Flagellin A Toll-Like Receptor 5 Agonist as an Adjuvant in Chicken Vaccines

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Chicken raised under commercial conditions are vulnerable to environmental exposure to a number of pathogens. Therefore, regular vaccination of the flock is an absolute requirement to prevent the occurrence of infectious diseases. To combat infectious diseases, vaccines require inclusion of effective adjuvants that promote enhanced protection and do not cause any undesired adverse reaction when administered to birds along with the vaccine. With this perspective in mind, there is an increased need for effective better vaccine adjuvants. Efforts are being made to enhance vaccine efficacy by the use of suitable adjuvants, particularly Toll-like receptor (TLR)-based adjuvants. TLRs are among the types of pattern recognition receptors (PRRs) that recognize conserved pathogen molecules. A number of studies have documented the effectiveness of flagellin as an adjuvant as well as its ability to promote cytokine production by a range of innate immune cells. This minireview summarizes our current understanding of flagellin action, its role in inducing cytokine response in chicken cells, and the potential use of flagellin as well as its combination with other TLR ligands as an adjuvant in chicken vaccines.

A significant sector of world agriculture is formed by the poultry industry. The major problem faced by the industry is a loss of productivity due to infectious diseases. Therefore, proper monitoring and active health management of the birds are required (1–3). Currently, active immunization using live virus vaccines is a routine practice. An effective vaccine not only needs a good antigen but also requires an appropriate adjuvant to enhance the immunogenicity of the antigen. Newer-generation vaccines, including recombinant vaccines, mostly fail to produce a strong immune response (4). Such vaccines need adjuvants which can augment the antigenicity of the antigen so that an enhanced immune response can be achieved.

Traditionally used adjuvants are inorganic compounds, bacterial products, and complex mixtures of surface-active compounds, mineral oil, and synthetic polymers (5, 6). Adjuvants based on alum and mineral oil are the most commonly used adjuvants. Freund’s complete adjuvant is an effective mineral oil-based adjuvant, but it shows high levels of adverse local painful reaction and tissue damage at the injection site and may cause systemic disorders in chicken (7, 8). Alum suffers from weak adjuvant activity as well as being associated with the induction of IgE antibody response and may cause allergic reactions (9). Recent developments in innate immunity mark a new era of TLR-based adjuvants which can substantially enhance the immune response to vaccines (10). The innate immune system recognizes unique conserved molecular patterns of pathogens (pathogen-associated molecular patterns [PAMPs]) through pattern recognition receptors (PRRs) (11). Recognition through PRRs alerts the immune system to mount a quick response to limit the spread of infection (12). TLRs are among the types of PRRs. In mammals, 13 TLRs have been reported, with each recognizing and responding to different pathogen molecules (13). Different ligands of TLRs include pathogen molecules such as lipopolysaccharide (LPS) (TLR4), flagellar protein and peptidoglycans (TLR1, TLR2, TLR5, and TLR6) (14, 15), viral double-stranded RNA (dsRNA) (TLR3) (16), bacterial and viral unmethylated cytosine-guanosine-containing oligonucleotides (CpG-ODN) (TLR9), and single-stranded RNA (ssRNA) (TLR7 and TLR8) (17–19). Recently, TLR11 and TLR12 have been shown to recognize profilin in Toxoplasma gondii infection whereas TLR13 senses the rRNA sequence CGGAAGACC (20–22).

To date, 10 TLRs have been identified in chicken and include TLR1A and TLR1B, TLR2A and TLR2B, TLR3, TLR4, TLR5, TLR7, TLR15, and TLR21 (23–25). Further, TLR21, which is a functional orthologue of mammalian TLR9, recognizes CpG-ODN whereas LPS and flagellin are recognized by TLR4 and TLR5, respectively (26–30). Mammalian counterparts of TLR8 and TLR9 seem to be defective in chicken, although chicken TLR3 appears to recognize dsRNA in a manner similar to that seen in mammals (31, 32). TLR15 has been shown to detect yeast proteins (33).

