

## Mini Review

# Cytokines as Effective Elements of the Avian Immune System

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### Abstract

The immune system is generally divided into two main branches: the adaptive (specific) and the innate (non-specific) immune responses. Components of the immune system (i.e. adaptive and innate) include T-lymphocytes, B-lymphocytes, macrophages, Natural Killer (NK) cells, heterophils, basophils, diverse humoral communication factors such as eicosanoids and cytokines (e.g., interleukins, interferons, tumour necrosis factor) and effector molecules (e.g. immunoglobulins, complement, lysozymes, nitric oxide). Additionally, some tissues in the body are dedicated to support the immune system such as dendritic, reticular and stromal cells. Regulation, interaction, and communication between various elements of the specific and the innate immune responses result into birds possessing a highly sophisticated immune system, capable of protecting against invading pathogens.

In addition to the various types of cells that are involved in the innate immunity, there are soluble physiological elements that play a role in defending the host from invading foreign substances and pathogens. Cellular and soluble elements coordinate in a sophisticated network of interactions to mount an effective innate and adaptive immune responses capable of eliminating foreign antigens. These soluble physiological elements include: complement proteins, lysozyme, acute phase proteins, and cytokines. The current paper sheds light on cytokines as effective elements of the avian immune system.

**Keywords:** Avian; Cytokines; Immune System; Lysozymes

### Introduction

The innate immunity reflects the inherent non-specific response that provides the first line of defence, just after the exposure to a pathogen. It is characterised by broad specificity where cells and other components of the immune system identify classes of molecules and pathogens, rather than specific antigens. Anatomic barriers, phagocytic cells, physiological components, and inflammation are the main elements of the innate immune response. In common with other animals, birds have evolved an immune system that can respond to and protecting against pathogens such as *Escherichia coli*, *Salmonella* sp. *Pasteurella multocida*, coronavirus, and avian Influenza A virus [1-3].

There are several white blood cells that play a role in the non-specific avian immune response. Most of these cells are capable of engulfing extracellular particles by phagocytosis, endocytosis or receptor-mediated endocytosis. Others can produce substances that play a role in the inflammation response and allergic reaction.

For example, activated eosinophils can produce lipids and proteins that have antiviral activity and induce degranulation of other cells such as mast cells and basophils [4,5].

The complement system is an important element of the innate immune system that also triggers the adaptive immunity. It was reported by Pandit et al. [6] and Skeeles, et al. [7] that the complement system can provide protection against viruses in the early innate defence in birds. Similar haemolytic complement activity was also reported against parasites and bacteria in birds [8-10].

### Complement System

Complement components are proteins and glycoproteins that are mainly synthesized in the liver. Other cells such as the tissue macrophages and the blood monocytes are involved in the complement production. These proteins are circulating in the blood in an inactive form. Once activated upon exposure to an antigen, they enter an enzymatic biochemical cascade that helps in the elimination of antigens by lysis of cells, opsonisation, binding

to specific complement receptors on cells of the immune system, and/or immune clearance of immune complexes [5,11,12].

There are three different pathways by which the complement cascade is activated, namely the classical pathway, the alternative pathway and the mannan-binding lectin pathway. The classical pathway is antibody-dependent, so it is more related to the adaptive immunity. It is initiated by antigen-antibody binding to form immune complexes or when an antibody binds to an antigen on the surface of a pathogen. Activation of complement component 1, often simply called C1 (a protein of the immune system) then occurs when it binds to such antibodies of type IgM and some classes of type IgG. On the other hand, the alternative and the mannan-binding lectin pathways are more related to the innate immunity because they are antibody-independent. The former is initiated by complement component 3 (C3 complement), activation upon exposure of foreign substances on the cell wall of pathogens like bacteria, while mannan-binding lectin pathway is activated when a lectin binds to a mannose residue on the cell wall of pathogens such as *Salmonella*. This lectin is a serum acute phase protein that is produced because of the inflammatory response in the site of inflammation. The Membrane Attack Complex (MAC) is produced upon activation of the complement system in the three pathways. This complex mediates lysis of the cell wall of bacterial pathogens. There are serum proteins as well as proteins on the surface of self-cells that control the activity of the complement system to ensure that host cells are not attacked [5-8,10,13-19].

In addition to playing a role in the innate immune response, the complement system plays a role in activation and regulation of the adaptive immune response [11,13,14]. Studying the different avian haplotypes that are related to immunocompetence has shown that the complement levels are genetically inherited, and that lower disease resistance is associated with lower levels of complement components [11,13,20,21].

