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RESEARCH ARTICLE

Evaluation of a Dendritic Cell–Targeted Chimeric EtIMP1 Vaccine Against *Eimeria tenella* in Chickens

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A B S T R A C T

Coccidiosis caused by *Eimeria tenella* (*E. tenella*) poses substantial economic threats to poultry production globally. To enhance vaccine efficacy, this study developed a chimeric subunit vaccine targeting dendritic cells (DCs) via DEC-205 receptor, fusing *E. tenella* immune-mapped protein 1 C-terminal region (EtIMP1C) to a single-chain variable fragment (scFv) specific for chicken DEC-205 (scFvDEC205). In vitro-expressed recombinant proteins were administered to chickens; immunized birds were challenged with *E. tenella* to assess vaccine efficacy. Results demonstrated that chimeric subunit vaccine elicited significantly higher IgG and IgA titers ($P< 0.05$) and enhanced production of IL-2, IL-4, IL-12, and IFN-γ compared to controls. Chimeric subunit vaccine vaccinated birds exhibited improved weight gain, reduced cecal lesion scores, and decreased oocysts shedding (63.4% lower than infected controls). *In vitro* assays validated the specific targeting capability of scFvDEC205 toward immature DC. These findings demonstrate that DEC-205-targeted delivery of EtIMP1C significantly enhances both humoral and cellular immune responses against *E. tenella*. The scFvDEC205-EtIMP1C fusion protein represents a promising candidate for developing effective subunit vaccines against avian coccidiosis.

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INTRODUCTION

Coccidiosis is a parasitic disease in chickens caused by protozoa of the genus *Eimeria (E.)*, which specifically target the intestinal tract (Mohsin *et al.*, 2021a; Mohsin *et al.*, 2022; Al-Hoshani *et al.*, 2023). Upon infection, these parasites penetrate intestinal epithelial cells, initiating inflammation and causing significant damage to the digestive system. This can disrupt nutrient absorption and feed efficiency, and in severe cases, it may result in death (Chen *et al.*, 2020; Al-Hoshani *et al.*, 2023). Common symptoms include intestinal inflammation (enteritis) and stunted growth, making coccidiosis a critical concern for poultry health and a major contributor to economic losses worldwide (Chen *et al.*, 2020; Mohsin *et al.*, 2021c; Al-Hoshani *et al.*, 2023). Recently, seven *Eimeria* species have been identified as infective to chickens. Among these, *E. tenella* and *E. necatrix* exhibit the strongest pathogenicity (Chen *et al.*, 2020; Abbas and Alkheraije, 2023). Conventional control methods relying on chemotherapeutic agents have become increasingly ineffective due to widespread drug resistance development and concerns regarding residual drug accumulation in poultry products (Lin *et al.*, 2020; Hussain *et al.*, 2021; Abbas and Alkheraije, 2023). These challenges necessitate the development of alternative control strategies, with particular emphasis on effective vaccine development.

Immune mapped protein-1 (IMP-1) is a newly discovered protein in *E. maxima* and is recognized as a highly conserved antigen in apicomplexan parasites (Blake *et al.*, 2011; Mohsin *et al.*, 2021b). Subsequent investigations revealed that immunization with *E. tenella* IMP-1 (EtIMP1) resulted in a 60% reduction in oocyst shedding (Yin *et al.*, 2013). Further characterization identified the C-terminal region (EtIMP1C) as maintaining equivalent immunogenic properties to the

full-length protein (Yin *et al.*, 2014), establishing as a viable target for subunit vaccine formulation. The critical role of dendritic cells (DCs) in initiating adaptive immunity through antigen presentation has been well established (Banchereau and Steinman, 1998; Mazzoccoli and Liu, 2024; Shao *et al.*, 2025). Protective immunity depends on cross-presentation of parasite antigens by DCs to CD^{8+} T cells via MHC class I molecules (Parker, 2017; Chen *et al.*, 2025). Among various DC receptors, DEC-205 (a member of the mannose receptor family and C-type lectin superfamily) has shown particular promise for antigen targeting (Hossain and Wall, 2019; Colaço *et al.*, 2024). This receptor, predominantly expressed on immature DCs, mediates efficient antigen uptake (Kim and Kim, 2019; Colaço *et al.*, 2024). Recent advances in vaccinology have demonstrated the efficacy of DEC-205-targeted antigen delivery against diverse pathogens (Feng *et al.*, 2020). While conventional monoclonal antibodies have been widely employed, single-chain variable fragments (scFvs) offer distinct advantages, including enhanced tissue penetration and reduced immunogenicity, which are attributed to their compact size and absence of Fc regions (Rodriguez-Nava *et al.*, 2023).

