Research News For U (RNFU) ISSN: 2250 –3668, Vol. 10, 2013
Available online http://www.doublehelixresearch.com/RNFU

Cytokines as potent therapeutic agent and vaccine adjuvant in poultry

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Introduction

Cytokines are soluble, low molecular weight polypeptides and glycopeptides produced by a broad range of cell types that may be hematopoietic and non-hematopoietic in origin, and have suppressive or enhancive effects on cellular proliferation, differentiation, activation, and motility. Cytokines regulate immune responses by mediating a multitude of effects ranging from activation and differentiation of immune cells to enhancing the immune function and production of other cytokines (Hilton et al., 2002). Though the vaccines and antibiotics are the main components to control the diseases, use of antibiotics restricted due to the emergence of drug resistant bacteria which leads to a huge loss in a wide population than the impact of the disease caused by those micro organism. So it intensifies the usage of natural and environmental friendly alternative to control the disease by using cytokines as vaccine or adjuvant. Recent progress in the recombinant technology and gene delivery system enhance the use of these recombinant cytokines as a good alternative.

There are several control measures against various infectious diseases includes vaccines, antimicrobials and chemicals. By conferring specific protection to pathogen after immunization, vaccines provide a long lasting immunity. Immunization using live attenuated vaccine is most efficient, but reassortment with field isolates and mutations between high and low pathogenic species restricting the use of live vaccines. Though the recombinant proteins and inactivated micro organism are less immunogenic, use of adjuvants solve the problem to a considerable level. Unfortunately most of the adjuvants of veterinary use is oil-based which causes adverse local reaction with few exceptions of licensed adjuvants like MPL (Monophosphoryl Lipid A), AS04 (alum with MPL) and AS03 (squalene based adjuvant) (Rahman et al., 2012), chemokines and cytokines are the alternatives and can be used as DNA vaccines. Cytokines determine both the type and the extent of an immune response that is generated following an infection by a pathogen or after vaccination. Depending on the combination of cytokines produced, a protective immune response can be generated as either an antibody-mediated (Th2) response, or a cell-mediated (Th1) response (Mosmann et al., 1996) . Therefore, cytokines are excellent candidates for developing naturally

occurring therapeutics and vaccine adjuvants (Heath et al., 1992). Expressed sequence tag (EST) library is a recent advance in the cloning of avian cytokine genes.

Avian immune system

Innate immune effector cells such as killer cells, heterophils and other lymphocyte subsets (γδ T cells) and its interactions with APCs particularly dendritic cells are better characterized. In chickens, like in mammalian system, Th1 cytokine responses (IFN-γ, IL-12, and IL-18) predominate in response to intracellular pathogen and Th2 cytokine responses (IL-4, IL-13, IL-19) predominate in response to extracellular pathogens. They lack lymph nodes but have avian specific bursa of Fabricius. Availability of avian genome sequences and post-geomic technologies have allowed the identification of various immune molecules specific to avian species.

TLR- recognizes unique PAMPs and signal to induce pro-inflammatory cytokine production there by triggering innate as well as adaptive immunity so that it can be used as effective adjuvant to initiate better immune response. The recent release of chicken genome allowed us to identify a number of potential orthologous chicken TLR and its similarities and differences with human. One of the disadvantages is the variation of TLR expression in different lines of chicken as higher expression greater the immunological response and vice versa.

Chicken cytokine

When compared to mammal, chicken contains a small number of cytokine family genes. Recent research development leads to identification of several new cytokine receptor genes in the chicken genome which raises the intriguing prospect that additional cytokines remain to be discovered. Chickens are good model system to study the effectiveness of cytokine as therapeutic agent and vaccine adjuvant due to its more homology with the mammalian in respect to the response to disease and vaccination. Very few bioassays like ELISAs developed for avian cytokines, molecular techniques like qRT-PCR commonly used to study the cytokine responses in diseases.

