Immune Response in Mammals and Chickens

3

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Abstract

The ability to distinguish between self and potential pathogens is a key feature of the immune response. There are many common mechanisms of action of the innate and adaptive immune systems shared across species as evolutionarily distinct as chickens and mammals. Toll-like receptors which detect pathogens are present in both species, but differ in number and function. The central roles of dendritic cells, Helper T cells and B cells as well as the cytokines released in response to pathogens are also shared. Lymphoid tissues in chickens are distinct from those in mammals and the maturation of B cells occurs in the bursa of Fabricius, an organ specific to avian species. The generation of antibody diversity occurs by gene rearrangement in mammals and by gene conversion in chickens. Chickens have three classes of immunoglobulins while mammals have five. The main serum immunoglobulin in mammals is IgG while that in chickens is IgY. The structural differences in these molecules is outlined.

Keywords

Innate · Adaptive · B cell · T cell · Chicken · Mammal

3.1 Evolutionary Context

The environment harbours a wide range of potential pathogens and all living organisms have of necessity developed defences against microbes and other toxic substances. An important parameter in this fight for survival is the recognition of

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non-self. The basis of this recognition, in both unicellular and multicellular organisms, is cellular receptors that detect the molecular motifs that are conserved among pathogenic microorganisms (Buchmann 2014). It is also essential that the immune system does not attack its own tissues otherwise this would result in self-destruction such as in autoimmune disorders (Goodnow et al. 2005). During the development of immune cells, there is a selection process in which those immune cells that react strongly with self-antigens are killed or suppressed which is referred to as self-tolerance. As many proteins are conserved across mammalian species, this underlies the difficulty in producing antibodies in closely-related species and is one of the major advantages of antibody generation in chickens due to their evolutionary divergence from mammals (Chap. 4).

Primitive animals relied on a basic innate defence mechanism (Yatim and Lakkis 2015), but as species evolved and developed more complex organs the immune system needed to respond and the adaptive immune system appeared about 450 million years ago (Kasahara et al. 2004). However, many of the basic mechanisms used in innate immunity have been retained in adaptive immunity. Antibody production for research or biomedical applications has traditionally been in mammalian species (mice and rabbits). This chapter will focus on the general mechanisms involved in antibody production in mammalian systems with the differences between mammals and chickens being highlighted. Due to constraints of space, the scope of the chapter is limited to the cellular and molecular signals involved in the production of immunoglobulins (antibodies) with some reference to cytotoxic T cells. The physical barriers to pathogens and roles of other classes of leukocytes involved in the immune response in mammals (Chaplin 2010) and during early development of the chick (Hincke et al. 2019) will not be discussed here. The other arm of the immune system which is activated in innate and adaptive immunity is complement (Zhang et al. 2017) is also beyond the scope of this chapter.

3.2 Innate and Adaptive Immune Response

A fully effective immune response relies on the interplay of the innate and adaptive immune systems; the innate immune system provides some immediate non-specific protection against invaders and primes the adaptive immune system. The adaptive immune system exhibits high specificity for a given antigen, albeit with a delay for a number of days before full activation, and memory which ensures a swift response on re-exposure to the same antigen. As a general introduction, many features of the innate and adaptive immune systems of mammals and chickens are listed in Table 3.1 and will be discussed in more detail. It is worth noting that with increasing complexity and body size during evolution, secondary lymphoid organs assumed importance (Yatim and Lakkis 2015) and cytokine and chemokine production by dendritic cells results in the necessary interaction between the components of the innate and adaptive immune system.

Table 3.1 General features of the innate and adaptive immune systems of mammals and chickens

Immune system	Innate	Adaptive
Mode of action	Non-specific	Very high specificity
	No memory	Memory
Time of action	Immediate	Delay of 4–7 days
Cells involved	Macrophages, Dendritic Cells, Neutrophils[a] Heterophils[b]	Macrophages, Dendritic Cells, Neutrophils[a] Heterophils[b]
	Natural Killer (NK) cells	Natural Killer (NK)cells
	γδ T cells	γδ T cells
		B cells
		T cells
Receptor types	Toll Like Receptors (TLRs)	MHC Class I, MHC Class II
		T cell receptors
		B cell antigen receptors
Receptor Genes	Germ-line gene elements	Gene re-arrangement[c] Gene conversion[d]
Co-stimulatory molecules (activating)	Dendritic cell: CD40, CD80/86	Dendritic cell: CD40, CD80/86 T cell: CD28, CD40L B cell: CD80, CD40
Cytokines released	IL-1, IL-6, IL-8, and TNF, IL-12, Type I Interferons	IL-2, IL-4, IL-5, IFNγ, TNF, IL-6,
Other immune systems activated	Complement	Complement
	Defensins	Defensins
	Cathelicidins	Cathelicidins