The current study developed an innovative chimeric vaccine by conjugating EtIMP1C with scFv specific for chicken DEC-205. We assessed its protective efficacy in chickens against *E. tenella* challenge through comprehensive evaluation of immunological parameters and clinical outcomes. These findings provide valuable insights for developing next-generation subunit vaccines against avian coccidiosis. This platform holds specific and critical relevance for broilers, which represent the vast majority of poultry reared for meat and whose 6 to 8-week production cycle imposes unique constraints on vaccination strategies. The development of a subunit vaccine capable of eliciting robust, short-term protection during this critical window therefore addresses a pressing need in the poultry industry and will be a key application target for this DEC-205-targeted vaccine strategy.

MATERIALS AND METHODS

Parasite Propagation and Experimental Design: One-day-old, male, Hy-Line brown layer chickens were purchased from Fuzhou Poultry Breeding company (Fuzhou, China). They were reared in a coccidia-free room and had free access to food and water. *Eimeria tenella* oocysts were propagated in 2–3-week-old chickens. Oocysts were collected from feces 6–8 days post-infection (3×10^4 oocysts per bird), isolated, purified, and sporulated following established protocols (Yin *et al.*, 2013; Mohsin *et al.*, 2021b). Animal procedures were approved by the Fujian Agriculture and Forestry University Ethics Committee (No. 2023-6-20).

Cloning and Expression of EtIMP1C: Primers for the EtIMP1C gene were designed with EcoRI and XhoI restriction sites and synthesized by Sangon Biotech (Shanghai). The gene was cloned into the pEASY-Blunt vector (TransGen Biotech, Beijing) and confirmed by sequencing. Protein expression was verified via Western blot. For recombinant protein production, EtIMP1C was inserted into pET-28a (Novagen, Germany), and scFvDEC205 (anti-chicken DEC-205 single-chain variable fragment) was codon-optimized. Plasmids pET28a-scFvDEC205 and pET28a-scFvDEC205-EtIMP1C were transformed into *E. coli* for expression. His-tagged proteins were purified using a Hi-Trap metal chelating column (GE Healthcare, USA) and analyzed by Western blot.

Generation and Maturation of Chicken Bone Marrow-Derived Dendritic Cells: Bone marrow cells were extracted from 4–12-week-old SPF chickens and isolated via Histopaque-1119 density gradient centrifugation. Cells were cultured in RPMI-1640 medium supplemented with recombinant chicken GM-CSF (50ng/mL) and IL-4 (25ng/mL) for 7 days at 39°C under 5% CO_2. Medium was refreshed every 2 days, removing non-adherent cells. Maturation was induced on day 6 using LPS (200ng/mL). Cell morphology was assessed via inverted microscopy.

Flow Cytometry and Phenotypic Analysis: chBM-DCs were collected on days 1, 6, and 7 (post-LPS maturation). Surface markers (MHC II and CD11c) were stained with FITC- and PE-conjugated antibodies (Abcam plc, Cambridge, UK) and analyzed via flow cytometry.

Conjugation and Immunofluorescence Verification: The scFvDEC205-EtIMP1C conjugate was purified via dialysis and metal chelating chromatography. Conjugation efficiency was confirmed by Native-PAGE and Western blot. For immunofluorescence, immature chBM-DCs were incubated with scFvDEC205, scFvDEC205-EtIMP1C, or EtIMP1C (5μg/mL), fixed, permeabilized, and stained with FITC-labeled secondary antibody. Binding was visualized via fluorescence microscopy.

Immunization and Challenge Infection: Five groups of 12 chickens each were immunized such as pET28a-scFvDEC205, pET28a-EtIMP1C, pET28a-scFvDEC205-EtIMP1C, challenged control (PBS+infection), and unchallenged control (PBS). Each chicken received 200μg of the respective protein via thigh muscle injection, administered three times at two-week intervals. Fourteen days of post-final immunization, all groups except the unchallenged control were challenged with 3×10^4 *E. tenella* oocysts.

