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Revolutionizing Poultry Vaccination: Flagellin A and TLR5

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ABSTRACT

Chicken raised in commercial settings are susceptible to many environmental diseases. Therefore, to prevent infectious diseases, the flock must be regularly vaccinated. To combat infectious diseases, birds must be administered vaccines along with effective adjuvants that improve protection and do not cause unanticipated negative effects. In light of this viewpoint, there is a greater need for improved vaccination adjuvants that work. Efforts are underway to improve vaccine effectiveness by using appropriate adjuvants, especially those based on the Toll-like receptor (TLR). Pattern recognition receptors (PRRs) that can identify conserved pathogen compounds include TLRs. Numerous investigations have confirmed flagellin's efficacy as an adjuvant and its capacity to stimulate the production of cytokines by various innate immune cells. This mini review compiles our current understanding of flagellin action, its role in inducing a cytokine response in chicken cells, and its potential use as an adjuvant in chicken vaccines, both by itself and in combination with other TLR ligands.

Keywords: Flagellin, Toll-like receptors, Cytokines, Vaccine, Infectious Diseases

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Introduction

The poultry industry is an important part of global agriculture. Infectious disease-related productivity losses are the industry's main issue. As a result, careful observation and proactive management of the birds' health are necessary. Active immunization using live viral vaccines is now commonplace. A suitable adjuvant is necessary to increase the immunogenicity of the antigen in addition to a high-quality antigen for a vaccination to be successful. Recombinant vaccines and other vaccinations of the more recent generation typically don't elicit a robust immune response [1]. Adjuvants that can increase the antigen's antigenicity are necessary for these vaccinations to boost the immune response. Adjuvants that have been employed historically include inorganic substances, bacterial products, and intricate blends of mineral oil, synthetic polymers, and surface-active chemicals. The most often used adjuvants are those based on mineral oil and alum. Freund's complete adjuvant is a mineral oil-based adjuvant that works well, but it causes a lot of tissue damage at the injection site and negative local unpleasant reactions, which might lead to systemic problems in chickens [2]. Alum is linked to the activation of the IgE antibody response, has poor adjuvant effectiveness, and can result in allergic responses. A new era of TLR-based adjuvants, which can significantly improve the immune response to vaccinations, is heralded by recent advances in innate immunity. The innate immune system can recognize unique conserved molecular patterns of pathogens, commonly referred to as pathogen-associated molecular patterns [PAMPs], thanks to pattern recognition receptors (PRRs) [3]. The immune system is alerted to prevent the spread of infection by recognition through PRRs. One of the PRR kinds is TLRs [4, 5]. Furthermore, although TLR5 and TLR4 identify flagellin and LPS, respectively, TLR21, a functioning orthologue of mammalian TLR9, identifies CpGODN. While chicken TLR3 seems to identify dsRNA similarly to that shown in mammals, the mammalian counterparts of TLR8 and TLR9 appear to be impaired in chicken [6]. In the case of Newcastle disease virus (NDV) infection, for instance, cellular immunity is essential due to the intracellular phase of viral pathogenesis. Because of this, an agent that can trigger both kinds of immune responses must be used. Although the TLR21 ligand CpG-ODN is an effective adjuvant, its usage has been restricted because of its short half-life in vivo, which causes it to absorb by irrelevant tissues, and its brief biological activity. Even though modification lengthens its half-life, it still degrades slowly and is not immune to nuclease action. Other drawbacks of the alteration include poor cellular absorption, non-specificity, toxicity, and serious adverse effects after prolonged use [7]. TLR5 ligand flagellin, a crucial structural protein of Gram-negative flagella and another microbial component, has shown significant potential as an adjuvant and is a potent inducer of the release of cytokines and chemokines, according to a study [8].