To combat infectious bacterial and viral diseases, depending upon the causative agent, humoral as well as cell-mediated immune responses may be required. Clearance of bacterial diseases may require robust humoral immunity (34, 35). Viral diseases, apart from humoral immunity, require cell-mediated immunity. For example, cellular immunity is crucial in Newcastle disease virus (NDV) infection because the viral pathogenesis includes an intracellular phase (36). This necessitates the use of an agent that can elicit both types of immune response.

CpG-ODN, a TLR21 ligand, has been reported to be an effective adjuvant, but its use has been limited due to its adsorption by nonrelevant tissues and transient biological activity due to a short half-life in vivo (6). Though modification increases its half-life, it does not render it completely resistant to nuclease activity and it still undergoes slow degradation (37). Poor cellular uptake, nonspecificity, toxicity, and severe side effects upon long-term...
MyD88-deficient TLR signaling (48). When macrophages from such MyD88-deficient mice were exposed to bacterial components, they failed to activate TLR signaling. Analysis of MyD88-deficient mice revealed the important role of MyD88 in TLR signaling (48). When macrophages from such MyD88-deficient mice were exposed to bacterial components, they failed to produce inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), IL-1β, and IL-12 (49, 50). Upon stimulation, MyD88 binds to the cytoplasmic portion of a TLR and recruits IRAK-4 (interleukin-1 receptor-associated kinase), IRAK-1, and TRAF6 (TNF receptor-associated factor) to the receptor. IRAK-4 then phosphorylates IRAK-1. TRAF6 with phosphorylated IRAK1 releases from the receptor and activates TAK1 (51). TAK1 (TGF-β-activated kinase 1) then phosphorylates the IkB kinase (IKK) complex and mitogen-activated protein (MAP) kinases. Activation of the IKK complex results in degradation of IkB and activation of NF-κB. Both NF-κB and MAP kinases then cause induction of genes involved in inflammatory responses. These genes, after activation, produce different types of inflammatory cytokines and chemokines which in turn direct the immune response (Fig. 2) (52).

**TLR SIGNALING AFFECTS MANY IMMUNOLOGICAL PROCESSES TO ENHANCE THE IMMUNE RESPONSE**

TLR ligands seem to affect an array of processes to augment the immune response (Fig. 3). In this regard, production of cytokines upregulates the expression of major histocompatibility complex (MHC) as well as various costimulatory molecules in the antigen-presenting cells (APCs), and upregulation of CD70 and CD40 molecules results in the activation of T and B cells (53, 54). TLR signaling and phagocytosis are distinctive features of macrophage-mediated innate immune responses to bacterial infection. Many studies have reported an enhanced rate of phagocytosis due to TLR signaling. Engagement of TLRs induces MyD88-dependent signaling through the activation of IRAK-4 and p38, causing upregulation of expression of a number of genes associated with phagocytosis (55–57).

Detection of microbial components by TLR enhances efficiency of vaccination by upregulating the cross-presentation and cross-priming ability of APCs (58). TLRs have also been reported to be present on CD4+ T cells, suggesting that microbial components may directly induce activated CD4+ T cell survival without any help from the APCs (40). This effect is mediated directly through the activation of the NF-kB pathway following TLR ligation (59, 60). These studies indicated a function of TLRs in the activation of adaptive immune cells and the involvement of effector memory T cells in innate immunity.

**FLAGELLIN: A TLR5 AGONIST**

**Structure.** Flagellin is a globular protein that arranges itself in a hollow cylinder to form the filament in bacterial flagellum and is present in large amounts in nearly all flagellated bacteria (61, 62). Flagellar protein consists of the four domains D0, D1, D2, and D3. The D0 and D1 domains are mostly helical in structure and have a highly conserved sequence of amino acids at the N and C termini of the primary sequence of protein across the species. The D2 and D3 domains form the hypervariable region (Fig. 4). Systemic deletion studies of flagellin protein have revealed that the TLR5-activating region of flagellin is located within the conserved domains of the protein (29). Deletion of the first 99 amino acids from the N terminus of the *Salmonella enterica* serovar Typhimurium flagellin monomer prevented TLR5 recognition, while deletions that removed amino acids 416 to 444 within the C terminus of flagellin were also sufficient to abolish the recognition of protein by TLR5 (29). The variable D3 domain is present at the surface of the flagellar filament, and it has been reported to have immunos-
timulatory activity. This makes this domain important for innate immune recognition (63, 64).