Immune Response Analysis: Peripheral blood mononuclear cells (PBMCs) were isolated two weeks after the second immunization, incubated with antibodies PE-CD3, APC-CD4, and FITC-CD8a (Southern Biotechnology Associates, USA), in the dark at room temperature and then detected with flow cytometer. Serum antibodies (IgG, IgA) (Bethyl Laboratories, USA; 1:10,000 dilution) against EtIMP1C were measured via ELISA. Cytokines (IFN-γ, IL-2, IL-4, IL-10, IL-12) were quantified in post-immunization sera using commercial ELISA kits (CusaBio, Wuhan, China).

Statistical Analysis: Data are presented as mean ± standard deviation (SD). Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test using SPSS software (version 25.0, IBM, Armonk, NY, USA). Each experiment was repeated with

at least three biological replicates. Differences were considered statistically significant at P< 0.05.

RESULTS

Expression, Purification, and Identification of Recombinant Proteins: The recombinant proteins scFvDEC205, EtIMP1C, and scFvDEC205-EtIMP1C were expressed in *E. coli* as His6-tagged fusion proteins and purified via Ni^{2+} affinity chromatography. Western blot analysis revealed distinct bands at approximately 28kDa (scFvDEC205 and EtIMP1C) and 58kDa (scFvDEC205-EtIMP1C) (Fig. 1).

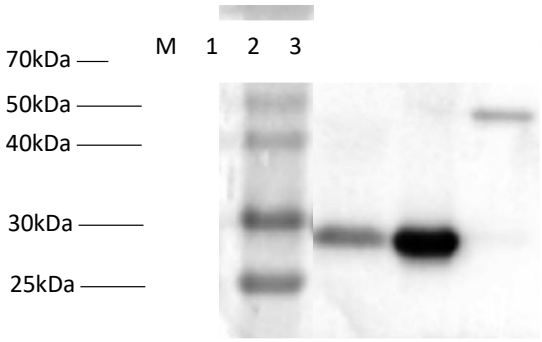

Fig. 1: Protein expression analysis. Western blot was performed to detect the expression of pET28a-scFvDEC205 (lane 1), pET28a-EtIMP1C (lane 2), and pET28a-scFvDEC205-EtIMP1C (lane 3).

Isolation, Culture, and Characterization of Chicken Bone Marrow-Derived Dendritic Cells (chBM-DCs): Primary chicken bone marrow-derived monocytes exhibited an oval morphology with scattered distribution, while some cells displayed spindle-shaped features (Fig. 2A). During culture, cells gradually aggregated, transitioning to elongated forms with branching protrusions (Fig. 2B–C). By day 6, LPS stimulation (200ng/mL, 24h) induced mature dendritic cell morphology, characterized by thin, elongated processes (Fig. 2D).

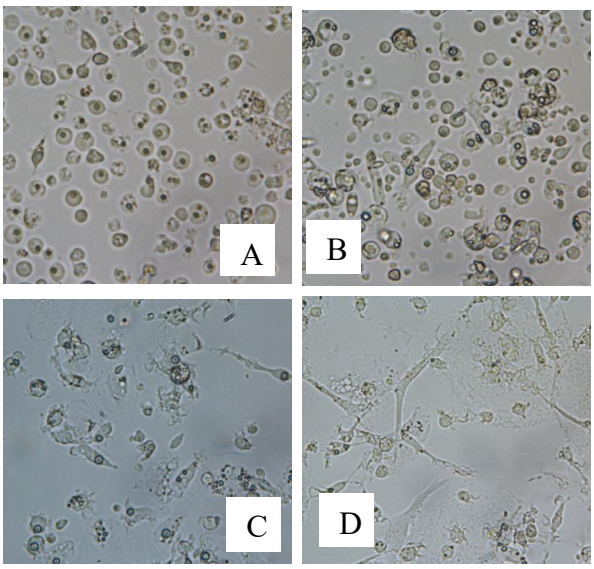

Fig. 2. Morphological changes of chicken bone marrow-derived dendritic cells (DCs) during differentiation (×400 magnification). Panels A–D represent DCs on days 1, 3, 5, and 7 of culture, respectively.

Flow cytometry analysis demonstrated increased expression of MHC-II and CD11c over time: immature chBM-DCs (day 1) showed 31.87% double-positive cells, rising to 86.88% by day 6 and 92.09% post-LPS stimulation (Fig. 3). These results confirmed differentiation of monocytes into functional chBM-DCs.

