IgY-Technology Applied to Studies of Toxoplasma gondii Infection

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Abstract
In this chapter, we describe relevant aspects of immunoglobulin Y (IgY) technology for Toxoplasma gondii applications, including comparison of avian IgY antibody with mammalian IgG antibody, egg yolk IgY production and isolation procedures, important applications for IgY antibody, and state of the art and perspectives for IgY-technology in T. gondii studies. T. gondii is a worldwide public health problem. IgY-technology provides an alternative antibody (IgY) to mammalian Immunoglobulin G (IgG) antibody. IgY-technology involves the chicken immunization, yolk IgY isolation, antibody characterization, and purified IgY application to several kinds of methods. Immunized chicken transfers a specific IgY from blood to egg yolk. Phylogenetic distance between chickens and mammals influences the generation of antibody repertoires recognizing an antigen profile. IgY is not bound to rheumatoid factor or mammalian complement protein and thus avoids the false-positive results. Yolk IgY isolation is carried out by simple procedures that are accessible for any laboratory and, also, for IgY isolation at large-scale production. IgY-technology provides antibodies for proteomic studies, diagnostic assays, and immunotherapy. Although IgY-technology is promising, there is a reduced number of investigations with IgY and T. gondii. Future perspectives involve the use of IgY-technology for the screening of new T. gondii antigens for diagnostics, therapy, or vaccine, development of innovative techniques for toxoplasmosis diagnostics and may be an immunotherapy for toxoplasmosis.

Keywords: antibodies, chicken, toxoplasmosis, immunoassay, immunotherapy
1. Introduction

Toxoplasma gondii is the toxoplasmosis agent in human and animals and is a worldwide public health problem. Alternative approach that offers possibility to improve diagnostics, treatment, or control of toxoplasmosis should be accepted as a protective approach to hosts at risk of infection by T. gondii. Immunoglobulin Y (IgY)-technology was designed in 1980s with the aim to reduce the suffering procedures to obtain a high-quality antibody, replace animal model for antibody production and refine the kind of applications for purified antibodies. IgY-based antibody production derived from chicken egg yolk antibody is named immunoglobulin Y (IgY). Hens transfer IgY from blood to egg yolk protecting their offspring. Isolation of yolk IgY avoids bleeding the chicken and reduces the number of painful procedures. Hens can lay approximately one egg each day during a period of 2 years. IgY antibody is functional and is equivalent to mammal IgG antibody. Both avian and mammalian antibodies have structure similarity. They have two identical light chains and two identical heavy chains bounded to each other by disulfide bonds. In the amino terminal extremity, they have the fragment antigen-binding (Fab). In the carboxy terminal region, they have the fragment crystallizable (Fc) that is associated to biological antibody functions as interaction with cells or molecules from immune system. Virtually, any substance can be the target of an antibody response and the response to even a single epitope comprises many different antibody molecules, each with a subtly different specificity for the epitope and a unique affinity, or binding strength. The total number of antibody specificities available for an individual is known as the antibody repertoire. The number of antibody specificities present at a particular time is limited by the total number of B cells in an individual, as well as by each individual’s previous encounters with antigens. Albeit, they are equivalent in function and structure, the phylogenetic distance and the way to generate antibody specificities produce difference in epitope recognition repertoire between avian and mammalian host. Additionally, modifications in amino acid sequence of IgY antibody result in advantages, comparing to IgG, as follows: IgY does not bound to rheumatoid factor, Fc receptor or mammalian complement proteins that can avoid false-positive results, and many others. Isolation of yolk antibodies begins by removing lipid fraction from yolk and obtaining a water-soluble fraction, and further procedures including salt-based precipitation, gel filtration, or affinity chromatography provide a high-purity IgY-enriched fraction. Purified IgY antibody can be applied to several kinds of techniques including screening new antigens by phage display, enzymatic or florescence immunoassays, diagnostics, and, also, passive immunization for therapy against gastrointestinal pathogens in humans and animal hosts. Future perspective using IgY-technology as a tool for T. gondii investigation should include development of new antigen libraries as an innovative source of possible targets for diagnostics, therapy, or vaccination. In addition, create new diagnostic platforms for diagnosing T. gondii infection. Finally, the potential protective effect of IgY to be used in prevention or toxoplasmosis therapy by passive immunization should be explored. In this chapter, we describe relevant aspects of IgY-technology to T. gondii investigations, including comparing avian IgY antibody with mammalian IgG antibody, egg yolk IgY production and isolation procedures, important applications for IgY antibody, and state of the art and perspectives for IgY technology in T. gondii studies.
2. Overview in IgY technology