### **Lysozyme**

Lysozyme is a hydrolytic protein that destroys the bacterial cell wall by digesting the sugar mucopolysaccharides, causing lysis of the pathogen. It is present in mucosal secretions such as saliva and tears [22-27]. Burns [28] reported the presence of lysozyme activity against *Micrococcus lysodeikticus* in the poultry serum, saliva, gut contents, faecal washes, urine and in tears. In the same study, lysozyme was also found to be present in the mucus-producing cells of the alimentary tract as well as the secretions of the Harderian gland and the lacrymal gland. Moreover, the secretory cells in the lumen of the tympanic cavity of the middle ear epithelium in the chicken *Gallus gallus* was shown to secrete lysozymes [29,30]. Interestingly, Immunoglobulin A (IgA) was noticed in several tissues that contained lysozyme. This would suggest a role of IgA in the lysozyme activity against bacteria [22,25,27,28,30,31].

### **Acute Phase Proteins**

Acute Phase Proteins (APPs) are a group of serum proteins, the concentrations of which are significantly affected by infection and inflammatory status [32-34]. The production of APPs is influenced by the pro-inflammatory cytokines, infectious agents and injury. Accordingly, these proteins can be used as biomarkers for infectious diseases and inflammation [33,35-38].

### **Cytokines**

These are low molecular weight proteins secreted by a wide range of cells, including leukocytes, and are known to regulate and control type and intensity of the immune response as well as other biological functions. These proteins are produced immediately after infection or vaccination and act by binding with high affinity to specific receptors on the cell membrane of the host or foreign body and inducing an intracellular signalling pathway which results in either stimulation or inhibition of a physiological response. Cytokines are considered to be important elements in both the innate and the adaptive immunity that act by stimulating an interactive network of biological responses, including effects on other cells and on cytokines themselves [5,39-44].

The progress in detection and discovery of the avian cytokine genes repertoire gave a great opportunity to categorise and determine the function of avian cytokines [45]. Classifying cytokines is really a debating issue as they could be classified according to their biological effect, the cells producing them or the cells that they affect. Most often, classification of cytokines is based upon the effect or activity they drive. Accordingly, they can be classified into: pro-inflammatory cytokines, interferons, and colony stimulating factors [46-48].

Pro-inflammatory cytokines are cytokines that are produced by both phagocytic and non-immune cells at the site of inflammation or acute phase response due to disease, infection or tissue trauma. The acute inflammatory reaction initiates the production of cytokines that include: interleukin-1 (IL-1 $\beta$ ), IL-6, IL-12 and Tumour Necrosis Factor-alpha (TNF- $\alpha$ ). These cytokines act on the site of inflammation by increasing permeability of the vessels and by inducing the production of another cytokine called IL-8. This is a chemokine that stimulates chemotaxis [39,46,49-51]. IL-1 $\beta$  is known to be an active pro-inflammatory cytokine that induces an acute inflammatory response by activating cells like macrophages and T-lymphocytes to produce other kinds of cytokines and chemokines [52]. Laurent, et al. [53] have reported that expression of IL-1 $\beta$  mRNA in the gut of chickens was increased 80-fold seven days after infection with *Eimeria tenella*. IL-6 is known to be a multifunctional cytokine that activates B- and T-lymphocytes and induces macrophage haematopoiesis [50,54-58]. In addition, IL-6 has a role in regulating B-cell differentiation to the effector antibody producing plasma cells [59]. *In vitro* IL-1 $\beta$

and IL-6 activity was increased in macrophage supernatants from birds infected with poultry enteritis and mortality syndrome [60], indicating their role in a bird's infection. IL-8 is a chemokine that attracts and induces the accumulation of neutrophils in the site of inflammation. The term chemokine refers to a specific class of cytokines that mediates chemoattraction (i.e. chemotaxis between cells). This accumulation usually causes damage to the tissues. Also, IL-8 attracts peripheral blood monocytes and fibroblasts [40,41,47,61,62]. In addition, there is evidence of the presence of an avian homologue of the mammalian TNF- $\alpha$  which is secreted by macrophages, T-lymphocytes and NK cells. TNF- $\alpha$  plays an important role in the systemic inflammation, cytotoxicity to tumour cells, and apoptosis. Fever and septic shock are usually associated with TNF- $\alpha$  activity [62-64].

Other chicken cytokines that are believed to be involved in the inflammatory reactions are IL-15 and IL-16. IL-15 has a role in the cell-mediated immune response by activating T- and B-lymphocytes as well as NK cells. Also, IL-15 is known to play a role in heterophil activation [39,46,55,65]. Kaiser [59] has proposed the role of IL-15 in inducing autoimmune disease in chickens as a result of increased immunity that results in inflammation and damage. IL-16 was also cloned in chickens, this cytokine is known to play a role in attracting T<sub>H</sub>-lymphocytes, monocytes and eosinophils. Interestingly, Concanavalin A (con A)-stimulated splenic T<sub>H</sub> cells in adult chickens, immunized with *Salmonella enteritidis*, can produce a lymphokine called *Salmonella* Immune Lymphokine (SILK). Prophylactic treatment with this lymphokine can provide protection against *Salmonella enteritidis* in one day old chicks [66,67]. Crippen et al. [66] also reported that the rP33 domain of SILK is an active part that is capable of inducing *in vitro* antimicrobial effects of heterophils against *Salmonella enteritidis* by triggering degranulation of these lymphocytes.