TLR5 Signaling

The innate immune system's TLRs, which detect microbial infections and trigger host defense mechanisms against them, have recently come into their own. Type 1 interferons (IFN) and other inflammatory cytokines are produced as a result of a series of events that are started when a ligand binds to its particular TLR. Two different pathways make up TLR signaling: one that is dependent on MyD88 and the other that is not [9]. All TLRs, except TLR3, employ the MyD88-dependent route, but TLR4 uses both. The MyD88-dependent pathway is triggered when flagellin attaches to TLR5. Analysis of mice lacking MyD88 demonstrated the critical function of MyD88 in TLR signaling, as shown in Fig. 1. These MyD88-deficient mice's macrophages were unable to produce inflammatory cytokines such as interleukin-1, IL-6, IL-12, and TNF. when they were exposed to bacterial components. When a TLR is stimulated, MyD88 attaches itself to its cytoplasm and attracts IRAK-4 (interleukin-1 receptor-associated kinase), IRAK-1, and TRAF6 (TNF receptor-associated factor) to the receptor. IRAK-1 is subsequently phosphorylated by IRAK-4. TRAF6 releases from the receptor and activates TAK1 when paired with phosphorylated IRAK1 [10]. IkB is degraded, and NF-B is activated when the IKK complex is activated. Thus, both MAP kinases trigger genes linked to inflammatory responses. These genes help direct and shape the acquired immune response by releasing a variety of inflammatory cytokines and chemokines when they are activated [8].

TLR Signaling Affects many Immunological processes to Enhance the Immune Response

The antigen-presenting cells (APCs) express more major histocompatibility complex (MHC) and other costimulatory molecules when cytokines are produced, and T and B cells are activated when CD70 and CD40 molecules are upregulated, as shown in Figure 2. The innate immunological responses of macrophages to bacterial infections are characterized by TLR signaling and phagocytosis; multiple investigations have shown that TLR signaling increases the rate of phagocytosis [11]. By increasing the cross-presentation and cross-priming capacity of APCs, TLR-based microbial component detection improves immunization efficacy. Furthermore, TLRs have been discovered on CD4 T cells, suggesting that microbial elements could directly support activated CD4 T cell survival without the need for APCs. After TLR ligation, the NF-B pathway is activated, which directly mediates this action. These investigations demonstrated that effector memory T cells play a role in innate immunity and that TLRs activate adaptive immune cells [12].

The Flagellin: as a TLR5 Agonist.

Structure: A globular protein called flagellin, which is present in large amounts in practically all flagellated bacteria, arranges itself in a hollow cylinder to create the filament in the bacterial flagellum [13]. Four domains make up the flagellar protein: D0 to D3. The core protein sequence's N and C termini contains a highly conserved amino acid sequence that is present in the D1 and D0 domains, which are primarily helical in shape. Within the hypervariable area are the D2 and D3 domains. Innate immune recognition depends on the variable D3 domain of the Salmonella enterica serovar Typhimurium flagellin monomer, which is located at the flagellar filament surface and has been demonstrated to have immunostimulatory activity [10, 14]. A stop codon mutation prevents these people from identifying flagellated germs and, as a result, from mounting a proinflammatory response that is mediated by TLR5-flagellin signaling. This explains why

these people are more vulnerable to contracting Legionella [15]. Mice lacking TLR5 are more prone to urinary tract infections caused by Escherichia coli, indicating that TLR5 controls the innate immune response in the urinary tract [16]. Additionally, TL5 action has been found in the immune cells of chicken, including monocytes, NK cells, Langerhans cells, heterophils, and B and T cells of the adaptive immune system [17].

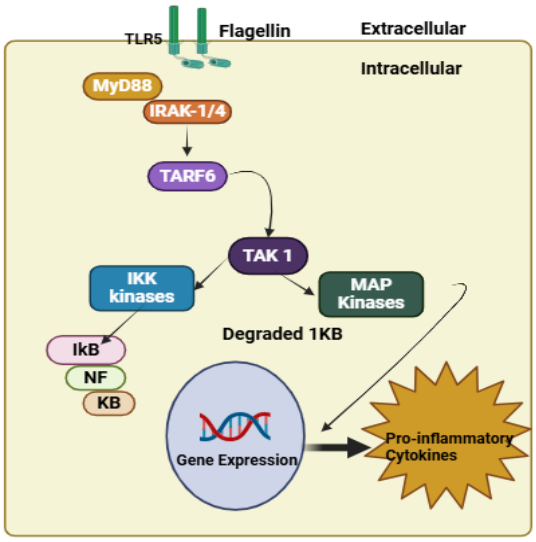

Figure 1: IRAK-1/4 and TRAF-6 are activated when flagellin binds to TLR, activating the adapter protein MyD88. This then causes NF-B to become active, which in turn causes proinflammatory cytokines to be produced