[a]Absent in chickens
[b]Heterophils may play a similar role to neutrophils in chickens
[c]Generation of antibody diversity in most mammals
[d]Generation of antibody diversity in chickens

3.3 Innate Immune Response in Mammals and Chickens

The innate immune responses in mammalian and avian species have many common features but also some differences in the receptors and cellular responses which will be discussed in more detail. During the primary innate immune response, macrophages, dendritic and NK cells recognize the pathogens by their pathogen-associated molecular patterns (PAMPs) that bind to pattern-recognizing receptors (PRRs), such as Toll like receptors which then elicit an appropriate response (Takeda et al. 2003) (Fig. 3.1). PAMPs include the lipopolysaccharide from Gram-negative bacteria, peptidoglycan, bacterial flagellar proteins, and viral genomic material including DNA and RNA. The range and action of the chicken Toll like receptors will now be explored.

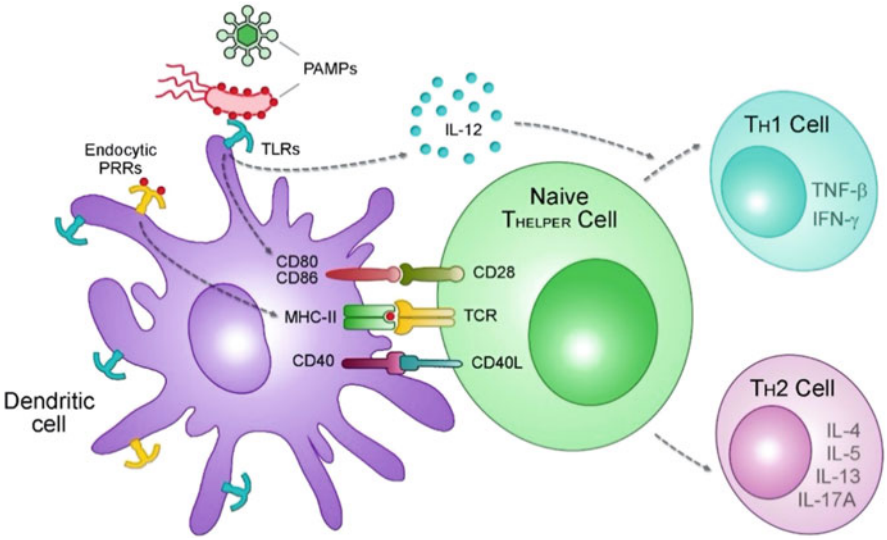

Fig. 3.1 Mature dendritic cells present antigens to naive T cells. Toll like receptors (TLRs) present in dendritic cells bind PAMP molecules present on pathogens (e.g., microbes, viruses) or even defective (e.g., tumour) cells. The antigen is processed and presented attached to MHC Class II molecules (MHC-II). Binding to the TLR upregulates the expression of co-stimulatory molecules such as CD80 and CD86 and IL-12 is released. A naïve T cell with the appropriate T cell receptor then binds to the mature dendritic cell, gets activated and the adaptive immune system is engaged. Endocytic **PRRs**, pattern-recognizing receptors. Figure courtesy of IgYTechnology.com (www.igytechnology.com)

3.3.1 Toll Like Receptors (TLRs)

Birds have ten TLRs while 13 mammalian TLRs have been identified (Boudinot et al. 2014) however, chicken TLRs differ to some extent from those expressed in mammals (Brownlie and Allan 2011). Each of the TLRs detects a distinct repertoire of highly conserved pathogen molecules. TLRs are also expressed on non-immune cells such as epithelial and endothelial cells, and fibroblasts and this distribution helps to promote a rapid immune response (McClure and Massari 2014). For a more detailed review of PRRs and TLRs in a number of other avian species, the reader is directed to a review by Chen et al. (2013).