2.1. Comparing avian IgY antibody with mammalian IgG antibody

2.1.1. General history

In the early 1893, Klemperer published his observation that there must be neutralizing proteins (i.e., antibodies) in the yolk of eggs laid by immunized chickens. In 1980s, when animal welfare came to be regarded as a serious ethical claim for the scientific community, the extraction of specific antibodies from the egg yolk was considered as an alternative to the blood-taking methods. In this context, phylogenetic distance between chickens and mammals, mechanisms of antibody repertoire diversification, and the way in which chickens deposit IgY immunoglobulin in the egg yolk provide a number of advantages compared to mammals as animal model for antibody production. In an additional manner, the one-step purification of antibodies from egg yolk in large amounts provides a virtually continuous supply; the epitope repertoire recognition of IgY antibodies potentially grants access to new specificities; the absence of cross-reactivity with mammalian epitopes avoids false-positive interference and improves the performance of immunological assays. The generation and application of avian antibodies has caused a surge of interest in a wide variety of applications within the life sciences [1].

2.1.2. Structure characterization of IgY and IgG antibodies

Structurally, avian IgY antibody is similar to mammalian IgG antibody (Figure 1). Two identical light chains and two identical heavy chains that are bounded by disulfide bonds compose the antibody’s molecular structure [2]. Both chains are formed by the sequences of amino acids (approximately 110 amino acids) named domains which are numerically ordered from the amino terminal region (NH$_3^+$) to the carboxy terminal region (COO$^-$). The amino terminal domain, named variable domains, presents a variable amino acid composition sequence by the occurrence of mutational events during the B lymphocyte-produced immune response. For both light chain and heavy chain, variable domains (VL and VH) have three regions with higher variation rate in amino acid sequence named complementarity determining region (CDR). Complementarity determining regions (CDRs) are completely bounded to epitopes (antigenic determinant) during a specific humoral response. In the direction to carboxy terminal extremity, after the variable domain, there are the constant domains. The light chain has one constant domain (CL); however, the number of constant domains for the heavy chain varies with antibody isotypes. IgY heavy chain, named upsilon (Greek letter $\upsilon$), has four constant domains (CH), and IgG heavy chain, named gamma (Greek letter $\gamma$), has three domains (CH) [2, 3].

The fragment antigen-binding (Fab) is positioned in amino terminal extremity and is composed by the entire light chain (VL and CL) and both VH and CH1 domains. The IgY fragment crystallizable (Fc) is formed by CH2, CH3, and CH4 domains and IgG Fc by the hinge region is formed by CH2 and CH3 domains. There was proposed that IgY CH2 domain must be
converted to IgG hinge region, which provides a higher mobility to the mammalian Fab than to its avian equivalent. Regarding heavy chain, the chicken has two other classes of antibodies (Immunoglobulin M [IgM], heavy chain µ, and IgA, heavy chain α) and mammals have other four classes of antibodies (IgM, heavy chain µ; Immunoglobulin A [IgA], heavy chain α; Immunoglobulin E [IgE], heavy chain ε; and Immunoglobulin D [IgD], heavy chain σ) [2, 3].

2.1.3. Generation of antigen recognizing repertoire in chicken and mammals

In the genome of a germ-line cell, the genetic information for an immunoglobulin (Ig) polypeptide chain is contained in multiple gene segments scattered along a chromosome. During the development of bone marrow-derived lymphocytes, these gene segments are assembled by recombination that leads to the formation of a complete gene [4]. The vertebrate B-cell repertoire is capable of generating up to 10(9) different antibody molecules. To generate diversity, mammals depend on combinatorial and functional variations that occur during the gene rearrangement events to produce complete heavy and light chain Ig genes. This gene rearrangement process goes continuously in the bone marrow, where each developing B cell assembles a unique heavy and light chain Ig gene from families of functional V (variable), D (diversity), and J (joining) gene segments. In contrast, chickens have only single functional V and J segments for the heavy and light chain loci, and chicken Ig gene rearrangement occurs only during a brief period of embryonic development. A specialized organ involved in avian B-cell development, the bursa of Fabricius, provides the microenvironment necessary for the amplification of B cells that have undergone productive Ig gene rearrangements. Within the bursa, B cells also acquire somatic diversity among the rearranged V gene segments of the