Individuals become susceptible to Legionnaires’ disease when a stop codon mutation occurs in TLR5 (65). Occurrence of a stop codon mutation makes such individuals unable to recognize flagellated bacteria and, hence, unable to mount a proinflammatory response mediated through TLR5-flagellin signaling. This accounts for the greater susceptibility of such individuals to Legionella infection (65). Absence of TLR5 in mice makes them more susceptible to *Escherichia coli* urinary tract infection, suggesting that TLR5 regulates the innate immune response in the urinary tract (66). Furthermore, TLR5-deleted mice have been reported to develop spontaneous colitis whereas another study suggested that the flagellin-induced pulmonary inflammatory response is TLR5 dependent (67, 68). Taken together, these studies strongly suggest that the functional status of the TLR5-mediated flagellin response in the host determines the host susceptibility to the infectious diseases.

In chicken, TLR5 has been reported to be expressed in lungs, kidney, colon, spleen, testes, and heart (23). TLR5 expression has also been detected in the immune cells of chicken such as heterophils, monocytes, Langerhans cells, NK cells, and T and B cells of the adaptive immune system (69–71).

Flagellin as an adjuvant in chicken. In chicken, many of the previous studies (Table 1) performed with flagellated bacteria such as *S. enterica* serovar Typhimurium have also revealed the potential of flagellar protein flagellin in activating the immune system of the host. In this context, infection of chicken TLR5-expressing HeLa cells with *S. enterica* serovar Enteritidis activated high levels of NF-κB in a dose- and flagellin-dependent manner (72). In another study, aflagellar *S. enterica* serovar Typhimurium *flgM* induced less IL-1β and IL-6 production than was seen with wild-type flagellated bacteria in chicken and the aflagellar bacteria had a greater ability for systemic infection (23). *S. enterica* serovar Gallinarum, a nonflagellated bacterium, shows reduced invasiveness and elicits reduced levels of cytokines and chemokines. de Freitas Neto et al. (73) produced flagellated strains of this bacterium and infected chicken kidney cells (CKC) with these strains
The flagellated strain \( S. \) enterica serovar Gallinarum Fla\(^+\) induced higher levels of CXCL12, inducible nitric oxide (iNOS), and IL-6 mRNA expression in CKC than were seen in the nonflagellated parent strain. In other TLR5-flagellin interactions, chicken peripheral blood mononuclear cells (PBMCs) produced more IL-1\( \beta \), IL-6, CXCL12, and CCL2 in response to \( S. \) enterica serovar Enteritidis infection (74). Pan et al. (75) also investigated the effect of flagellin-deficient mutant \( S. \) enterica serovar Typhimurium on the immune system of the chicken \textit{in vivo}. Mutant (flagellin-deficient) strains showed a greater ability to establish systemic infection and elicited reduced levels of IL-1\( \beta \) and CXCL12 compared to the wild-type bacteria. An absence of TLR5-flagellin-mediated production of various proinflammatory cytokines and chemokines may account for the enhanced ability of the flagellin-deficient mutant to cause systemic infections (23, 75). Taken together, all these studies indicated that the \textit{Salmonella} flagellin protein is involved in triggering the innate immune responses.

A number of studies have demonstrated the effectiveness of flagellin as an adjuvant in chicken immune cells \textit{in vitro} (Table 2). Heterophils showed an increased oxidative burst and a significant upregulation of various proinflammatory cytokines (IL-1\( \beta \), IL-6, and IL-8) and chemokines (CXCL12) in response to flagellin (76, 77). Isolated chicken monocytes stimulated with flagellin showed upregulated nitric oxide production, which indicates enhanced macrophage function (78). Furthermore, chicken macrophage-like cells (HD11), chicken kidney epithelial cells (CKC), and chicken embryo fibroblasts (CEF) have been shown to upregulate production of IL-1\( \beta \) when stimulated with flagellin (23). Keestra et al. (72) reported that the increased proinflammatory response was due to TLR5-flagellin-mediated enhanced production of NF-\( \kappa \)B in HeLa cells expressing chicken TLR5.