Structurally, TLRs are integral membrane glycoproteins consisting of an extracellular ligand binding domain with leucine-rich repeats, a transmembrane domain and an intracellular Toll/interleukin 1 receptor (Chen et al. 2013). Chicken Toll-like receptor 3 (TLR-3) contributes to the induction of the innate immune response against viruses and is constitutively expressed in all chicken tissues; it is present in intracellular vesicles (endosome and endoplasmic reticulum) where viruses undergo uncoating during infection; it upregulates Type I interferon and inflammatory cytokine production (Table 3.1) (Chen et al. 2013). Chicken TLR-7 is located in intracellular vesicles and is involved in the interaction with RNA viruses; TLR

15 located on the plasma membrane and TLR21 in intracellular vesicles are involved in sensing both viruses and bacteria. Chickens, unlike ducks, lack the retinoic acid inducible gene 1 (RIG-1) however, this is compensated by the presence of melanoma differentiation-associated gene 5 (MDA5) which is necessary for the induction of type I interferon; the lack of RIG-I necessary for Interferon-ß production may explain the susceptibility of chickens to highly pathogenic influenza (Chen et al. 2013).

3.3.2 Cytokine Production and Host Defence Peptides

Comparative studies of cytokines in chickens and mammals suggest close functional capabilities although at the level of the genome and primary protein structures they are very different (Giansanti et al. 2007). The range of pro-inflammatory cytokines produced in response to binding to TLRs include interleukin-1 (IL-1), IL-6, IL-8 and tumour necrosis factor-alpha (TNF-α) (Table 3.1) (Gao et al. 2016; Sun et al. 2019; Alkie et al. 2019; Awad et al. 2019). The presence of TNF-α which was long thought to be absent in chickens has recently been confirmed (Rohde et al. 2018). Transcription factors, such as interferon regulatory factor-7 (IRF-7) and nuclear factor kappa B (NFκB), drive the transcription of genes encoding these cytokines (Goossens et al. 2013; Karnati et al. 2014; Markowska et al. 2017; Hoang et al. 2017; Liu et al. 2018). In addition, the maturation of antigen-presenting cells is promoted and the co-stimulatory molecules CD80 and CD86 are up-regulated (Table 3.1) (Mogensen 2009). Simultaneously to the humoral response, a cellular innate response is elicited by activation of tissue-homing cells such as natural killer cells, macrophages, and dendritic cells (Igyártó et al. 2006; Jansen et al. 2010; de Geus et al. 2012; Neulen and Göbel 2012; Sun et al. 2017; Jahromi et al. 2018; Feng et al. 2019; Lin et al. 2019; Yasmin et al. 2019). The NK cells produce IFN at early stages of a viral infection and later target the virus-infected cells to destroy them by apoptosis (Song et al. 2017).

Host defence peptides (HDPs), which are families of effector molecules found in mammals, insects and plants are also present in chickens (Table 3.1); the readers are referred to a review of their role, evolution and biological activities (Cuperus et al. 2013). Avian host defence peptides are present in nearly every organ and tissue supporting their importance in the innate immune response. Within the defensin family, β-defensins are the only defensins found in avian species so far. HDPs, as well as exerting a direct antimicrobial action, also function in immunomodulation and wound healing (Cuperus et al. 2013).

There are a number of other cell types (NK cells, γδT, NK-T, and B1) which are active in both the mammalian innate and adaptive immune response (Getz 2005). Cell types confirmed to be present in the chicken are as follows: NK cells (Jansen et al. 2010); γδT (Pieper et al. 2011); and NK-T (Rogers et al. 2008); γδT cells occur at a much higher frequency in chickens compared to mammals (Sowder et al. 1988). The common characteristics of mammalian γδT, NK, NK-T cells is their ability to recognize stressed cells in the absence of antibodies and MHC protein interactions.

Response to lipid and polysaccharide antigens occurs in these cells in a pathway independent of MHC Class I and II proteins but by a similar mechanism (Chaplin 2010). B1 cells produce natural antibodies and can respond without T-cell activation and show no evidence of somatic hypermutation of their receptor genes (Yatim and Lakkis 2015).

3.4 Adaptive Immune Response

The adaptive immune system normally has a small number of cells with specificity for any individual pathogen, toxin or allergen. The responding cells (T and B cells) must proliferate after encountering the antigen in order to have sufficient cells to mount an effective immune response. An example of the interplay between the innate and adaptive immune response is the role of dendritic cells; these cells present the antigen bound to MHC Class II molecules which then activates a T cell which displays a TCR which can bind that antigen (Fig. 3.1). Pathogens are complex and are able to interact with a large number of lymphocytes; each B lymphocyte is activated to proliferate and differentiate into plasma cells; each plasma cell only produces a single type of antibody but as a number of plasma cells are stimulated the overall response is polyclonal. Any protein when degraded by the endocytic pathway (Fig. 3.1) will result in a number of short fragments of about 15 amino acids, so a single protein will also give rise to a polyclonal response. A comparison of the lymphoid tissues of mammals and chickens and the origin and roles of B and T cells will now be discussed.

