which can be obtained from one chicken (see tab. 1) the use of avian antibodies offers further advantages. The molecular structure of avian antibodies shows significant differences in comparison to mammalian antibodies (see fig. 3, Shimizu et al., 1992). In addition, the phylogenetic distance between the two vertebrate classes Aves and Mammalia may also contribute to a reduction of cross-reactivity with mammalian Igs. That, in general, can be of advantage concerning background staining in several diagnostic systems and may be of particular interest in case of interference by rheumatoid factors (RF, see contribution of A. Rieger, page XX) or complement activation. Such interferences can be avoided by the use of avian antibodies in these diagnostic systems since avian antibodies are unable to activate the mammalian complement system and do not show cross-reactivity with RF (Larsson et al., 1993). Consequently, the combination of avian with mammalian antibody in many immunological systems can be recommended and this might develop into a field of application for IgY. In contrast to a „silent“ mammalian immune system against certain antigens there is now growing evidence that it is possible to elicit an antibody-response in chickens in these critical cases. This, in particular, concerns highly conserved antigens (Goueli et al., 1990; Gassman et al., 1990; Rosol et al., 1993; see the contribution of Dr. Gerl, page XX). Thus, the immunisation of chickens frequently may be an alternative if an antigen fails to activate an immunogenic response in mammals.

Apart from the advantages of the IgY antibodies as discussed above there are some points of criticism. One of the them is the argument that IgY failed to bind to protein A or G, an important property of IgG with respect to a simple IgG-isolation. According to our opinion this is no substantial deficit since there exist sufficient simple procedures for IgY-extraction. Beyond that, the search for a „protein A/G-like“ IgY isolation procedure is subject of the project „Avian antibody“ supported by a grant of the BMBF (Bundesministerium für Bildung und Forschung). Another point is the different precipitation behaviour of IgY. In principle, the IgY antibodies are able to precipitate an antigen similar to mammalian IgG, but optimal precipitation can be seen by increasing the salt concentration in the buffer solution. That may hamper the application of IgY in turbidimetric or nephelometric systems (Dr. Larsson, personal communication).

In summary, there are numerous arguments and practical indications (see this supplement volume) for supporting a broader use of IgY antibodies. During the ECVAM workshop, mentioned above, some aspects of IgY-technology were discussed by representatives of several European universities, enterprises and governmental agencies resulting in recommendations and cooperation of several laboratories concerning this subject. It is expected that the results of the workshop (which will be published in ATLA) and the activity of the participants will contribute to an increasing interest and provide information for potential users of IgY-technology in Europe. We believe that this alternative method is well suited to integrate the interests of animal protection, science and commercial production (fig. 4). However, we also have to stress that there is no justification to insist on a complete substitution of mammalian antibodies. The IgY antibody differs from IgG not in the sense of a higher or lower diagnostic quality but in a different philosophical sense altogether. Thus, the advantages of IgY may be utilised to improve diagnostic tools in combination with mammalian IgG and, in addition, the use of IgY sometimes offers new approaches for scientific problems. However, when starting an antibody production we should take into account the animal welfare and consequently it should be considered whether the immunisation of a chicken instead of a mammal could provide the desired results.

Figure 3: This figure compares simplified molecules of IgG and IgY. Demonstrated are differences concerning the number of constant domains of the heavy chain as well as carbohydrate chains. The zig-zag line in the molecule model symbolises the hinge region. There is a remarkable difference between IgG (rabbit) and IgY (chicken) since this region is much more flexible in mammalian immunoglobulin (the figure is a simplified schematic view according to a figure shown by Shimizu et al., 1992).

Figure 4: The figure symbolised the idea that the IgY-technology has the potency to integrate interests of animal welfare concerning the three R, interests of science (characteristics of the avian immune system) and economic interests (amount of antibody to be obtained from one chicken).
References


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Freunde und Förderer der Anwendung alternativer Antikörper e.V.

Ein Verein stellt sich vor


In diesem Supplementband ist erschöpfend dargestellt, daß die IgY-Antikörper im Vergleich zu mammären Antikörpern (IgG) für viele Anwender Vorzüge aufweisen. Die Forschung auf diesem Gebiet ist innovativ und eröffnet Möglichkeiten, die mit mammären Antikörpern nicht zu erreichen sind.

Für Spenden zur Unterstützung dieser Forschung sind wir Ihnen dankbar (s. untenstehendes Konto). Wenn Sie Mitglied werden möchten, senden wir Ihnen gerne eine Satzung des Vereins zu.

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