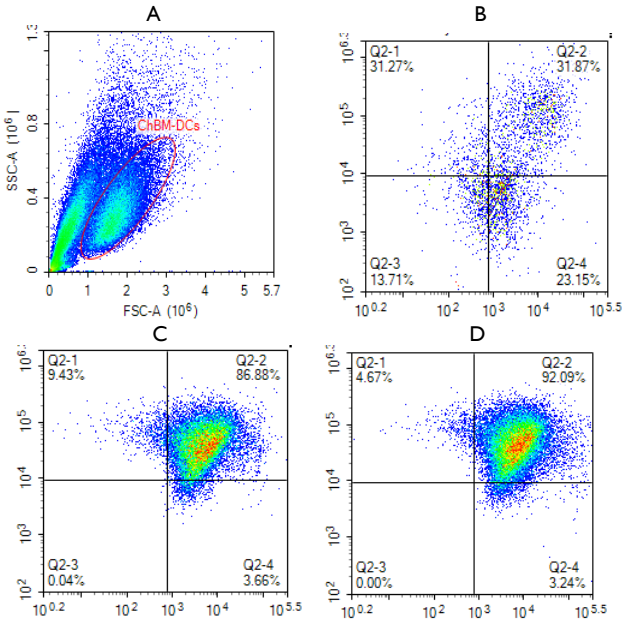

Fig. 3. Characterization of chicken bone marrow-derived dendritic cells (chBM-DCs) by flow cytometric analysis. Flow cytometry analysis demonstrated increased expression of MHC-II and CD11c over time (A-D).

Binding of Recombinant Proteins to Immature chBM-DCs: Indirect immunofluorescence assays revealed that scFvDEC205 and scFvDEC205-EtIMP1C bound efficiently to immature chBM-DCs, evidenced by green fluorescence (Fig. 4), Western blot analysis of membrane proteins detected a band >250kDa for scFvDEC205-EtIMP1C but not for EtIMP1C alone (Fig. 5), supporting target-specific conjugation.

Flow Cytometric Analysis of T Cell Populations: The pET28a-scFvDEC205-EtIMP1C group exhibited a significant increase in CD^{4+} and CD^{8+} T cell populations compared to controls (P<0.05), with CD^{4+} T cells showing the most pronounced expansion (Table 1). This suggests enhanced antigen and immune activation.

Table 1: Flow cytometry was used to analyze the percentages of T lymphocyte subsets. Values are expressed as mean ± SD. Means in the same column with different letters were significantly different between treatment groups (P<0.05)

Group	$CD4^+$ (%)	$CD8^+$ (%)
PBS	8.22 ± 2.25^c	3.05 ± 0.73^b
pET28a-EtIMP1C	9.08 ± 1.52^b	3.13 ± 0.77^b
pET28a-scFvDEC205	9.00 ± 1.56^b	3.37 ± 1.05^b
pET28a-scFvDEC205-EtIMP1C	14.12 ± 1.17^a	6.51 ± 1.48^a

Humoral Immune Responses: ELISA analysis of serum antibodies post-immunization demonstrated elevated IgG and IgA levels in all experimental groups. The pET28a-scFvDEC205-EtIMP1C group showed significantly higher IgG titers than controls (P<0.05) (Fig. 6A). IgA levels in the pET28a-EtIMP1C and pET28a-scFvDEC205 groups increased gradually but remained lower than the fusion protein group (Fig. 6B).