Following are few cytokines and its role in avian immune system has been studied well in recent days (Table 1). Cytokines characterized as Th1 cytokines (IL-2. IFN-γ, TNF and Lymphotoxin (LT) (Mosmann et al., 1996) which are involved in CMI and Th2 cytokines (IL-4, IL-5, IL-6, IL-10 and IL-13) involved in regulation of humoral immunity. Except IL-6, all cytokines cloned in chicken are being categorized as Th1 like. Similarly based on the function, chicken cytokines classified as pro-inflammatory (IL-1β, IL-6, il-8), Th1(IFN-γ, IL-2, IL-8), Th2 (not described), Th3/Tr1 (TGF-β) and others (IFN-α, IFN-β, IL-15, IL-16, chemokines).Some of cytokines also been cloned in other avian species including turkey, Japanese quail, pheasant and guinea fowl. Several laboratories involved in avian cytokine research recently formed the avian cytokine group (ACG) for the exchange of resources for research purpose and also developed a web site (www.geel.li.csiro.au/aviancytokines).

Table 1. Avian cytokines and its role in immune system

Cytokine	Host	Role in immune system	Reference
IFN-α	Chicken, Duck	Antiviral effect synergistically with IFN-γ	Sekellick et al., 1998

IFN-β	Chicken	Induction of fibroblast to secrete chemokines	Weining et al., 1988
IFN-γ	Chicken, Pheasant, Japanese Quail, Duck	Immune enhancer and vaccine adjuvant	Lowenthal et al., 1997
IL-1β	Chicken	Pro-inflammatory effect	Weining et al., 1998
IL-2	Chicken	T cell growth factor	Schnetzler et al., 1983
IL-6	Chicken	Th2 type response	Schneider et al., 2001
IL-15	Chicken	NK cell and $CD8^+$ memory cells activation and	Carson et al., 1994
IL-16	Chicken	IFN-γ production by Th1, NK and NK T cells	Okamura et al., 1995; Kohno et al., 1997
IL-18	Chicken	IFN-γ production	Kaiser et al., 2001
SCF	Chicken, Japanese quail.	Stem cell growth	Zhou et al., 1993
MGF	Chicken	Growth of avian myeloid precursor cells	Leutz et al., 1984
TGF β	Chicken	Immature thymocytes to mature $CD3^+$	Mukamoto and Kodama et al., 2000)

Use of cytokine as immunotherapeutic agent and adjuvant in poultry sector

Exogenous use of cytokines to restrict the infectious agent has three options: use as adjuvants with vaccines, to induce protection against the response of pathogen and their ability to stimulate the ontogeny and activation of neonatal host defenses (Kogut et al., 2000).

As immunotherapeutic agent

Commercial poultry is having more exposure to common pathogens, vaccination and antibiotic therapy may not economical and feasible against each of these pathogens. So use of non-specific potentiators (cytokines) may be a solution to protect the stock from the diseases. Cytokines extensively used experimentally and clinically in mammalian therapeutics and now it is growing in avian sector. Incorporation of IL-18 along with attenuated vaccine strain 9R leads to higher production of IFN-γ and macrophage activation which subsequently clears Salmonella from macrophages as they survive with in macrophages (Jones et al., 2001). Cross protection between serotypes in contrast to Salmonella vaccine observed when cytokine administered in poultry (Cooper et al., 1994). Similarly cytokine mediated functional activation of heterophils lasts about 5 days with the development of resistance to salmonellosis (Ziprin et al., 1989). Similarly experimental injection of IFN-α reduced the level of NDV disease in poultry (Marcus et al., 1999).

As vaccine adjuvant

Adjuvant is an agent that increases immunogenic response of a immunogen when incorporated in to a vaccine formulation which will accelerate, extend or enhance the magnitude of specific immune response. Though aluminium hydroxide (alum) adjuvants commonly used in veterinary and human vaccines, due to its ability to induce weak response to recombinant proteins, poor induction of CMI particularly cytotoxic T-cell response and mediation of IgE mediated allergic

reactions, it is considered as weak adjuvant. In the case of oil-based adjuvant like CFA, induction of inflammation and ulceration at the site of injection spoil the meat quality in poultry industry and fever and sensitivity reaction render them not useful as poultry producers need good and effective adjuvant that prop up protection without causing any pain or distress to birds when administered with vaccine.

