Journal of Immunological Methods 342 (2009) 58–63

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Enhancement of anamnestic immunospecific antibody response in orally immunized chickens

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ARTICLE INFO

Article history:
Received 30 September 2008
Received in revised form 21 November 2008
Accepted 25 November 2008
Available online 25 December 2008

Keywords:
IgY antibodies
Oral immunization
Immunological memory
Animal welfare

ABSTRACT

Production of immunospecific egg yolk antibodies (IgY antibodies) in egg laying hens through oral immunization is an attractive alternative to conventional antibody production in mammals for economic reasons as well as for animal welfare reasons. Oral immunization results in a systemic humoral response, but oral booster immunizations lack efficiency. The aim of the present study was to develop immunization schemes in which the concentration of immunospecific IgY would increase following oral booster immunizations. Two groups of egg laying hens (5 in each group) were immunized orally (each immunization event consisted of dosing on three consecutive days) with Bovine Serum Albumin (BSA) in combination with RhinoVax® (RV) using different immunization schemes. A 3rd group served as a reference and received BSA emulsified in Freund's Incomplete Adjuvant (FIA) by subcutaneous injection three times and one oral dose with BSA+RV. The eggs of the chickens in this group had a significantly higher immunospecific anti BSA IgY-concentration than did any of the eggs from the orally immunized chickens. One of the immunization regimes (immunizations in weeks 1, 7 and 18) clearly included a booster effect of the immunization in week 18, demonstrating the presence of memory cells following the two initial oral immunizations. Considering that oral immunization results in approximately ten times lower concentrations of immunospecific antibodies in the egg yolk, compared to traditional subcutaneous immunization schemes, the oral immunization routines have to be further refined to compete with parenteral immunization protocols.

1. Introduction

Mice and rabbits are the most commonly used species for polyclonal antibody production (UK HO statistics, 2007). The traditional way of production is parenteral administration of antigen (immunogen) combined with an adjuvant followed by blood sampling and harvest of antibodies from serum over a given period of time? Jensenius et al. (1981) demonstrated that antibodies could be purified in generous amounts from the egg yolk of immunized chickens, thus eliminating invasive blood-sampling procedures. Since egg laying hens produce large amounts of egg antibodies during the period of laying, the productivity is much greater than from a similarly sized mammal. Older egg laying hens produce more antibody than do young egg layers (Bollen and Hau 1999). More than 2 g of IgY can be isolated per month from the eggs of one egg laying hen, while only about 200 mg IgG can be isolated from a rabbit. The chicken antibodies are thus ten times less expensive to produce (Bollen et al., 1996). The production of polyclonal antibodies in chicken is therefore an attractive alternative to conventional antibody production in mammals (Hau and Hendriksen, 2005).

Abbreviations: AU, Arbitrary Units;BSA, Bovine Serum Albumin;CTB, Cholera Toxin B-subunit;ELISA, Enzyme Linked Immunosorbent Assay; ECVAM, European Centre for the Validation of Alternative Methods;FIA, Freund's Incomplete Adjuvant;IFER, The International Foundation for Ethical Research;IgY antibodies, Egg yolk antibodies;OPD, O-phenylenediamine dihydrochloride-substrate;PBS, Phosphate Buffered Saline;RV, RhinoVax®.

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doi:10.1016/j.jim.2008.11.017

By replacing the traditional routes of administration (subcutaneous, intramuscular, intradermal injections) with voluntary oral feeding of the antigen, all stress on the chickens associated with capture, handling, restraint, and injection could be eliminated. Not surprisingly the European Centre of the Validation of Alternative Methods (ECVAM) recommends that yolk antibodies should be used instead of mammalian antibodies for animal welfare reasons (Schade et al., 1996, 2005). There are also practical reasons why oral immunization combined with harvest of antibodies from the egg yolk is an attractive alternative. Provided that conventional animals can be used, these procedures are no different from other agricultural production procedures. Consequently, the chickens do not have to be housed in facilities approved for housing laboratory animals. Furthermore, there is no requirement for personal and project licenses and staff with specialist competence in laboratory animal science. Therefore, future production of polyclonal antibodies would no longer be a regulated procedure according to the EU's directive on protection of animals used for experimental and scientific purposes (Council Directives, 1986) and the European Convention ETS 123 (1986).