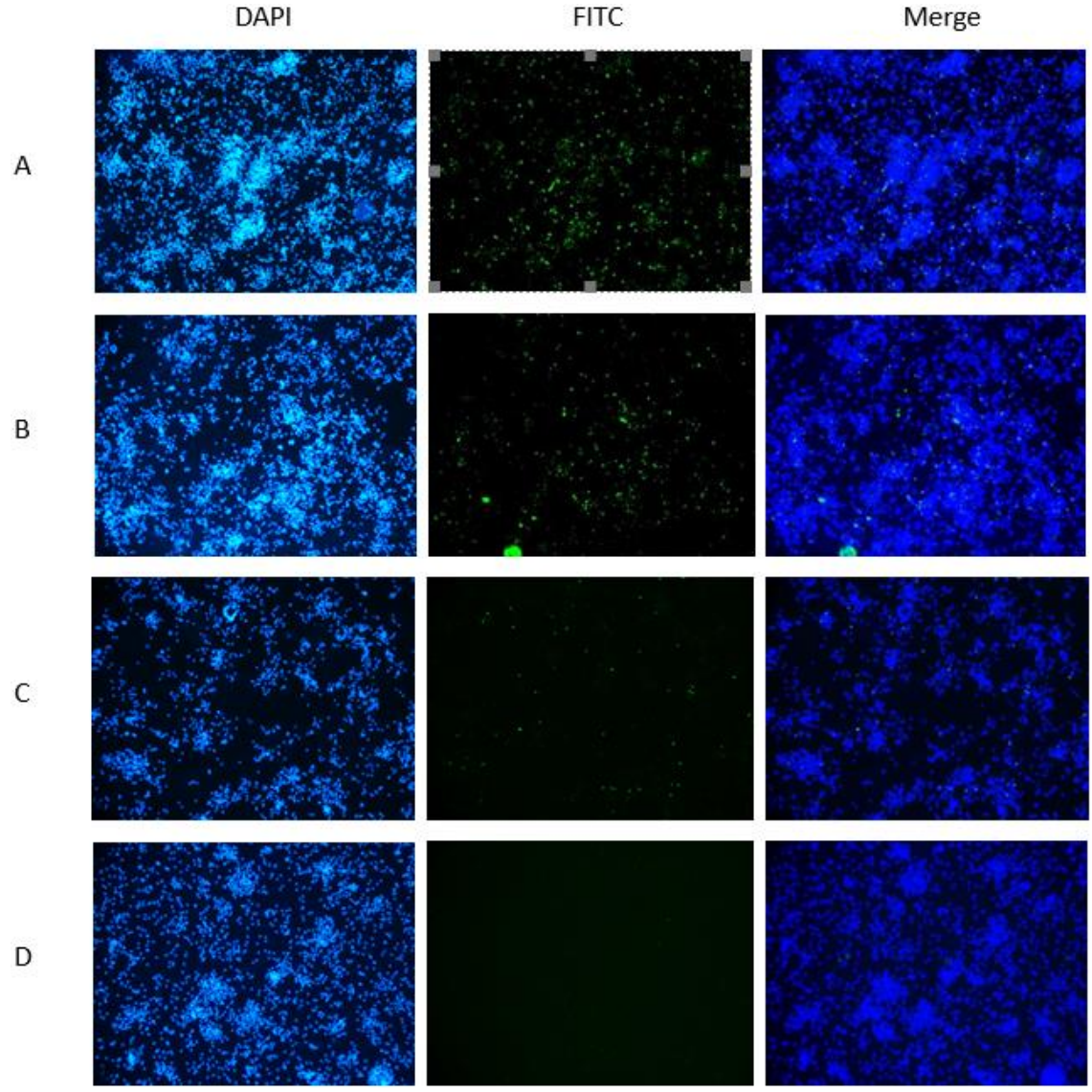

Fig. 4: Indirect immunofluorescence identification of immature chBM-DCs specifically binding to the target protein (×100). A, scFvDEC205; B, scFvDEC205-EtIMP1C; C, EtIMP1C; D, negative control.

Table 2: Results of oocyst output and average weight gain (AWG) following challenge infection of chickens with the *E. tenella*. The control group was not inoculated with coccidial oocysts, and therefore, no oocyst reduction rate is applicable. Values are expressed as mean ± SD. Means in the same column with different letters were significantly different between treatment groups (P<0.05).

Groups	AWG	(%) RWG	Average amount of Oocysts (×10^7)	Oocyst reduction (%)	ACI
Blank	144.63±10.16^a	100	0	-	200
E. tenella challenged	59.88±9.75^d	40.25	4.20±0.24^a	0	131.55
pET28a-EtIMP1C	92.22±19.16^b	59.15	3.32±0.27^b	21.02	152.83
pET28a-scFvDEC205	72.58±26.18^{bc}	55.94	3.63±0.23^{ab}	13.56	148.15
pET28a-scFvDEC205-EtIMP1C	112.28±12.52^b	76.48	1.54±0.16^c	63.44	173.28

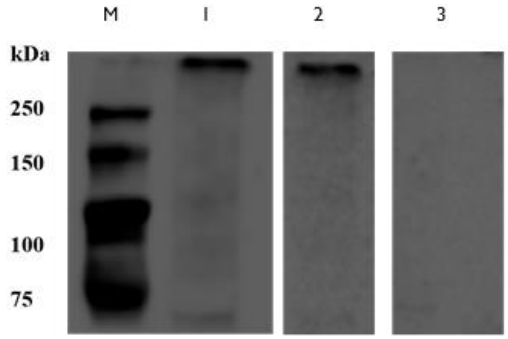

Fig. 5: Western blot analysis of membrane proteins detected a band >250kDa for scFvDEC205-EtIMP1C (Lane 1, 2) but not for EtIMP1C alone (Lane 3).