Figure 1. Schematic general structure of the egg yolk antibody (IgY) and the mammalian IgG antibody.
heavy and light chain Ig loci. Somatic diversification of chicken V gene segments occurs by intrachromosomal gene conversion, a DNA recombination process which involves unidirectional transfer of nucleotide sequence blocks from the families of V region pseudogenes into the functional rearranged VH and VL genes [5].

2.1.4. Molecular characterization of IgY and IgG antibodies

Phylogenetic studies have shown that the IgY antibody, homolog of mammalian IgG, has similarities with both mammalian IgG and IgE antibodies. IgY is the predominant isotype in sera, produced after IgM in the primary antibody response, and it is the main isotype produced in the secondary immune response. IgY has different biochemical properties from those of mammalian IgG antibodies and shares homology while functioning with them. Due to the lack of the hinge region, IgY has limited flexibility to its Fab resulting in to precipitate antigens at physiological salt concentrations. Chicken IgY in serum is monomeric, with molecular weight (MW) 165–206 kDa, the mean serum concentration average 5–7 mg/mL and in yolk approximately 25 mg/mL [2]. Mammalian IgG is the Ig found in highest concentration in blood and plays a major role in antibody-mediated defenses. It has a MW of about 180 kDa. Its serum level is approximately 8–16 mg/mL in human and 17–27 mg/mL in cattle [3]. The amount of antibodies obtainable from rabbit is approximately 200 mg, IgG/bleeding (40 mL blood) with 5% of specific antibody, and from chicken is approximately 100 mg/egg (5–7 eggs/week) with 2–10% of specific antibody. IgY sampling is not invasive, which confers advantage on IgG; also, yolk antibody does not interfere with rheumatoid factor or activate mammalian complement [1].

2.2. IgY production

The IgY technology–based layout to produce polyclonal antibodies is displayed in Figure 2. First step is the chicken immunization followed by the yolk IgY extraction, the antibody characterization, and finally IgY applications in various kinds of assays. Chickens are a suitable model to produce IgY by immunization with nucleic acid [6], protein [7], lipid, and carbohydrates [8]. In addition, an immune humoral response must be elicited by immunization with recombinant proteins [9] or peptides [10]. To improve the specific IgY response it is demanded the use of adjuvants like Freund’s adjuvants for immunization procedures, which is the most frequently used adjuvant. Intramuscularly way is a common way for chicken immunization to produce specific IgY. Alternatively, subcutaneous way provides satisfactory amounts of specific antibody [1].

2.3. IgY purification

Regarding its constitution, yolk can be viewed as an oil-water emulsion with a watery portion containing proteins and a dispersal portion of so-called yolk-granules and lipid-drops [11]. The concentration and distribution of immunoglobulins Y (IgY) in the serum and egg are 6 and 25 mg/ml, respectively. The concentrations of specific antibodies in the yolk are similar to the antibody profile of the serum. To isolate yolk IgY, distinct procedures may be used alone
or in combination according to the following criteria: amount, purity, and biological activity. The yolk antibody extraction begins by separating yolk and egg white. After removal of the vitellin membrane, the yolk is put into a measuring cylinder. The next steps in IgY extraction are devoted to removing the lipid content and obtaining a water soluble phase (WSP) antibodies enriched [12]. The consequent purification procedures vary widely in type and combination and from laboratory to laboratory. The degree of IgY purity depends on desired application; for example, for oral administration, the whole yolk can be used, and on the other hand, for a reagent, isolated IgY from the mixture of yolk molecules is necessary [1].