3.4.1 Lymphoid Tissues in Mammals and Chickens

The major chicken lymphoid organs involved in the adaptive immune response are the bone marrow, spleen and the bursa of Fabricius which is found only in birds (Table 3.2) (Boehm et al. 2012).The chicken's immune system lacks lymph nodes but has mucosa associated lymphoid tissues (MALT) that are scattered in the subcutaneous tissue and through the mucosal epithelia, lamina propria and serosa

Table 3.2 Comparison of the lymphoid tissues of mammals and chickens

	Mammals	Chicken	Observations
Bursa of Fabricius	No	Yes	B cell selection and antibody generation
Thymus	Yes	Yes	T cell selection
Lymph nodes	Yes	No	Site of development of adaptive immune response
Spleen	Yes	Yes	Site of development of adaptive immune response
Bone marrow	Yes	Yes	Haematopoiesis
Mucosa-associated lymphoid tissue	Yes	Yes	Regulating mucosal immunity

membranes (Hammer 1974). The Harderian gland, located in the orbit of the eye, forms part of the head-associated lymphoid tissue, has high levels of B-dependent lymphoid cells and plasma cells and is a key immune organ in chickens (Saelao et al. 2018). Birds do not have neutrophils (Table 3.1) but heterophils function in a similar manner to mammalian neutrophils (Chen et al. 2013). The spleen, a capsulated tissue found close to the stomach, mounts the immune response to blood borne pathogens. Immune cells (dendritic cells, macrophages, heterophils and lymphocytes) homing into the parenchyma of lymphoid organs function by trapping pathogens and removing them from an infected host (Zhang et al. 2015; Silveira et al. 2019).

Overview of the immune system of mammals and chicken comparing relevant aspects of their lymphoid tissues and immune response (Keestra-Gounder et al. 2013; Kaufman 2015, 2018; Taha-Abdelaziz et al. 2018).

3.4.2 B Cells, T Cells and MHC Class II Proteins

The roles of avian B and T cells in the production of antibodies has been reviewed (Ratcliffe and Härtle 2014; Smith and Göbel 2014). The development of B and T cells in the chicken begins with the migration of multipotent progenitors from the bone marrow to the bursa of Fabricius (B cells) and the thymus (T cells). The bursa begins development about embryonic day 5 and is populated by pre-bursal cells between embryonic days 8–14 (Ko et al. 2018). Chickens have three distinct B cell stages: pre-bursal, bursal and post-bursal B cells (Ko et al. 2018) which differs from B cell development in mammals. Overall, the function of the bursa is to generate naïve B cells which are then exported to the periphery, probably the spleen (Lassila 1989). Gene conversion (Sect. 3.6) occurs only in the bursa of Fabricius. As sexual maturity approaches, the bursa begins to regress and the adult depends on the post-bursal stem cells in the bone marrow as the source of B cells (Davani et al. 2014). In mammals, B cells mature in the bone marrow into committed B cells.

The T cell repertoire of the chicken and mammals develop by similar mechanisms. T cells are present in the chicken thymus by embryonic day 15 (Bucy et al. 1990) and by the 18th day of embryonic development the chicken is capable of producing both an innate and adaptive response to pathogens (Schilling et al. 2018 and references therein). As an aside, this feature is exploited in large-scale poultry production with the administration of *in ova* vaccination for multiple pathogens. The population of T cells are divided in two subpopulations named Cytotoxic T cells (CTL or $CD8^+$) and Helper T cells (Th or $CD4^+$) (Erf 2004). Binding of a Cytotoxic T cells through its T cell receptor to a MHC Class I peptide complex induces death of a cell infected by endogenous pathogens such as viruses (Gimeno and Schat 2018). T cells, in a process called immunosurveillance, are also involved in the detection and elimination of tumour cells. There is a comprehensive review on this arm of the immune system (Erf 2004) but it will not be discussed further here. There is a further distinction within Helper T cell populations as naïve T helper cells can differentiate into Th1 or Th2 type cells. Th2 cells are associated with antibody production and synthesis of IL-4, IL-5, IL-13 and IL-17A (Degen et al.