IgY is transported to the yolk from the circulation, in which compartment it is termed IgG, probably by receptor-mediated endocytosis (Cutting and Roth, 1973), and is the only immunoglobulin detectable in the yolk, while IgA and IgM are present in the white (Rose et al., 1974). The concentration of IgY in egg yolk is independent of egg size but proportional to the maternal serum IgY concentration (Woolley and Landon, 1995, Bollen and Hau 1997).

Oral immunization with antigens in solution leads to oral tolerance in most mammals but rarely so in the chicken (Klipper et al., 2000) in which a humoral response is often mounted. The use of selected adjuvants results in an increased circulating antibody response. RhinoVax® (RV) (a pegylated mono/di-glyceride, formerly known as Softigen®) and glutaraldehyde-conjugated CTB (Cholera toxin B-subunit) have both been shown to possess strong immunopotentiating effects when administered orally in combination with antigen to chickens (Persdotter Hedlund and Hau, 2001). Mayo et al. (2003) demonstrated a 4–10 times higher immunospecific antibody concentration in chickens immunized with relatively high BSA concentration in combination with either RV or CTB compared with chickens immunized without an adjuvant. CTB is in itself very immunogenic and its adjuvanticity has been demonstrated to be correlated with its immunogenicity (Mayo et al., 2005).

Our recent studies have indicated that a change of immunization procedure from single administrations to administration daily for three or five consecutive days may result in higher concentrations of immunospecific antibody. Three-day oral immunization schemes — using BSA as the antigen — proved as effective as a five day administration scheme (Mayo et al., 2006). Although memory is established after oral immunizations (Mayo et al., 2006), repeated oral immunizations often fail to result in the boosting effect, which is the classical response in animals immunized parenterally (Hajishengallis et al., 1996; Kaden et al., 2004).

The aim of the present study was to optimize oral immunization schemes to ensure the presence of high concentrations of immunospecific antibody following repeated administrations of antigen in combination with the adjuvant RV.

2. Materials and methods

2.1. Animals and husbandry

A total of 20 (one chicken died spontaneously during the study) White Leghorn (Line 44) layers (*Gallus domesticus*) from GAL AB breeding stock housed at Söderby gård, Uppsala, Sweden were used for the present study. They were kept in single metal cages (h: 65 cm, l: 48 cm, d: 41 cm). The room temperature varied between 18 and 21°C and the light cycle was 8 h dark and 16 h light. Crushed food pellets containing 15% protein (Johan Hansson, Uppsala, Sweden) and drinking water were available *ad libitum*. The chickens were 20 weeks old at the beginning of the study.

2.2. Immunization protocol

Five chickens were randomly allocated to each of the four study groups (Table 1). Groups 1 and 2 received immunization with BSA administered by gavage into the crop. This procedure was for convenience termed oral immunization. Group 1 during four treatment periods and group 2 during three periods. Group 3 received three consecutive subcutaneous injections with BSA followed by one oral treatment period with BSA. Group 4 received distilled water orally during three treatment periods. A treatment period consisted of three consecutive days of oral immunization. The immunogen mixture, which consisted of 200 mg BSA (Sigma-Aldrich)/ml dissolved in MilliQ H_2O with 20% RhinoVax®, the dose volume was 0.5 ml per day. Group 3 chickens received subcutaneous injections with a mixture of 20 mg BSA/ml in 50% Freund's Incomplete Adjuvant (FIA) (Sigma-Aldrich) total volume 0.2 ml, and served as positive controls. In week 18, group 3 chickens received BSA+RV as an oral booster. Group 4 was used as a negative control group and the chickens in this group received water by gavage at relevant

Table 1
Immunization scheme for all four groups

Group no.	Week 1	Week 4	Week 7	Week 18	Chickens/group
1	p.o. BSA+RV	p.o. BSA+RV	p.o. BSA+RV	p.o. BSA+RV	5
2	p.o. BSA+RV	p.o. H_2O	p.o. BSA+RV	p.o. BSA+RV	5
3	s.c. BSA+FIA	s.c. BSA+FIA	s.c. BSA+FIA	p.o. BSA+RV	4[a]
4	p.o. H_2O	p.o. H_2O	p.o. H_2O	–	5

BSA = Bovine Serum Albumin, RV = RhinoVax®, FIA=Freund's Incomplete Adjuvant, p.o. = oral administration, s.c. = subcutaneous administration.
[a] One chicken died during phase 2.