2.3.1. Removing lipid from yolk

A high quality removing lipid method is the first step to efficiently isolate IgY antibodies from egg yolk. Several natural gums, as carrageenan and xanthan gum, were found to be effective for removal of yolk lipoprotein as a precipitate, resulting in a water-soluble fraction with very low lipid concentration [13, 14]. Optionally, organic solvents (chloroform) have been used as a mean to remove yolk lipid content at the initial step of IgY isolation [1, 14]. The water-dilution method is a widespread procedure for the yolk lipid removal, which provides IgY-enriched water-soluble fraction [14–16].

2.3.2. Isolating IgY by precipitation methods

The extraction of yolk antibodies by the use of polyethylene glycol precipitation (PEG, MW 6000) was, firstly, developed by Polson et al. [17]. The IgY-extraction by means of PEG-precipitation is very cost-effective and results in highly specific antibody with stable titers up
to 1:1,000,000 [18]. Afterwards, PEG 6000 was widely accepted as a standard procedure. Pulverized PEG 6000 is added to WSP until the final concentration of 3.5% is stirred, and the protein precipitate is pelleted by first centrifugation. Finally, PEG is added to supernatant until it brings the final concentration to 12%. The mixture is stirred thoroughly and a second centrifugation PEG method can result in a high IgY antibody concentration per egg; however, it may not be homogeneous when analyzed by SDS-polyacrylamide gel electrophoresis [19]. To improve the quality of extraction, after PEG 12% precipitation, saturated ammonium sulfate solution can be added to IgY enriched fraction [20]. Additionally, by following precipitation procedures, the IgY antibodies may be further purified by DEAE cellulose ion-exchange column chromatography [21] or ionic-liquid based aqueous biphasic systems [22]. IgY-PEG 6000 can be extracted by using three consecutive precipitation steps: 3.5, 8.5, and 12% is a recent adaptation to Polson et al. [17] technique and produces a high amount of specific egg yolk antibodies [23].

Proteins can be precipitated with sodium sulfate (Na₂SO₄, NaS) or ammonium sulfate [(NH₄)₂SO₄, AmS]. Since the precipitation of certain proteins from a mixture by NaS or AmS depends on the concentration of the salt, this procedure can be used not only for isolation of desired proteins but also for elimination of the undesired ones. A precipitate is obtained through centrifuging, taken up in a certain amount of desired buffer or distilled water, and finally dialyzed [1]. NaS and AmS precipitations are economical and environmentally correct procedures for yolk antibody extraction [24]. The major problem in separating IgY is to remove the high concentrations of lipids in egg yolk, which can be obtained by water dilution method [15], followed by IgY salt-based precipitation in the antibody-enriched fraction [25]. NaS or AmS precipitation provides fractions with low protein content compared to original yolk, but with a high specific activity [26]. Salt-based yolk antibody precipitation is promising to be a more efficient and useful purification method for the large-scale IgY from egg yolk preparation [16, 27].

The isolation of IgY from yolk using of combination of different methods results in a high amount of specific antibodies with elevated purity. Sequential precipitation with 31% ammonium sulfate and 12% polyethylene glycol (PEG) produces IgY antibodies with above 95% purity and there is no loss in immunoreactivity [28]. The salt-based egg antibody precipitation combined with thiophilic acid chromatography provides a simple and efficient mean of IgY from egg yolk [29, 30]. Association between AmS and ethanol to IgY precipitation results in a high quantity of antibodies with great purity [31]. Specific antisnake venom IgY with neutralizing effect for therapeutic proposes can be isolated by AmS precipitation followed to ion exchange chromatography [32, 33]. The inhibiting growth activity of IgY anti-Streptococcus mitis was preserved by extracting yolk antibodies by precipitation with PEG 6000 and AmS and further isolation by DEAE cellulose ion exchange column chromatography [34]. Similarly, growth inhibition of Staphylococcus aureus and Escherichia coli was obtained with yolk antibodies isolated by NaS and AmS precipitations [35].