2005; Walliser and Göbel 2018). Helper T cells recognize exogenous antigen presented on macrophages, dendritic cell or B cell MHC class II molecules.

In the humoral response, the major class of MHC molecules involved in the production of antibodies are Class II. MHC Class II molecules are capable of antigen internalization, processing and surface display (Fig. 3.1). Mammals have 260 genes for the MHC and this polymorphism gives rise to six distinct binding grooves each of which can bind a diverse range of antigens (Chaplin 2010). Birds have approximately 19 MHC genes (Kaufman et al. 1999).

3.4.3 Antibody Secretion by B Cells

Mammalian and chicken T and B cells have receptors which are highly diverse, however each individual cell has a receptor of a single specificity. T cells recognize the antigen displayed in the MHC complex through their T cell receptor (Fig. 3.1). In mammals, to adequately distinguish between self and non-self, two proteasome subtypes have evolved, the immunoproteasome and the thymoproteasome. In the chicken these proteasome subtypes are lacking, which raises the question whether these animals have evolved compensatory mechanism for presentation of antigens to T cells (Kasahara and Flajnik 2019).

The major class of mammalian T cells displays the αβTCR. Chickens on the other hand may have frequencies of up to 50% γδ T cells (Sowder et al. 1988); the functions of these cells have not yet been fully determined. The activation of mammalian and chicken B cells follow similar routes. B cells can undergo antigen-dependent proliferation and differentiation into plasma cells (secrete Ig) and memory cells (Fig. 3.2). They can also act as antigen-presenting cells: antigens upon binding to the B cell receptor are internalised and displayed by MHC Class II molecules which can interact with the T cell receptor (Fig. 3.2). There is an absolute requirement for interactions between co-stimulatory molecules and the release of cytokines before B-cells proliferate (Fig. 3.2). Proliferation induces an antibody class switch (Sect. 3.5) that ultimately leads to plasma cells secreting specific antibodies against the antigenic target. Memory B cells are also generated which is the basis of vaccination (Fig. 3.2).

A subset of the cytokines that are released during the innate and adaptive immune response are listed in Table 3.1 (Chaplin 2010). These soluble proteins ensure a coordinated interaction of the cells of the immune system (Turner et al. 2014). Type I interferons block the spread of viruses and stimulate NK cells. IL-12 produced by activated dendritic cells induces the expression of the CD80/86 co-stimulatory molecules. Tumour Necrosis Factor (TNF) is involved in cell migration to the lymphoid organs. An example of the activation of IL-2 and IL-4 during B cell T cell contact is illustrated in Fig. 3.2.