Recent studies aimed at exploring the potential of flagellin as an adjuvant have reported a mixed induction of Th1 and Th2 immune responses in chicken cells (79, 80). We have found that when chicken PBMCs were stimulated with recombinant flagellin, both Th1 and Th2 cytokines (Th1–IL-12 and Th2–IL-4) were detected along with proinflammatory cytokine IL-6 (79). The same pattern of cytokine production (Th1–IL-12, gamma interferon [IFN-\( \gamma \)], and Th2–IL-4 and –IL-13) was observed in chicken splenocytes (80).

Many \textit{in vivo} investigations were undertaken looking at the promising results of various \textit{in vitro} studies with purified or recombinant flagellin in chicken immune cells (Table 3). In this regard, flagellin administered \textit{in vivo} enhanced the infiltration of heterophils into the site of injection, which suggests that flagellin is a potent stimulator of a heterophil-mediated innate immune response \textit{in vivo} and can protect against \textit{Salmonella} infections in chickens (81). Although the exact mechanism behind this phenomenon was not clearly documented, it was reasoned to be mediated by the TLR5-flagellin-induced production chemotactic factor IL-8 (76).

Use of flagellin in recombinant vaccine can elicit a greater immune response (82). Chaung et al. (82) investigated the adjuvant effects of the monomeric and polymeric forms of \textit{Salmonella} flagellin in specific-pathogen-free (SPF) chickens immunized intramuscularly or intranasally with inactivated avian influenza virus H5N2 vaccines. Results showed that flagellin cooperating with

![FIG 4](https://example.com/flagellin_graph.png) Structure of flagellin with four major domains indicated (NCBI; GenBank accession no. KF589316).

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**TABLE 1** Summary of TLR5-mediated adjuvant action of flagellated \textit{Salmonella} strains

<table>
<thead>
<tr>
<th>Study model</th>
<th>Result(s)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Chicken kidney cells (CKC)</td>
<td>Flagellated strains ( S. ) Enteritidis and ( S. ) Gallinarum (Fla(^+)) induced higher levels of CXCL12, iNOS, and IL-6; less mortality with flagellated strains</td>
<td>73</td>
</tr>
<tr>
<td>Chicken (in vivo)</td>
<td>Flagellin-deficient mutants exhibited enhanced ability for systemic infections; lower levels of IL-1( \beta ) and CXCL12 than wild type</td>
<td>75</td>
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<tr>
<td>Chicken PBMCs</td>
<td>TLR5-flagellin-mediated upregulation of IL-1( \beta ), IL-6, CXCL12, and CCL2 expression in ( S. ) Enteritidis-infected cells</td>
<td>74</td>
</tr>
<tr>
<td>CKC and HD11</td>
<td>Flagellated ( S. ) Enteritidis and ( S. ) Typhimurium induced more IL-6, CXCL12, and iNOS than nonflagellated ( S. ) Pullorum and ( S. ) Gallinarum</td>
<td>105</td>
</tr>
<tr>
<td>HeLa cells expressing chicken TLR5</td>
<td>Induced more NF-( \kappa )B in response to ( S. ) Enteritidis infection in a dose- and flagellin-dependent manner</td>
<td>72</td>
</tr>
<tr>
<td>Chicken (in vivo)</td>
<td>Reduced levels of IL-1( \beta ) and IL-6 and enhanced ability to establish systemic infection after challenge with aflagellar ( S. ) Typhimurium</td>
<td>23</td>
</tr>
<tr>
<td>Intestinal epithelial cells</td>
<td>Flagellin-deficient ( S. ) Typhimurium mutant (( fliC ), ( fliB ), and ( fliD )) failed to elicit IL-8 expression</td>
<td>52</td>
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</table>
the 64CpG adjuvant significantly induced influenza virus-specific antibody titers of plasma IgA in the vaccinated animals. The nasal IgA levels in the flagellin-coadministered avian influenza virus-vaccinated chickens were significantly elevated compared to levels observed with the H5N2 vaccine alone. Okamura et al. (34) used recombinant flagellin protein from S. enterica serovar Enteritidis and tested its potential in a vaccine candidate against homologous challenge in chickens. After immunization with recombinant flagellin or administration of phosphate-buffered saline, the chickens were challenged with S. enterica serovar Enteritidis. The vaccinated birds showed significantly decreased bacterial counts in the liver and cecal contents compared to those administered phosphate-buffered saline (PBS).