Cytokine Production: Serum cytokine analysis revealed significantly elevated levels of IFN-γ, IL-12, IL-2, and IL-4 in the pET28a-scFvDEC205-EtIMP1C group (P<0.05) (Fig. 7). While the pET28a-EtIMP1C group induced IL-12 and IL-2 production, the response was less comprehensive than the fusion protein group (Fig. 7).

Protection against *Eimeria tenella* Challenge: Chickens immunized with pET28a-scFvDEC205-EtIMP1C exhibited partial protection, higher relative weight gain, reduced oocyst excretion, and improved coccidial indices compared to controls (Table 2).

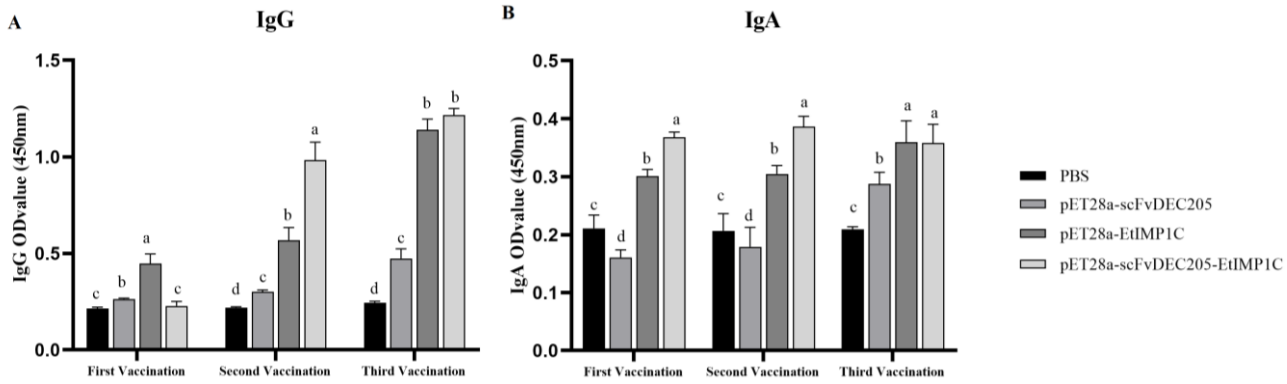

Fig. 6. IgG (A) and IgA (B) antibody titers in chicken serum samples. In the same column, different letters indicate significant differences between groups (P<0.05).