2.3.3. Isolating IgY by gel filtration

The separation of IgY antibodies according to their molecular weight through gel filtration is a further stage of purification of yolk antibodies but rarely reported except in the desalting of
preparation through columns instead through a dialysis [1]. Gel filtration by Sephacryl S300 and Sephadex G50 or G75 provides a high-purity IgY separation but further protein concentration procedures for separated antibody samples are desired [16, 36, 37]. On the other hand, the ion-exchange chromatography is a widespread method to separate proteins from mixtures and it is also used for immunoglobulins isolation. IgY antibodies are bound electrostatically to an ion-exchange matrix with a reverse charge. According to the strength of binding, the antibodies are detached by increasing concentrations of ions of the buffers [1, 32, 33, 34].

2.3.4. Isolating IgY by affinity chromatography

The isolation of yolk IgY antibodies by affinity chromatography consist in attach an antibody ligand on a matrix. The extraction of mammalian IgG-isotypes by affinity chromatography can be obtained by immobilized S. aureus A protein or B protein from the Streptococci of the C- or G-group. IgY antibodies bind neither with protein A nor with protein G [1]. Recently, protein M (a transmembrane protein from human mycoplasma) has been demonstrated as a promising ligand for purifying polyclonal, monoclonal, or engineered IgY antibodies [38]. Synthetic IgY-ligands were developed to improve yolk antibody isolating mainly by reducing the purification steps and increasing the amount and purity of recovered IgY [39, 40]. Attaching the IgY-induced antigen to matrix provides another way to extract a high-purity IgY by affinity chromatography [41, 42]. The chromatographic thiophilic interaction provides a simple one-step method capable of recovering IgY at concentration close to 100% of initial yolk antibody amount [29, 43].

2.4. IgY applications

2.4.1. Proteomic

IgY antibodies are suitable for a wide application protocols like proteomic, diagnostics, and therapy. The immunodepletion for humans or animal fluids by using specific IgY-attached columns enables the detection of new protein spots, increases resolution, and highly improves the intensity of low-abundance proteins by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) [44, 45]. Other possibility for the use of IgY to proteomic studies is screening random peptide phage display library to detect new epitope candidates to be applied for diagnostic methods to widespread parasitic human diseases [46]. Additionally, using phage-displayed chicken single-chain antibody fragment libraries provides useful diagnostic and research reagents. Libraries constructed, using mRNA from an immune source, are enriched in affinity-matured sequences. Repertoires focused on a number of defined targets can be constructed using lymphocyte mRNA from chickens immunized with a mixture of several different antigens from Plasmodium falciparum, Trypanosoma sp., or human viruses [47, 48].

2.4.2. Immunoassay

Development of IgY-based immunoassay is a widespread field to apply yolk antibodies. Enzyme-linked immunosorbent assay (ELISA), western blotting (WB), immunofluorescence
(IF), and immunohistochemistry (IHC) methods are the most common applications for IgY [1]. Relevant to consider that antigenic targets recognition by the use of antibodies can show some differences according to the method used. Regarding the conformational epitope structure, the performance of antibodies recognizing a given epitope by WB can represent nothing about the same antibody performance in ELISA with the same antigen [49]. IgY antibody shows advantages comparing to the mammal IgG use in immunoassay. In this context, the rheumatoid factor (RF) is a major source of interference in many immunoassays using mammalian polyclonal or monoclonal antibodies by interacting with IgG and causing false-positive results. IgY antibodies do not react with RF avoiding false-positive results in ELISA [50].

2.4.2.1. Enzyme-linked immunosorbent assay

IgY-based ELISA does not demand expressive modifications from the most common procedures and reagents used to carry out this protocol [16, 51]. Egg yolk IgY can be labeled with horseradish peroxidase for use in immunoenzymatic assays [52]. Some possibilities for application of yolk antibodies-based ELISA are: screening molecules related to drug-drug interactions [53]; sandwich ELISA to capture bacterial toxins [54]; screening animal diseases by using recombinant antigens [55]; characterization of maternal IgY transferred to egg yolk [56]; coproantigen to capture ELISA for intestine human parasite [57]; screening antibiotic residues in food samples [58]; detection of blood circulating helminth antigens by immunomagnetic bead ELISA [59]; potential for clinical application diagnosing cancer antigen [60].