Recombinant vectors based on S. enterica serovar Typhimurium have also been used. Pan et al. (75) developed a recombinant S. enterica vector expressing the fusion protein (F) gene of NDV; experimental data revealed that recombinant vector induced higher levels of interleukin-1β (IL-1β), CXCL12, and TLR5 mRNAs in the cecum, the spleen, and the heterophils. Huang et al. (35) used flagellin as an adjuvant against Campylobacter jejuni and reported an increased Ig and decreased bacterial load in the birds. Further, flagellin has also been used as an adjuvant against Eimeria tenella. A recent study demonstrated the potential of flagellin as an adjuvant against parasitic infections (83). A fusion protein consisting of flagellin and immune-mapped protein-1 (IMP-1), an antigenic protein of Eimeria tenella, elicited a stronger immune response than recombinant IMP-1 with Freund’s complete adjuvant, showing that flagellin can also be used to enhance the immunogenicity of parasite antigens. Flagellin-antigen fusion proteins hold great potential and can be effectively used once the antigenic region of a pathogen is identified.

Flagellin as an adjuvant in combination with other TLR ligands. Recent studies have shown that when two or more TLRs are activated simultaneously, their pathways interact with each other and this cross talk results in either synergistic or antagonistic immune response (84, 85). TLR combinations can produce a stronger and selective immune response, and a number of studies have been undertaken in mice as well as in human immune cells (86–91). In chicken, many combinations have also been tried to establish the pattern of cytokine production and induction of the type of immune response (Table 4). In this context, costimulation with TLR3 and TLR21 ligands synergistically upregulated IFN-γ and IL-10 expression in chicken monocytes (92). A combination of CpG-ODN and poly(I·C) synergistically stimulated a proinflammatory immune response in chicken monocytes that included nitric oxide (NO) production and expression of iNOS and of proinflammatory cytokines and chemokines (93, 94). Flagellin has also been used in combination with other agonists. In a previous study, cross talk between TLR5 and TLR9 on human PBMCs resulted in a more robust production of IL-10 and IFN-γ but antagonized IL-12 production (86). In another study, we have reported that a combination of recombinant flagellin and LPS synergistically upregulated nitric oxide production and IL-12 and IL-6 expression but antagonistically downregulated IL-4 expression in comparison to recombinant flagellin alone. The results indicate that these agonists synergistically interact and enhance macrophage function and promote Th1 immune response in chicken PBMCs (79). Our study (79) also favored the use of combinations of these (LPS and recombinant flagellin) agonists, where Th1 type immunity is required, in small doses to alleviate the toxicity-related concerns associated with these agonists. These studies indicated that a single TLR ligand may not be effective enough to combat deadly infectious diseases and that instances occur in which a well-directed immune response is required; hence, in such cases, a novel combination of TLR ligands can be tried to achieve a more effective and selective immune (Th1 or Th2) response in chickens.