Interferons (IFN) are a group of cytokines that are produced by leukocytes and viral-infected cells because of stimulation of the immune system by viral infection and inflammation reactions. Knowledge about avian interferons was lacking molecular analysis of these proteins. Recently, purification, functional characterisation, cloning and sequencing of genes that encode chicken interferons from the cDNA library has proved the presence of two types of interferons in chickens [68-76]. These are: IFN- $\alpha/\beta$  (type I) that is produced by mononuclear cells and fibroblasts upon viral infection and IFN- $\gamma$  (type II) that is produced by T-cells (T<sub>H</sub>1) and NK cells upon stimulation by an immunogenic factor. IFN- $\alpha/\beta$  increase level of class I-MHC molecules on the surface of viral infected cells so that these cells are identified for cytotoxic T-cells (Tc). Also, these interferons have a role in affecting tumour cells by slowing down the cell cycle [41,55,59,62,77]. On the other hand, IFN- $\gamma$  is more known in modulation of the immune response and more involved in the inflammatory response of chickens, it is also known to play a role in macrophage activation [41,45,55,62,78-89]. Neutralization

of the immunological effects of IFN- $\gamma$  has been accomplished by Monoclonal Antibody (mAb) techniques [90]. In addition to IFN- $\gamma$ , T-cells (T<sub>H</sub>1) produce IL-2. This is a well identified cytokine in chickens and turkeys by cloning of genes encoding this interleukin from the cDNA library. IL-2 is known to have a role in the cell-mediated immune response as well as in macrophage activation [40,91]. Furthermore, cDNA was cloned for chickens and turkeys IL-18. Like IL-2 and IFN- $\gamma$ , IL-18 develops a cell-mediated immune response (T<sub>H</sub>1-type response) and activates macrophages. It also stimulates the production of IFN- $\gamma$ . It is reported that IL-18 is produced by Kupffer cells (liver macrophages) [59,62,92,93].

In addition, Growth Factors are a family of cytokines that have been shown to play a role in the development of immune cells. In birds, these include granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), granulocyte-macrophage-colony stimulating factor (GM-CSF), transforming growth factor- $\beta$  (TGF- $\beta$ ) and chicken myelomonocytic growth factor (cMGF). McGruder et al. [94] has reported that *in vitro* observations of chicken cytokine cultures indicated the biological activities of G-CSF and M-CSF in supporting the proliferation and differentiation of macrophages and granulocytes and the activity of GM-CSF in supporting differentiation of myeloid precursors to granulocytes and macrophages. On the other hand, TGF- $\beta$  is a growth factor that regulates the development of T-lymphocytes and has a role in the anti-inflammatory response [94,95,96]. The anti-inflammatory activity of TGF- $\beta$  was reported by Choi, et al. [97] when he showed that m-RNA expression of this cytokine was increased in caecal tonsil, spleen and duodenum after microbial infection in chickens. cMGF stimulates avian myeloid cells differentiation into mononuclear cells. Moreover, there are other growth factors that are described in chickens. Among these are: Fibroblasts Growth Factors (FGF), Platelet-Derived Growth Factor (PDGF) and Stem Cell Factor (SCF). PDGF and SCF have a role in healing from injury and in differentiation of stem cells, respectively [98,99].

Interestingly, modulation of the immune system could be achieved using purely cloned cytokines or cytokine adjuvants. This has many applications in the field of experimental and clinical research in poultry. For example, cytokines are used in neonatal poultry to support and accelerate the onset of the immune system. *In vivo* injection of recombinant cMGF avian cytokine has shown that this cytokine can increase the number of bone marrow progenitor cells that will be differentiated to immune cells in newly hatched chicks [100]. Lowenthal et al. [101] have reported that IFN- $\gamma$  is an effective vaccine adjuvant in birds injected with sheep red blood cells. This cytokine considerably elevates the immune response and triggers more IgG response, compared with the control birds. Also, IL-18 as an adjuvant in case of *Salmonella* infection of birds was observed to considerably increase T<sub>H</sub>1-type response, IFN- $\gamma$  and macrophage activation. Taken together, cytokines, along with

other component of the immune system, play critical role in the immune response.

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