2.4.2.2. Immunofluorescence assay

IgY antibodies can be applied to immunofluorescence and flow cytometry assays. IgY-based immunofluorescence experiment allows the detection of antigenic targets on cells and tissues samples [61]. Considering that IgY antibodies do not induce Fc-dependent activation of complement, they must be used to clarify the pathogenicity of mammalian autoimmune diseases by immunofluorescence assay [62]. Chicken IgY and rabbit IgG conjugations with fluorescein isothiocyanate (FITC) comparatively used to examine strains of *Campylobacter fetus* revealed that both conjugates have a high percentage rate of detection; IgY has less background due to unspecific fluorescence than IgG. Additionally, IgY is a cheap, bloodless, and very productive method [63]. FITC-labeled anti-*Mycobacterium avium* subspecies *paratuberculosis* can target the pathogen inside the cytoplasm of infected macrophages [64]. Frozen liver fragments were diagnosed with cytomegalovirus antigens by using IgY-based confocal microscopy confirming the yolk antibody as suitable reagent for immunofluorescence assay [65].

2.4.2.3. Flow cytometry assay

IgY antibodies are suitable reagents for flow cytometry and as well as certain monoclonal antibodies, for example, to study human and rabbit platelet physiology [66]. When using phage display-based single chain variable fragments (scFvs), polyclonal IgY production and further the flow cytometry assays it is possible to develop immunoreagents for the isolation and characterization of stem cells, molecular diagnostics and therapeutics of lung cancers [67, 68].
2.4.2.4. Immunochromatographic assay

The immunochromatographic assay (ICA) requires no instruments and has a detection time of less than 10 minutes and it is portable and easy to perform in the field. The development of IgY-based strip could be a promising on-site tool for screening infection or disease outbreaks. IgY-gold complexes depositing onto the conjugate pad as detector reagents showed high specificity [69, 70].

2.4.2.5. Using IgY for passive immunization

The passive immunization is a protective method, which has been tested for many years and shown to be effective [71]. Passive immunization with pathogen-specific egg yolk antibodies (IgY) is emerging as a potential alternative to antibiotics for the treatment and prevention of several human and animal diseases. Laying hens are an excellent source of high-quality polyclonal antibodies, which can be collected noninvasively from egg yolks. The use of IgY offers several advantages such as it is environmentally friendly, nontoxic, reduces the numbers of animals required for antibody production, stability in the orogastrointestinal tract, and its safety profile [71–74]. A major obstacle to its implementation is its relatively high cost, which is dependent, among other things, mainly on two factors: the efficacy of antibody production, and the use of specific pathogen-free (SPF) birds for antibody production to avoid the possible of pathogens transference from commercial layers. Alternatively, treatment of the extracted IgY with formalin can negate the need for SPF birds and shows no interference with the Fab specific antigen-binding or Fc-complement activation of the antibody [75].

Regarding the IgY concentration in egg yolk and blood of laying hens, a recent study provides evidence that there is a significant circaseptan rhythm in yolk IgY and circaquattran rhythm in serum IgY. Additionally, the serum IgY concentration reached to maximum in the morning, decreased to minimum during the daytime, and increased again at night revealing a significant circadian rhythm, which may reflect in yolk antibody concentration [76].

The microencapsulation with a methacrylic acid copolymer may be an effective method of protecting purified yolk IgY from gastrointestinal inactivation, enabling its use for oral passive immunotherapy [77]. The use of chitosan-alginate microcapsules to protect IgY from gastrointestinal environment conditions provides significant resistance to pepsin hydrolysis and may enable intact IgY to reach target microorganisms within the lower digestive tract [78]. Another approach protecting IgY from degradation in gastric pH can be the incorporation of antibodies to hydrogel containing acrylamide and acrylic acid with promising results for IgY oral delivery [79].

Oral administration of IgY antibodies has been tested for many years with promising results [80] to different pathogens as human rotavirus [81]; dental plaque formation by Streptococcus mutans [82, 83]; enteropathogenic E. coli [84]; Helicobacter pylori [85, 86]; Cryptosporidium parvum [87, 88]; canine parvovirus [89]; Porphyromonas gingivalis [90]; Pseudomonas aeruginosa [91]; shrimp’s white spot syndrome virus [92]; Eimeria acervulina [93]; E. tenella and E. maxima [94, 95]; H5N1 e H1N1 in mice [96]; Vibrio cholerae [97]; rotavirus and norovirus [98]; Campylobacter jejuni [99–101]; and botulinum neurotoxins [102]. Immunotherapy as a passive
immunization method to neutralize venom using purified IgY proved to be efficient for therapy protocol [103–107].