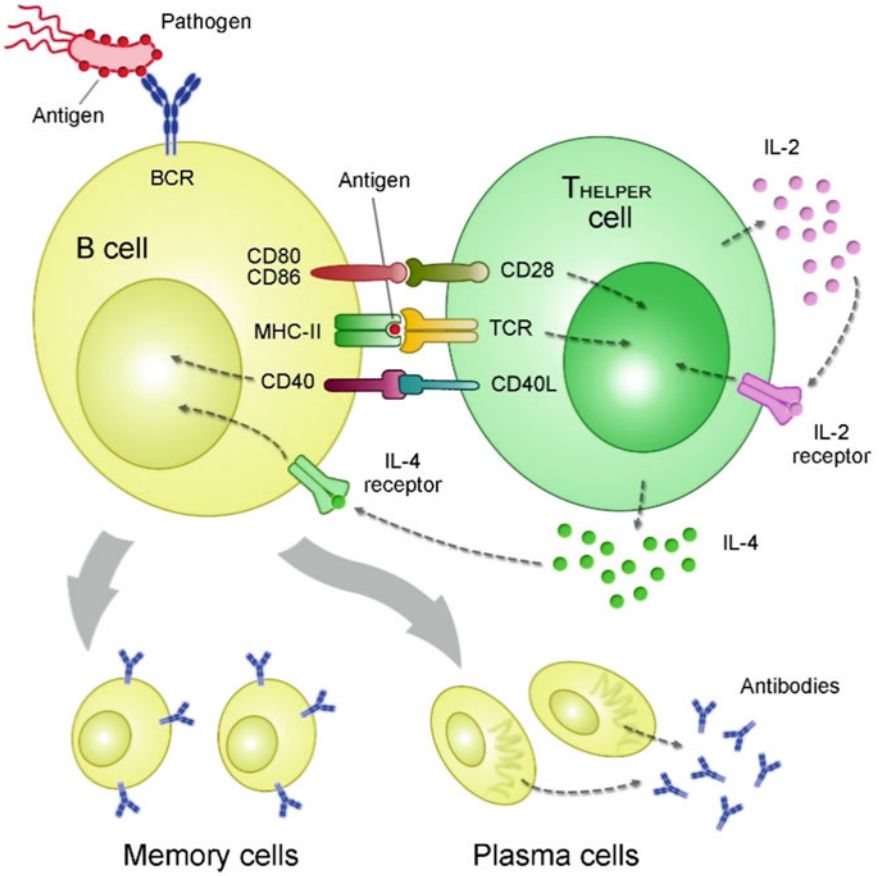

Fig. 3.2 Model of the interaction between B cells and Helper T cells. The B cell receptor (BCR) takes up the antigen and then displays it in the MHC Class II receptor (MHC-II) which reacts with a T cell receptor (TCR) which can interact with that specific antigen. Optimal T cell activation occurs when the costimulatory molecules CD80/CD86 interact with CD28 leading to the secretion of IL-2 and upregulation of the IL-2 receptor on the T cell. The activated T-helper cells upregulate CD40L and secrete IL-4 which binds to the receptor on the B cells. B cells differentiate into antibody secreting plasma cells and long-lived memory cells. Figure courtesy of IgYTechnology.com (www.igytechnology.com)

3.5 Genetic Basis for Diversity in Mammalian TCR and Immunoglobulins

Seminal events in the development of the adaptive immune system were two waves of extensive gene duplication which occurred as jawed vertebrates evolved and the acquisition of the recombination-activating genes (RAG) (Kasahara et al. 2004). RAG genes encode part of a protein complex which is involved in rearrangement

Table 3.3 Genes encoding mammalian TCR and Ig chains

	TCRα	TCRβ	Ig Heavy chain	IgGκ Light chain	Igλ Light chain
Variable (V) segments	70	67	65	44	38
Diversity (D) segments		2	27		
Joining (J) segments	61	13	6	5	5
Constant (C) segments	1	2	11	1	1

and recombination of genes encoding Ig and TCR molecules. These genes are not embedded in the genome but during lymphocyte development they arise by gene recombination where a fixed number of adjacent genes when rearranged give rise to a large number of unique antigen receptors, of the order of 10^{12}.

The genes for Ig and TCR contain segments known as variable (V), diversity (D) and joining (J) and constant regions. The number of gene segments reported for the mammalian light chain of Ig (Collins and Watson 2018) and heavy chain (Li et al. 2004) and TCR gene segments, not all of which are functional (Li et al. 2004), are shown in Table 3.3.

The main TCR is composed of the αβ chains which are somatically assembled from the V, D, J elements initiated by the RAG1 and RAG2 proteins which cleave the DNA near the V, D and J segments which is then re-joined by DNA repair enzymes. An additional enzyme, Terminal deoxynucleotidyl Transferase (TdT) adds nucleotides into some of the junctions providing extra diversity (Chap. 4). The action of these enzymes delivers huge diversity, but also frequently non-functional genes on cells which are then removed in the thymus. Autoreactive cells are also removed. In fact, fewer than 10% of the developing T cells survive these selection processes (Klein et al. 2014). TCRs do not undergo affinity maturation nor exhibit rapid evolution during infection.

In mammals, a similar process of somatic rearrangement of the gene elements occurs in Ig involving RAG genes and the Ig molecule is composed of two identical Heavy and Light chains. The combination of the two chains, imprecise joining of segments and somatic hypermutation all increase diversity (Mishra and Mariuzza 2018). In the initial response antibodies of the IgM class are expressed (Table 3.4) but during clonal expansion class switching occurs by recombination with other constant chain genes to give the following classes and subclasses: IgM, IgD, IgG (IgG_1, IgG_2, IgG_3 and IgG_4), IgA (IgA_1 and IgA_2) and IgE (Calonga-Solís et al. 2019). IgG_1 is the main immunoglobulin in serum having a plasma concentration of approximately 9 mg/mL and a half-life of 21 days (Almagro et al. 2017).

Affinity maturation occurs during the secondary immune response as IgM is switched to IgG; this results in Ig of higher affinity for the antigen (Mishra and Mariuzza 2018).