Advantages of flagellin as an adjuvant in chicken vaccine. Flagellin has emerged as a potential adjuvant candidate for vaccines due to a number of advantages associated with it. It has been shown to be effective at very low doses (81, 82), and previous immunity does not alter its adjuvant activity, as it has high affinity for TLR5 (95, 96). Being protein in nature, it can be manipulated and epitopes can be fused with its N or C terminus as well as in the

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<th>TABLE 2</th>
<th>Flagellin as an adjuvant in chicken: applications in vitro</th>
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<tr>
<td>Cell category</td>
<td>Response(s)</td>
</tr>
<tr>
<td>Chicken PBMCs</td>
<td>Upregulation of both IL-12 and IL-4 as well as IL-6</td>
</tr>
<tr>
<td>Chicken splenocytes</td>
<td>Induction of a mixed Th1- and Th2-like response</td>
</tr>
<tr>
<td>Chicken heterophils</td>
<td>Upregulation of IL-6 and CXCL2</td>
</tr>
<tr>
<td>HeLa cells with chicken TLR5</td>
<td>Upregulation of TLR5-flagellin-mediated NF-κB expression</td>
</tr>
<tr>
<td>Chicken monocytes</td>
<td>Induction of nitric oxide production</td>
</tr>
<tr>
<td>Chicken heterophils</td>
<td>Increased oxidative burst; significant production of proinflammatory cytokines and chemokines</td>
</tr>
<tr>
<td>CEF, CKC HD11 cells</td>
<td>Significant upregulation of IL-1β</td>
</tr>
<tr>
<td>Chicken heterophils</td>
<td>Significant upregulation of IL-1β, IL-6, and IL-8 and increased oxidative burst</td>
</tr>
</tbody>
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<tr>
<th>TABLE 3</th>
<th>Flagellin as an adjuvant in chicken: applications in vivo</th>
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</thead>
<tbody>
<tr>
<td>Antigen</td>
<td>Result(s)</td>
</tr>
<tr>
<td>Immune-mapped protein-1 (IMP-1) of Eimeria tenella (EtIMP1)</td>
<td>Flagellin-fused EtIMP1 elicited a stronger protective immune response; reduced mortality</td>
</tr>
<tr>
<td>Avian influenza virus</td>
<td>Increased IgY and IgA</td>
</tr>
<tr>
<td>Salmonella enterica serovar Enteritidis</td>
<td>Increased IgA and decreased bacterial counts</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>Increased Ig and decreased bacterial load</td>
</tr>
<tr>
<td>Salmonella enterica serovar Enteritidis</td>
<td>Reduced mortality</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>Increased Ig and decreased bacterial load</td>
</tr>
</tbody>
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Minireview

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Table 4: Adjuvant effect of combinations of TLR agonists on chicken immune cells

<table>
<thead>
<tr>
<th>TLR combination</th>
<th>Cell category</th>
<th>Result(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR5 and TLR4</td>
<td>Chicken PBMCs</td>
<td>Synergistic upregulation of NO, IL-6, and IL-12; antagonistic downregulation of IL-4</td>
<td>79</td>
</tr>
<tr>
<td>TLR3 and TLR21</td>
<td>Chicken RBCs(^a)</td>
<td>Synergistic upregulation of type 1 IFNs</td>
<td>102</td>
</tr>
<tr>
<td>TLR3 and TLR21</td>
<td>Chicken monocytes</td>
<td>Synergistic upregulation of IFN-γ and IL-10</td>
<td>103</td>
</tr>
<tr>
<td>TLR4 and TLR21</td>
<td>Chicken thrombocytes</td>
<td>Synergistic upregulation of IL-1β, IL-6, and IL-8</td>
<td>104</td>
</tr>
<tr>
<td>TLR5 and TLR21</td>
<td>Chicken monocytes</td>
<td>Synergistic upregulation of NO, IL-1β, IL-6, and MIP-1β</td>
<td>92</td>
</tr>
<tr>
<td>TLR3 and TLR21</td>
<td>Chicken monocytes</td>
<td>Synergistic upregulation of NO production</td>
<td>94</td>
</tr>
</tbody>
</table>

\(^a\) RBCs, red blood cells.