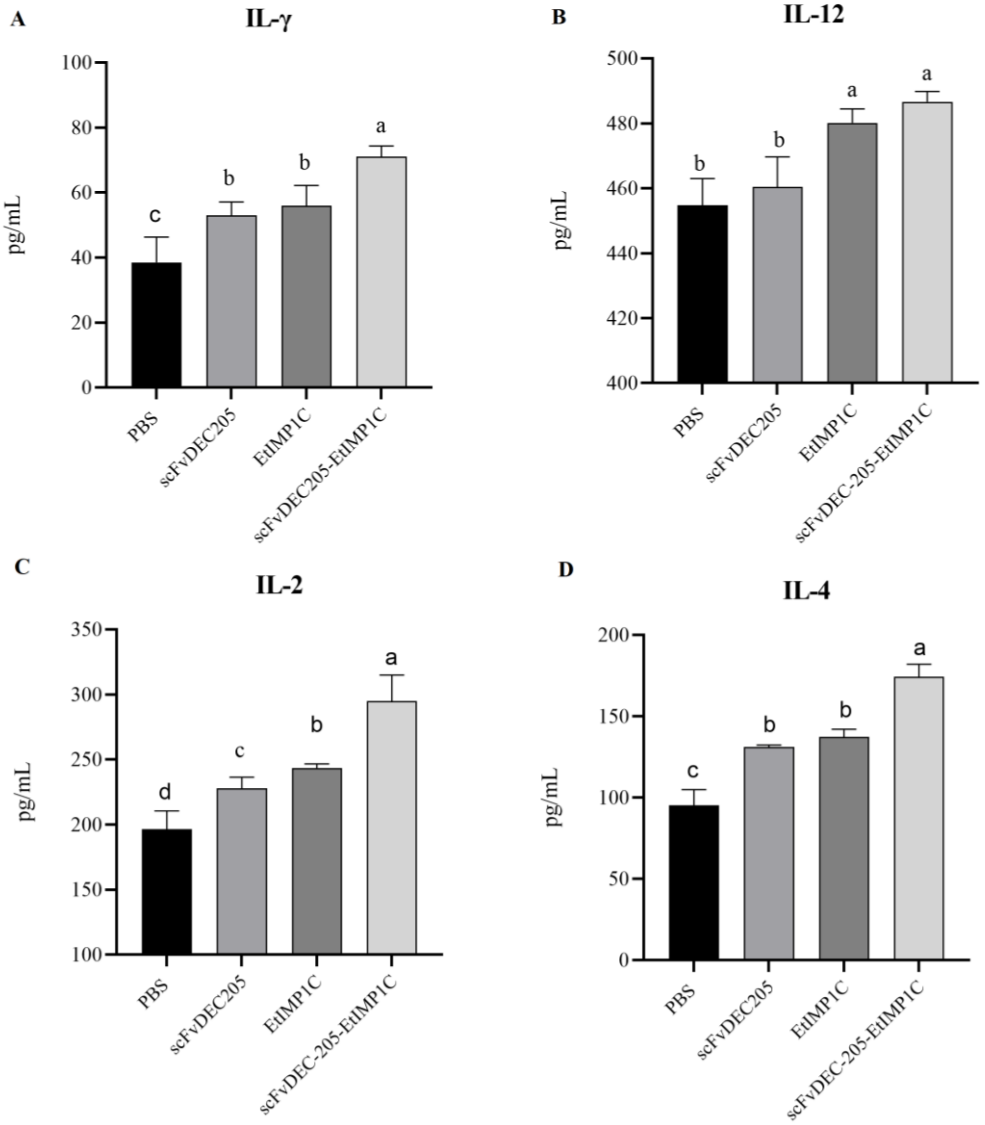

Fig. 7: The concentration of cytokines IFN-γ (A), IL-12 (B), IL-2(C) and IL-4 (D) in serum after the third immunization (pg/mL). In the same column, different letters indicate significant differences between groups (P<0.05)

DISCUSSION

The findings of this study demonstrate that targeting the *E. tenella* antigen EtIMP1C to chicken dendritic cells via DEC-205-specific scFv enhances both cellular and humoral immune responses, conferring significant protection against coccidiosis. The expression and purification of recombinant scFvDEC205-EtIMP1C fusion protein, along with its specific binding to chBM-DCs, validates the proper molecular design. These results align with previous studies showing that antigen targeting to dendritic cells through DEC-205 improves

immunogenicity, as reported in another study (Marschall, 2019). This is the first study to construct and evaluate a DEC-205-targeted subunit vaccine against *E. tenella* in chickens. The observed morphological changes and marker expression (MHC II and CD11c) during chBM-DC maturation are consistent with established dendritic cell differentiation protocols, though the use of IL-4 alongside chicken GM-CSF represents an adaptation from mammalian systems that proved effective in this avian model.

The expansion of CD^{4+} and CD^{8+} T cell populations following immunization with scFvDEC205-EtIMP1C suggests robust T cell activation, potentially due to improved antigen delivery and presentation. This aligns with findings in mammalian systems, where targeting DEC-205 enhances cross-presentation (Marschall, 2019). Notably, the stronger CD^{4+} response may reflect the role of this subset in coordinating immunity against apicomplexan parasites, as seen in studies with *Eimeria maxima* (Mtshali and Adeleke, 2020; Pu *et al.*, 2023). The elevated IgG and IgA titers in the fusion protein group further support the induction of a balanced Th1/Th2 response, corroborating reports that DEC-205 targeting can enhance antibody production(Iberg and Hawiger, 2019). The analysis of cellular immunity was primarily focused on quantifying CD^{4+} and CD^{8+} T cell populations. Future studies should incorporate analysis of T cell activation markers, memory phenotypes, and antigen-specific responses using techniques like intracellular cytokine staining upon antigen restimulation to more directly elucidate the mechanisms of enhanced immunity.