Table 3.4 Immunoglobulin classes in mammals

Immunoglobulin classes					
	IgM	IgG	IgA	IgE	IgD
Heavy chain	μ	γ	α	ε	δ
Antigen binding sites	10	2	4	2	2
% in serum	6%	80%	13%	0.002%	1%
Main functions	Main Ig of the primary immune response. Binds complement Monomer is a B cell receptor	Main serum Ig	In mucus, tears, saliva, colostrum	Ig related to allergic response	B cell receptor on naïve B cells

3.6 Genetic Basis for Diversity in Chicken TCR and Immunoglobulins

The generation of diversity of the TCR and Ig in the chicken occurs by two different mechanisms. The T cell repertoire is generated in a similar way to that of mammals and there is no evidence of somatic gene conversion mechanisms; a detailed review of the process can be accessed (Smith and Göbel 2014) and will not be discussed in detail here. In B cells, gene conversion is responsible for introducing sequence diversity in the Ig genes and appears to be as efficient as the gene rearrangement mechanism in mammals (Ratcliffe and Härtle 2014). The chicken contains only one light chain gene which is closely related to the mammalian λ light chain; it appears that the locus encoding the κ-type light chain was deleted during evolution (Reynaud et al. 1985); also, there is only one J segment. This contrasts with the situation in mammals (Table 3.3). RAG-1 and RAG-2 proteins involved in the deletion of coding nucleotides or the addition of palindromic (P) nucleotides induces diversity at the joining sites (Chap. 4); there is no evidence of the involvement of TdT in the chicken (Ratcliffe and Härtle 2014). There are three constant heavy chain segments encoding the μ, α and υ Ig heavy chains of IgM, IgA and IgY, respectively; this in contrast to the 11 heavy chain segments in mammals (Table 3.3). Variable, D and J segments encode the heavy chain domain; there is a single V and J segments and 15 D segments which however have low variability compared to those in mammals (Reynaud et al. 1991). Gene rearrangement in the heavy chain occurs in the same way as in the light chain. Additional diversity of the light chain is generated by gene conversion where a stretch of the rearranged L chain gene is replaced by a homologous sequence from one of 25 upstream pseudogenes; the length of the exchanged sequences can range from 10–300 nucleotides (Ratcliffe and Härtle 2014). Activation-induced cytidine deaminase (AID) is required to mediate repertoire diversity of B cell immunoglobulins genes by three closely associated pathways

Table 3.5 Immunoglobulin classes in chickens

Immunoglobulin classes			
	IgM	IgA	IgY
Heavy chain	μ	α	γ
Antigen binding sites	8 or 10	2, 4, 6	2
Serum concentrations (mg/mL)	1–2	0.35–0.65	5–7
Main functions	Main Ig of the primary immune response. Monomer is a B cell receptor	Mucosal protection (High in lachrymal fluid and cloaca)[a]	Main serum Ig; main maternal antibody in egg yolk

[a]Merino-Guzmán et al. (2017)

named somatic hypermutation, class switch recombination and gene conversion. Along with AID the transcription factor Bcl6 is a key to diversification by these three processes (Williams et al. 2016). Also, bursa of Fabricius-derived peptides are involved in local processes such as B cell differentiation and antibody production (Feng et al. 2019) Gene conversion in the heavy chain locus occurs by a similar mechanism. Greater detail of these gene rearrangements is given in Chap. 4 on the evolution of IgY. The contribution of affinity maturation to the development of specificity and effectiveness of chicken Ig is unclear (Ratcliffe and Härtle 2014). The characteristics of chicken Ig are given in Table 3.5 (Härtle et al. 2014).

3.7 Structure of IgG and IgY

IgG is the main serum antibody released during the mammalian humoral response. The relationship between the generation of antibody diversity and the structure of IgG is illustrated in Fig. 3.3a. The variable regions of the light and heavy chains (V_L and V_H) contain three sub-regions which are hypervariable and named the complementarity-determining regions (CDR1–3); these form the antigen binding domain (Fig. 3.3a). The three-dimensional alignment of the CDR of the light and heavy chains are shown in Fig. 3.3d. Each IgG has two identical antigen binding sites. The carboxy terminal region is constant for each class of antibody (fragment crystallizable, Fc) and is responsible for most of the effector functions including binding to Fc receptors and activating the complement system. The hinge region of the antibody, linking the antigen binding portions to the Fc region, is a flexible tether allowing some movement of the antigen binding arms. This is important for binding to antigens of different sizes.

