AVIAN CYTOKINES AND DISEASE PREVENTION: AN OVERVIEW

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Abstract: Cytokines are soluble low molecular weight (< 30 kDa) regulatory proteins that serve many functions in the body such as mediating and regulating immunity, inflammation and hematopoiesis. Cytokines are secreted by various cell types that play important role in the regulation of immune response, but the largest group of cytokines is involved in immune cell proliferation and differentiation. Cytokines have enormous potential to control infectious diseases in poultry. Recent advances in recombinant DNA technology and avian genetics have explored the application of avian cytokines in poultry medicine. The use of cytokines in poultry has become more feasible with the discovery of number of avian cytokine genes. This monograph will review the recent advances made in avian cytokines and their therapeutic potential.

Key words: Cytokine, Avian disease

INTRODUCTION

Poultry is an important component of livestock industry. Chickens are reared under intensive conditions and are prone to infection by opportunistic pathogens. Newly hatched chickens are highly susceptible to infection during first 2 weeks of life [1]. Several factors contribute to this problem, including immaturity of immune system, reduced maternal antibody protection and stress factors associated with intensive rearing of poultry [2]. A major problem faced by the poultry industry is the loss of productivity due to disease. Therefore, considerable resources are required in order to maintain the health status of these animals [3].

Various infectious diseases are mainly controlled by the combined use of vaccines, antibiotics and chemicals. Vaccines are designed to provide long term immunity and confer specific protection against a particular pathogen following immunization. Vaccination with live (attenuated) organism is more effective and provide long term immunity without the use of any adjuvants. However, the use of live vaccines imposes serious safety concerns. They can sometime cause disease, either due to genetic reversion or due to infection of immunocompromised host [4].

Inactivated vaccines require the use of adjuvant to develop a long term protective immune response. Unfortunately, most commercial adjuvants are oil-based and their use can result in adverse local reactions. Antibiotics have also been used to treat and control diseases in poultry [5]. However, the short term protection and continuous supplementation as feed-additive limit their usage [6]. Also, the extensive use of antibiotics in animal feed has resulted in the emergence of drug resistant bacteria in mankind [3]. Similarly, chemicals have been used to control bacterial and parasitic diseases in poultry but their long term use have also created problems. Some European countries have already banned the use of antibiotic and chemical feed additives in food
animals. With the imminent and widespread ban of in-feed antibiotics and chemicals, alternative measures need to be sought to ensure that the Poultry industry will not be adversely affected.

**Alternative to feed additive:** Alternative to feed additive will need to guarantee that food animals such as poultry are able to reach their growth potential; and should be safe, easy to administer and cost effective [5]. Chicken cytokines are also being examined as replacement for in-feed antibiotics in poultry. Cytokines are adverse family of proteins that regulate the immune response by mediating a multitude of effects ranging from activation and differentiation of immune cells to enhance the immune function and production of other cytokines [7]. Depending on the combination of cytokines produced, a protective immune response can be generated as either an antibody-mediated (Th2) or cell-mediated (Th1) response. Cytokines, therefore, represent excellent candidates as naturally occurring therapeutics as well as vaccine adjuvants [8].

**Avian cytokines in disease prevention:** Cytokines have enormous potential in the control of infectious disease in poultry. Their use as novel therapeutic agents in poultry farming is becoming more feasible with the recent advances in recombinant DNA technology and genome sequencing techniques of avian species [9]. A number of cytokine genes have now been cloned in several avian species. Nowadays several expression systems are available for the production of recombinant cytokine. Various avian cytokines have been successfully expressed in *E. coli*, yeast, CHO, COS, baculovirus, tobacco mosaic virus (TMV) and fowl adenovirus [3]. Based on their origin and activity, cytokines have been classified into various groups like interferons (IFNs), interleukins (ILs), transforming growth factors (TGFs), tumor necrosis factor (TNF), colony stimulating factor and chemokines [10]. This section will provide information regarding various chicken cytokines and their role in disease prevention.

**Interferons (chIFNs):** Interferons, first reported by Isaacs and Lindenmann [11], are multifunctional glycoproteins secreted by white blood cells and viral infected somatic cells in response to viral infection, immune activation, inflammatory stimulation and chemical stimulant. These secreted proteins have antiviral, anti-tumoral and immunoregulatory effects [12]. According to their origin and physico-chemical properties, IFNs can be grouped into three types: Type I, Type II and Type III [13].

**Type I IFNs:** The type I IFN of chicken occupies a privileged position in cytokine research as it was the first IFN to be discovered [14]. Type I IFNs are mainly produced by viral infected mononuclear cells and viral infected fibroblasts. In chicken, type I IFNs include IFN-α, IFN-β, IFN-κ and IFN-ω of which IFN-α and IFN-β have well defined anti-viral and anti-tumor effects. The therapeutic potential and anti-viral activities of chIFN-α have been demonstrated against various avian infectious diseases like infectious bronchitis virus [15], infectious bursal disease virus [16], new castle disease virus [17, 18], avian influenza virus [19], etc.

**Type II IFNs:** Type II IFNs (IFN-γ) are the most extensively characterized chicken cytokine and are derived from T-lymphocytes and natural killer (NK) cells. They represent the major macrophage activating factor (MAF), exhibits anti-viral activity and drive TH-1 immune response [20,21]. IFN-γ is a pleiotropic molecule having adjuvant [22] and growth promoting properties [23,24]. The immunoenhancing and therapeutic effects of chIFN-γ have been demonstrated using coccidiosis challenge models [24,25]. The ability of chIFN-γ to combat infection and enhance vaccine efficacy makes it an excellent candidate as a therapeutic agent and adjuvant.