The cytokine profile, particularly the increase in IFN-γ and IL-12, indicates a Th1-biased response critical for intracellular parasite clearance, consistent with observations in other *Eimeria* vaccine studies (Arce-Fonseca *et al.*, 2018; Chen *et al.*, 2025). However, the concurrent rise in IL-4 suggests a partial Th2 component, which may contribute to mucosal immunity via IgA a feature also noted in recombinant *Eimeria* antigen trials (Tan *et al.*, 2024). The superior protection conferred by scFvDEC205-EtIMP1C, evidenced by reduced oocyst shedding and cecal pathology, aligns with the correlation between cellular immunity and coccidiosis resistance (Gaghan *et al.*, 2022). While EtIMP1C or scFvDEC205 provided partial protection, their combination yielded synergistic effects, underscoring the importance of targeted delivery in subunit vaccine design. Measurement of cytokines in serum provides a snapshot of the systemic response. To definitively link cytokine production to antigen-specific T cells, future experiments should include in vitro restimulation of PBMCs or splenocytes from immunized birds with EtIMP1C, followed by quantification of cytokine secretion.

The injection-based regimen used in this study is labor-intensive and less suitable for mass vaccination in commercial poultry farms. Oral delivery of the vaccine is the ideal route for ease of application. However, a major challenge is antigen degradation in the gastrointestinal tract, which could significantly reduce efficacy and potentially hinder the scFv's ability to bind its target receptor. So, encapsulation technologies (e.g., in acid-resistant polymers, plant-based systems, or nanoparticles) would be necessary to protect the recombinant fusion protein during passage through the gut to its target immune sites in the intestinal mucosa. The typical broiler production cycle is short (6-8 weeks), and protective immunity must last at least for this duration. Our data shows that a significant protective effect (63.4% oocyst reduction, improved weight gain, and an ACI of 173.28) was measured at the time of challenge (14 days after the final immunization). The level of protection achieved by our targeted vaccine is comparable to or slightly superior to these earlier reports using the antigen alone, supporting the value of the targeting strategy (Yin *et al.*, 2013). This demonstrates a robust short-to-medium term response after the prime-boost regimen. While our study did not measure immunity beyond 6-8 weeks, it is critical need to evaluate the longevity of the immune response in the context of a broiler's lifespan. The strong, balanced Th1/Th2 response and high antibody titers induced by the DEC-205-targeted vaccine suggest the potential for a durable memory response. However, this needs to be empirically validated.

The production cost of recombinant protein subunit vaccines, especially those involving engineered targeting molecules like scFvs, is typically higher than producing attenuated oocysts for live vaccines. Costs include fermentation/purification, quality control for protein folding and stability, and formulation for targeted delivery. But the benefits of our vaccine could offset these costs through improved production performance. As shown in our results, the vaccinated group had a markedly higher average weight gain and a significant reduction in oocyst shedding and lesion scores. These can translate directly into better FCR and reduced subclinical losses.

Comparatively, this approach outperforms traditional live oocyst vaccines in safety while matching some DNA vaccine efficacy (Snyder, 2021), though challenges remain in scaling production. The study advances existing knowledge by demonstrating DEC-205 targeting chickens, a concept previously explored mainly in mammals. In commercial poultry production, co-infections with multiple *Eimeria* species are the norm, EtIMP1 is recognized as a highly conserved antigen across apicomplexan parasites. This conservation provides a strong molecular foundation for cross-reactive immunity. Our future work will evaluate this cross-reactive immunity, field efficacy and longevity of protection, as well as potential combinations with other *Eimeria* antigens.

Live oocyst vaccines provide a complex, poly-antigenic immune stimulus that closely mimics natural infection, often inducing robust and long-lasting protection. However, these vaccines have many limits: the risk of pathogenicity reversion, potential for transmission and recombination with field strains, and variable efficacy under different conditions. Future head-to-head challenge study comparing our vaccine formulation with live vaccines and another DC-targeting approach under identical conditions is the gold standard for efficacy benchmarking. Overall, these findings contribute to the growing evidence supporting dendritic cell-targeted vaccines as a promising strategy against avian coccidiosis.

Conclusions: DEC205-targeted vaccine represents a significant advance in avian coccidiosis control,

combining the precision of DC targeting with the immunogenicity of EtIMP1C. The balanced humoral/cellular response and strong protection observed position this platform as a promising alternative to conventional vaccines, with potential applications against other avian pathogens.

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Author contributions: GWY, RC, MM, LW, DFW: conceptualization, methodology, investigation, writing review and editing, funding acquisition. SKM, SHS, HYW, PPG, YTY, FA, and YA: Review and editing, Validation.

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