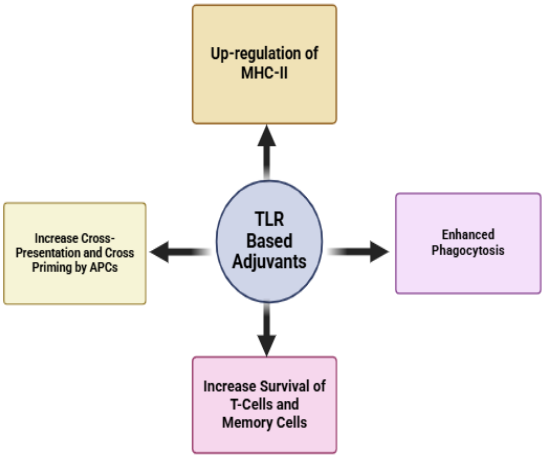

Figure 2: TLR ligands affect many immunological processes to elicit an elevated immune response

Flagellin as an adjuvant in chicken.

The flagellar protein flagellin can activate the host's immune system, according to numerous earlier experiments conducted on chicken using flagellated bacteria like S. enterica serovar Typhimurium [18]. Here, S. enterica serovar Enteritidis infection of chicken TLR5-expressing HeLa cells triggered high levels of NF-B in a manner that was reliant on both flagellin and dosage [9]. The flagellar protein flagellin can activate the host's immune system, according to numerous earlier experiments conducted on chicken using flagellated bacteria like S. enterica serovar Typhimurium. Here, the infection of chicken TLR5-expressing HeLa cells with S. enterica serovar Enteritidis produced elevated NF-B levels in a way that depended on both flagellin and dose. In comparison to wild-type flagellated bacteria, Aflatellar S. enterica serovar Typhimurium fliM was reported to produce less IL-1 and IL-6 in chicken and to have a greater capability for systemic infection. A nonflagellated bacterium called S. enterica serovar Gallinarum is less invasive and produces less cytokines and chemokines. Since TLR5-flagellin does not produce many proinflammatory cytokines, the flagellin-deficient mutant's greater ability to cause systemic infections may be explained [10].

Advantages of flagellin as an adjuvant in chicken vaccine.

The Flagellin's many advantages have made it a promising adjuvant candidate for vaccines. Its high affinity for TLR5 allows it to function as an adjuvant even at extremely low levels, and prior immunity does not change its properties [1]. Being a protein, it can be modified, and epitopes can be joined with the hypervariable region and its C or N terminus without affecting its ability to bind to TLR5. Using recombinant DNA technology, it may also be produced in vast quantities with ease. Another application for flagellin is DNA vaccination, in which an antigenic epitope is positioned adjacent to the gene encoding flagellin. Mice were given an expression vector that included the fliC gene to protect them from a deadly influenza A virus challenge [20]. According to the experiment's findings, immune responses to influenza A virus were significantly improved and expanded when mammalian cells expressed DNA-encoded TLR agonists. Furthermore, a recombinant viral vector carrying an antigenic gene can use flagellin. In this case, flagellin has been used successfully as an adjuvant in a recombinant vector that expresses viral proteins [21].

Conclusion

Flagellin affects several host cell types in a variety of immunological ways. The chicken immune system was stimulated by the TLR5 ligand flagellin, which produced an assorted Th2 and Th1 response with a tendency concerning a Th2 response. But when flagellin and LPS were combined, we found that the chicken PBMCs had a Th1 response. Additionally, a lot of other TLR agonists cause a Th1-dominated response, which makes flagellin a desirable choice in both situations. For instance, flagellin alone may be used when a higher antibody-mediated immune response is desired, and in situations where cell-mediated immunity is necessary, it may be combined with LPS. However, some additional parameters, such as the amount of antigen or flagellin, the cell subtypes, the vaccination route, and its combination with other agonists, may also affect the specific type of response. It is impossible to overlook the toxic issues surrounding the use of flagellin in birds, even if a large number of in vivo studies indicate that it is a powerful adjuvant in hens. This makes a thorough study of flagellin use in hens necessary. Combining flagellin with another TLR ligand may be a useful way to minimize toxicity issues; this will help to reduce the number of agonists used and, consequently, toxicity-related issues in chickens. As a result, Flagellin works well as an adjuvant. However, to employ flagellin and its combinations with other TLR ligands as adjuvants in future vaccine development against infectious diseases of chicken, comprehensive in vivo studies are necessary to evaluate long-term safety and determine factors like dosage and administration route.

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