Since immunological response (humoral and cellular) induced by adjuvants are controlled by cytokines, direct use of cytokine as vaccine adjuvant created interest in poultry vaccinologist. Use of IL-2 as potential adjuvant has been studied and shown to enhance the immune response when administered along with rabies inactivated vaccine (Nunberg et al., 1989) and herpes simplex vaccine (rouse et al., 1985) and also been demonstrated in vaccine model systems of cattle, guinea pig, pig and mice (Hughes et al., 1992, Pighetti et al., 1993). Similarly adjuvant efficacy of IFN-γ have been reported as effective adjuvant in mice when delivered along with malarial (Heath et al., 1989) and influenza vaccine (Cao et al., 1992). Similarly IL-1α and IL-1β also used as adjuvant in sheep. These studies open the way to consider the cytokine use as adjuvants in chicken too. As compared to the mammalian cytokines, chicken cytokines adjuvant activities are less well characterized. Genes of chicken myelomonocytic growth factor (cMGF), Stem cell factor (SCF), IL-8, type I IFN and type II IFN cloned and expressed by several workers. Combined administration of ChIFN-α, ChIFN-γ and ChIL-1β increased antibody responses to tetanus toxoid in chicken (Schijns et al., 2000). Chicken myelomonocytic growth factor (cMGF) enhances the growth of macrophages and granulocytes from avian bone marrow progenitor cells (Leutz et al., 1984). Enhancing of vaccine efficacy and the ability to combat infection by activating macrophages through IL-1 and nitric oxide intermediate secretion, ChIFN- γ act as excellent candidate as a therapeutic and adjuvant agent (Kayaga et al., 1989).

Table 2. Use of few avian cytokine as therapeutic and adjuvant agent

Cytokines	Host	Effects
IFN-α	Chicken	Level of NDV disease reduced in experimental infection with rIFN-α (Marcus et al., 1999)
	turkey	As adjuvant along with NDV DNA vaccine increased the antibody titre (Rautenschlein et al., 2000)
IFN-γ	Chicken	Increased immune response (Lowenthal et al.,1998)
	Turkey	Rapid antibody response in in-ovo IFN-γ NDV DNA vaccine (Rautenschlein et al., 2000)
Type I & II IFN	Chicken	Invitro suppression of MDV replication (Heller et al., 1997)
IL-1β	Chicken	No effect as adjuvant with Tetanus toxoid (Schijns et al., 2000)
Unidentified immune lymphokines (ILK)	chicken	Inhibits colonization of Salmonella in gut (Kogut et al., 1997)

Delivery of cytokine:

By protein degradation and excretion mechanisms, intravenously administered cytokines are rendered ineffective. Advances in the development of delivery system especially viral vectors allowed variety of cytokines individually or combinely administered and expressed in different species of animals. For larger high value animals like pig and cattle, recombinant cytokines cloned baculovirus, yeast or E.coli expression system successfully delivered via injection. But for chicken, it should be produced in large scale and should be administered single dose to be cost effective. Good option to achieve this requirement is live viral vectors which also eliminate the need of multiple boosters. For example, fowl adenovirus (FAV) expressing cytokine genes overcome the problem of short half-life of recombinant cytokines in vivo as it is expressed over a period of time till the virus live in the host and also can be administered through drinking water and via aerosol (Johnson et al., 2000). Combining of IFN gene with vaccine strain virus allow more virulent strain to be used as a vaccine strain as IFN production attenuate the virus while allowing a vigorous immune response (Giavedoni et al., 1992). One advantage in chicken delivery system than mammal is the unique opportunity of in-ovo injection of therapeutics and vaccines and this allows injection of cytokine as DNA vaccine and also possible for injecting using automated system. In recent days, studies going on to demonstrate the delivery of micro particle like liposomes encapsulated cytokines that induce immune response as vaccine supplementation or to treat pathogens.

Future challenge:

Chicken will be a good model system to study effectiveness of cytokine therapy in controlling animal diseases. One of the main drawbacks of cytokine therapy in poultry is the delivery as injection of recombinant cytokine proteins is not feasible in commercial poultry. Alternatives like bacterial and viral vector technology (oral or aerosol) or in-ovo injection of protein or targeted vector (through automated egg injection system) to specific sites such as respiratory tract or digestive tract has to be explored enormously. Administration of single or multiple cytokine with the immunogen is possible with the help of these new generation delivery mechanisms. As cytokines play very important role in infectious and non-infectious immunity development, its therapeutic role should be investigated very deeply. By increasing the understanding of adjuvant property of cytokines especially along with DNA vaccine as well as live viral vaccine, more effective vaccine and vaccination strategies can be formulated for the betterment of poultry as well as livestock sector. As most of the meat producing countries advising to use alternate methods to protect from diseases to avoid antibiotic resistance as well as chemical residues, cytokine may be the suitable alternative in livestock and poultry industry.

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