2.5. State of art the use of IgY for Toxoplasma gondii studies

Currently, there are a limited number of studies about IgY antibody production against T. gondii, either native or recombinant antigens. T. gondii is a worldwide public health and veterinary problem.

Hassl et al. [108] first described the production of anti-T. gondii IgY. They used total soluble antigens that are immunogenic to comparatively produce rabbit IgG and chicken IgY. The results indicated differences between the specificities of egg yolk IgY antibodies and rabbit IgG serum antibodies, although both animal species had been immunized with identical antigen preparations. Ferreira Junior et al. [16] have produced comparative mice IgG and chicken IgY against soluble antigens from tachyzoites of T. gondii. They purified yolk antibodies by water dilution method, sodium sulfate precipitation, and molecular weight exclusion chromatography. Specific antibody characterization was due to both indirect ELISA and Western blotting methods and applied to immunofluorescence assays by immunohistochemistry and immunocytochemistry assays. Using the Western blotting method, the antigenic bands recognition profile were different between mice and chicken antibodies. IgY antibodies detected parasites in cytoplasm of infected cells and in brain samples of infected mice.

Hoto et al. [109] experimentally infected turkeys with tachyzoites and oocyst, and chickens with oocyst from T. gondii and investigated the humoral response against recombinant T. gondii antigens (rGRA1, rGRA6, rGRA9, rSAG1, and r SUB1) in a line blot assay. They found that infection with oocyst induced a stronger, permanent long-lasting antibody response compared to tachyzoite-infected animals.

Studying the heat shock protein of T. gondii (TgHSP70), a parasite virulence factor that is expressed during parasite stage conversion, Barenco et al. [110] produced a polyclonal IgY against the recombinant TgHSP70 and tested it to detect native heat shock protein in brain samples from T. gondii-infection resistant (BALB/c) and susceptible (C57BL/6) mice after dexamethasone (DXM)-induced infection reactivation. In parallel, they investigated the TgHSP70-specific humoral response. They found higher specific antibody titers in serum samples of BALB/c compared with C57BL/6 mice. C57BL/6 mice presented high expression of TgHSP70 in the brain with the progression of infection and under DXM treatment. They conclude that these data suggest that the TgHSP70 release into the bloodstream depends on the death of the parasites mediated by the host immune response, whereas the increased TgHSP70 expression in the brain depends on the multiplication rate of the parasite.

More recent study with IgY and T. gondii was published by Cakir-Koc [111]. In this investigation, specific IgY antibodies were produced against the surface antigen 1 (SAG1) protein of T. gondii and the antibody activity was carried out by ELISA. Specific and higher amounts of IgY antibody against SAG1 were obtained. Regarding the advantages of IgY antibody and the importance of SAG1 for the diagnosis of toxoplasmosis, anti-SAG1 IgY would be a promising reagent in research, diagnostics, and immunotherapy against toxoplasmosis.
2.6. Future perspectives for the use of IgY for *Toxoplasma gondii* investigations

Although there are a reduced number of papers with IgY and *T. gondii*, the yolk IgY antibody has been demonstrated as an efficient tool for application for various kinds of different methods. Regarding phylogeny distance between chicken and mammals, which reflects differences in antigens profile recognition, IgY technology may be applied for the screening of new antigen libraries by random phage display method and the selection of candidate targets for therapy, diagnostics, or vaccine. In this context, polyclonal IgY and scFvs may be a promising alternative for proteomic studies. In the field of diagnostic methods, yolk IgY might be used as an alternative to mammalian IgG for rapid test to detect phase disease-associated circulating antigens, ELISA, flow cytometry test, and fluorescence techniques. Enzyme-labeled or fluorophore-labeled IgY as a primary antibody is employed to detect *T. gondii* antigens in fixed tissue samples or parasite-cultured cells. Speculating about a possible therapy for oral infection by *T. gondii*, IgY protecting the definitive and the intermediary hosts against infection may be an efficient tool for public health to control human and animal toxoplasmosis.

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