References:


Diehl L, den Boer AT, Schoenberger SP, van der Vaart EI, Schumacher TN, Melief CJ. 1999. CD40 activation in vivo overcomes peptide-induced peripheral cytotoxic T lymphocyte tolerance and augments anti-

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Clinical and Vaccine Immunology
Shishir Kumar Gupta obtained his graduate degree in Doctor of Veterinary Medicine (DVM) from Bombay Veterinary College (BVC), Mumbai, India. He joined the Indian Veterinary Research Institute (IVRI) for his Master’s degree in Animal Biotechnology and Immunology and worked on the potential use of TLR ligands and their combinations as adjuvant in new-generation recombinant chicken vaccines in the Recombinant DNA Laboratory of this institute. He received a fellowship from the Indian Council of Agricultural Research (ICAR) during his thesis research work. Currently, he is pursuing his Doctor of Philosophy (Ph.D.) degree from the same institute and working in the area of viral oncology. He is receiving a fellowship from CSIR (Council of Scientific and Industrial Research) for his Ph.D. research work.

Preeti Bajwa graduated in Biotechnology from the Chaudhary Charan Singh University and obtained her M.Tech. in Biotechnology with specialization in Genetic Engineering from the School of Biotechnology, Gautam Buddha University. During her Master’s studies, she enrolled in the Laboratory of Gut Inflammation and Infection Biology at the Regional Centre for Biotechnology, Gurgaon, India, for her research thesis. Her work involves understanding the mechanism of Salmonella-mediated alteration of host SUMOylation. Currently, she is working as a Senior Research fellow (SRF) in a DBT project at G.B. Pant University of Agriculture and Technology, Pantnagar, India. Her project is based on creation of a double-gene mutant of Salmonella enterica serovar Typhimurium and testing of its immune potential.

Continued next page
Rajib Deb obtained his Master’s degree in Animal Biotechnology from Indian Veterinary Research Institute (IVRI), Uttar Pradesh, India. His postgraduate research work was on development of a bicistronic DNA vaccine against *Mycobacterium paratuberculosis* using gamma interferon as an adjuvant. Presently, he is pursuing his Ph.D. in Animal Biotechnology from the same institute. His Ph.D. research work involves developing a chimeric DNA vaccine against infectious bursal diseases (IBD) of poultry that incorporates the flagellin (*fliC*) gene of *Salmonella enterica* serovar Typhimurium as an adjuvant. He is also presently employed as an Agricultural Research Scientist (ARS) under the Indian Council of Agricultural Research (ICAR), government of India.

Madhan Mohan Chellappa obtained his Master of Veterinary Science in Animal Biotechnology in 1997 and Doctor of Philosophy (Ph.D.) in Animal Biotechnology in 2003 from the Department of Animal Biotechnology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, India. In 2000 he was appointed Scientist in the Division of Veterinary Biotechnology, Indian Veterinary Research Institute (IVRI), India. He was awarded with a Department of Biotechnology (DBT) overseas fellowship by the government of India in 2006 for pursuing a postdoctoral program at the University of Maryland Biotechnology Institute and a BOYSCAST fellowship under the Department of Science and Technology in 2010 to pursue research work at the Institute of Animal Health, Pirbright, United Kingdom. Currently, he is holding the position of Senior Scientist at the Indian Veterinary Research Institute. His research interests include development of virus-like particles and new-generation vaccines and diagnostics against important poultry viral diseases.

Sohini Dey obtained her Master of Veterinary Science in Animal Biotechnology in 1997 and Doctor of Philosophy (Ph.D.) in Animal Biotechnology in 2003 from the Department of Animal Biotechnology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, India. In 2000, she was appointed Scientist in the Division of Veterinary Biotechnology, Indian Veterinary Research Institute (IVRI), India. She was awarded with a DBT overseas fellowship by the government of India in 2006 for pursuing a postdoctoral program at the University of Maryland Biotechnology Institute and a BOYSCAST fellowship under the Department of Science and Technology in 2010 to pursue research work at the Institute of Animal Health, Pirbright, United Kingdom. Currently, she is holding the position of Senior Scientist at the Indian Veterinary Research Institute. Her research interests include development of virus-like particles and new-generation vaccines and diagnostics against important poultry viral diseases.