**Type III IFNs:** Only one IFN-λ gene has been identified in the chicken that possesses anti-viral properties [26-28].

**ChIL-1β:** IL-1β is a proinflammatory cytokine produced by macrophages, NK cells, monocytes and neutrophils [29,30]. Mammalian IL-1β was synthesized as a procytokine (31-33 kDa) which is cleaved by caspase-1 (interleukin IL-1 convertase) to form mature IL-1β. In the chicken, the homologue of mammalian IL-1β was cloned in 1988 [31]. ChIL-1β has also been examined for its immunoadjuvant activities using tetanus toxoid as adjuvant [32].

**ChIL-2:** IL-2 was discovered in 1975 and was originally named as T-cell growth factor (TCGF). IL-2 is a monomeric glycoprotein of 15 kDa secreted
predominantly by activated T-lymphocytes. Functionally, IL-2 is a Th1 cytokine that leads to the activation of macrophages and the development of a cell mediated immune response [33]. The chIL-2 cDNA encodes a predicted protein of 143 aa, with a signal sequence of 22 aa and a mature protein of 121 aa [34, 35]. The immunomodulatory activity of chIL-2 has been studied in some cases [36].

**ChIL-6:** Chicken IL-6 is a multifunctional cytokine produced by T and B lymphocytes, endothelial cells, monocytes and fibroblast. This cytokine is involved in acute phase response, immune regulation and hematopoiesis [37]. The cloning of chIL-6 and the description of its biological activity is a significant advancement in the field of avian cytokine [38]. ChIL-6 activity has been found in several infectious diseases of chicken like Eimeria [39].

**ChIL-18:** Mammalian IL-18, a proinflammatory cytokine, was originally designated as IFN-γ inducing factor [40]. IL-18 is synthesized as a precursor molecule that is cleaved by caspase-1 into a bioactive mature form. IL-18 act synergistically with IL-12 to promote IFN-γ production. The chIL-18 cDNA was identified and cloned in 2000 which encode a protein having approximately 30% aa identity with mammalian IL-18 [41]. Several studies have documented the immunomodulatory and anti-viral properties of chIL-18 [42-45]. However, the properties and applications of chIL-18 in disease prevention remain largely uninvestigated.

**Transforming growth factor β (TGF-β):** TGF-β plays an important role in the development of T-lymphocytes and has important anti-inflammatory activity. Three isoforms of TGF-β have been cloned from chicken: TGF-β_1 [46], TGF-β_2 [47] and TGF-β_3 [48] which has 80%, 96-99% and 97-99% aa identity respectively with their mammalian homologue. Most studies in the chicken have been focused on the role of TGF-β in the embryonic development [49] and hematopoiesis [50]. Recently, the adjuvant effect of chicken TGF-β has been studied in relation to DNA vaccination against *E. acervulina* [51].

**Chemokines:** Chemokines are a group of small (8-14 KDa), structurally related proteins that regulate trafficking of various types of leukocytes through interactions with transmembrane G-protein coupled receptors [7]. They are divided into sub-families on the basis of arrangement of the conserved N-terminal cysteine residues: CXC, CC, C and CX3C. A total of 23 chemokines have been identified in the chicken [4]. CXC chemokines designated as cCAF/IL-8 have chemotactic activity for chicken peripheral blood mononuclear cells and plays a role in wound healing [52].

**Summary and future perspectives:** Control of infectious disease in poultry has depended largely on the use of antibiotics and vaccines. However, there are concerns arising from their usage in livestock especially poultry. The extensive usage of antibiotic has led to the emergence of drug-resistant bacteria in humans. The imminent banning of antibiotic usage in livestock feed has intensified the search for environment friendly alternative methods to control disease. Under such conditions, cytokines offers a natural approach to therapeutics particularly in relation to the enhancement of protective immune responses. Cytokines represent potent molecular regulators of the entire host physiology. The use of cytokine in poultry has become more feasible with the discovery of a number of avian cytokine genes.

For cytokines to be used as commercial therapeutics, certain aspects of the poultry industry should be taken into account. The delivery methods for therapeutic agents are of prime importance and are required to be safe, easy to administer and cost effective. Cytokines in poultry can be administered as soluble proteins, DNA plasmid and *in-ovo* administration. Administration of recombinant cytokine protein by injection is not feasible in commercial poultry. In 2000, Johnson and coworkers [53] studied the effectiveness of adenoviral delivery system. The administration of recombinant fowl adenovirus with incorporated chIFN-γ gene results in increase in bird weight [53]. Adenoviral delivery of therapeutics and immunomodulators can be possible through drinking water and in-feed administration. *In-ovo* administration of cytokines provides a simple and efficient method of delivery of soluble therapeutics. Guo and coworkers [22] administer chIFN-γ *in-ovo* as a treatment for chicken anaemia virus (CAV). However, for commercial utilization of *in-ovo* system in poultry industry, large scale production of cytokine has to be considered. Recent developments in live viral vectors and DNA vaccination technologies now provide realistic alternative. These new generation delivery mechanisms also allow the administration of single
or multiple cytokines. With the increasing number of chicken cytokines being cloned, cytokines could potentially provide the poultry industry with the most natural, non-antibiotic methods for increasing disease resistance.

**REFERENCES**