The characteristics of the five classes of mammalian Ig: IgM, IgA, IgG, IgD and IgE (Table 3.4) are based on the structure of heavy chain (Schroeder and Cavacini 2010). Glycosylation sites are shown as green circles (Fig. 3.3a); the degree of

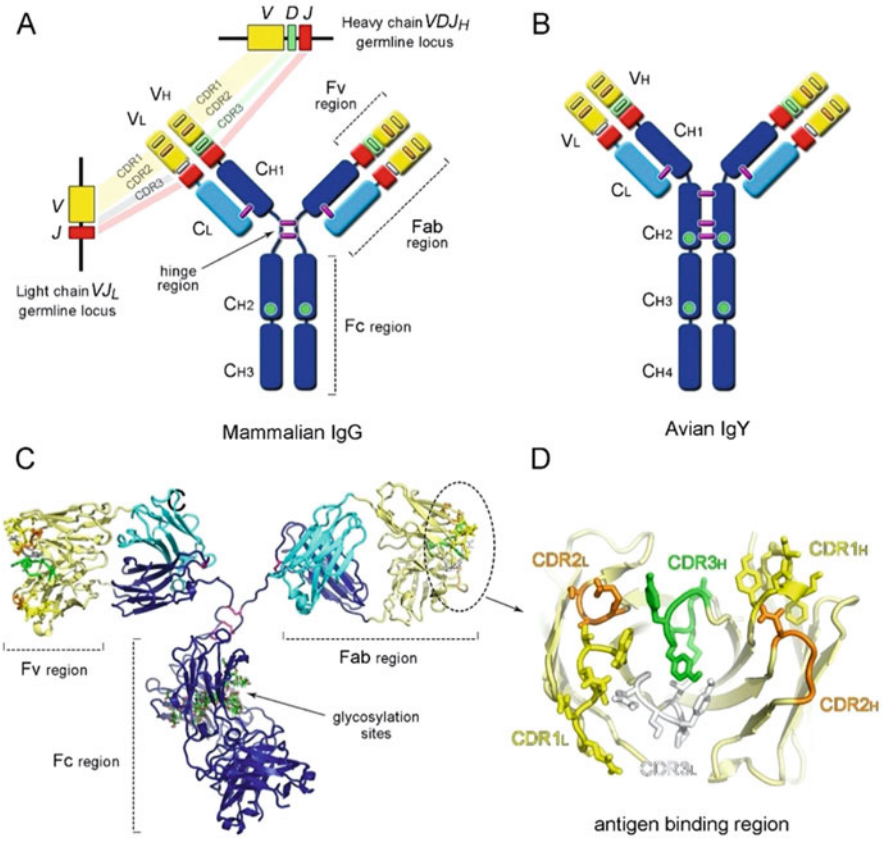

Fig. 3.3 Domain architecture and structure of mammalian IgG an avian IgY. (**a**) General domain architecture of mammalian IgG (MW: –150 kDa). Constant (C) and Variable (V) domains of the Heavy (H) and Light (L) chains of the antibodies are presented in different colours. Complementarity determining regions (CDR) are shown as yellow (CDR1), orange (CDR2) and white or green (CDR3) lines and interchain disulphide bonds as magenta lines; green circles indicate glycosylation sites. Fc (fragment crystallizable), Fab (fragment antigen-binding) and Fv (variable fragment) regions are indicated. (**b**) Similar representation of avian IgY (MW: –180 kDa), showing the additional CH domain (CH4). (**c**) Crystal structure of mammalian IgG (IgG2a) monoclonal antibody (PDB ID: 1IGT, (Harris et al. 1997)) in ribbon representation, following the same colouring scheme. (**d**) Close-up view of the antigen binding region showing the six CDRs, three from the light chain (L) and three from the heavy chain (H). Structure images prepared with PyMOL. Figure courtesy of IgYTechnology.com (www.igytechnology.com)

glycosylation may affect the activity of the molecule (Schroeder and Cavacini 2010) and circulatory half-life. IgG has been the antibody of choice for diagnostic and research tools.

IgY (Fig. 3.3b) has a similar structure to IgG with the following notable differences. It has an additional constant region (CH4), lacks a hinge region and has two glycosylation sites (Fig. 3.3b). The reader is referred to Chap. 4 for detailed

information on the evolution of IgY and its relationship to other mammalian classes and an exploration of the relationship of its structure to its biological activity (Chap. 5).

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