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Impact of *Echinococcus granulosus* Antigens on Monocyte Development and Dendritic Cell Differentiation

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ABSTRACT

Background: Different subtypes of dendritic cells (DCs) can induce different types of immune responses. Our previous study found that *Echinococcus granulosus* (E.granulosus) antigens (Eg. ferritin, Eg.mMDH and Eg.10) stimulated DC differentiation to different subtypes and produced different immune responses.

Objective: To further understand whether Eg.ferritin, Eg.mMDH and Eg.10 affect the DC-mediated immune response by promoting the differentiation of monocytes to DCs.

Methods: Bone marrow-derived monocytes were exposed to three antigens of *E. granulosus* on days 0, 3, 5, and 7. The percentage of monocyte-derived DCs (moDCs), DCs subsets, and the expression of surface molecules of DCs at different time points in different groups were assessed by flow cytometry. The levels of cytokines of IL-1β, IL-4, IL-6, IL-10, IL-13, IFN-γ, TNF-α, IL-12p70, IL-18, IL-23, and IL-27 in the cell culture supernatant were detected by multi-factorial detection technology.

Results: The percentage of moDCs revealed that none of the three antigens blocked monocyte differentiation to DCs. The monocytes of 7-day-old cultures showed increased sensitivity to these antigens. The Eg.ferritin induced more mature DCs, which expressed high levels of MHC II and costimulatory molecules, and secreted Th1 cytokines. Eg10 and Eg.mMDH induced lower degrees of DC maturation, however differentiated DCs were in a semi-mature state due to low expression of MHC II and costimulatory molecules and secretion of higher Th2 and lower Th1 cytokines.

Conclusion: Eg.ferritin promotes full maturation of DCs and induces Th1 immune response, whereas Eg.10 and Eg.mMDH induce semi-mature DCs producing higher levels of Th2 cytokines.

Keywords: Dendritic Cells, Echinococcus granulosus, Immune Response, Monocytes

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INTRODUCTION

Cystic echinococcosis (hydatid disease) is a parasitic zoonotic disease caused by *E. granulosus* spread globally. Vaccination is an effective way to inhibit and control the spread of *E. granulosus*. In recent years, many vaccine molecules have been reported, such as Eg95 (1), EgP29 (2), and Eg14-3-3 (3). However, due to the large body, multiple growth, development stages, and complex life history of *E.granulosus* (4), a single vaccine molecule can not control the development of the disease. In addition, due to the unclear immune mechanism between the parasite and the host, effective disease control is hindered (5). Therefore, it is important to better understand the immune mechanism in parasites and hosts, providing the foundation for vaccine research and application.

Monocytes are precursor cells of the myeloid system that affect immune defense, inflammation, and homeostasis by sensing the local environment, clearing pathogens and dead cells, and initiating adaptive immunity. Monocytes serve as a progenitor pool that has the potential for directional polarization of dendritic cells (DCs) and macrophages (6), and the process that determines their developmental fate is regulated by multiple factors and pathways (7). In this process, IL-6 inhibits the development of monocytes to DCs and promotes the formation of macrophages (8); however, IL-4 is important for monocyte development to DCs (9). Additionally, IL-10 and tumor necrosis factor alpha (TNF-α) regulate the monocyte development (10). DCs are a small group of white blood cells that are powerful antigen-presenting cells in the mammalian immune system, balancing innate and adaptive immunity. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 induce monocyte-derived bone marrow to form monocyte-derived DCs (moDCs) in vitro. Recently, researchers found that DCs exist in three states: immature, semi-mature, and fully mature (11). The state of DCs depends on the antigenic environment. In vitro, moDCs without any antigenic stimulation are in an immature state called immature DCs (iDCs), showing higher endocytic activity, express low costimulatory molecules, lack costimulatory signals, and secrete IL-10 and transforming growth factor beta (TGF-β) cytokines that induce tolerance immunity. When iDCs capture an invading pathogen or other foreign protein, surface maturation ligands such as CD80, CD86, and major histocompatibility complex (MHC) II are upgraded (12), leading to the secretion of cytokines IL-12p70 and IL-1ß, balances the inflammatory or immunostimulatory responses, and enhances T cell activation to fully form mature DCs (mDCs) (13). When iDCs are exposed to non-immunogenic or low-immunogenic antigens, they attain a semi-mature DC state, an intermediate maturity between iDC and mDC. Semi-mature DCs are divided into two subsets according to their advantages on a phenotypic or functional level to cause T cell anergy and facilitate tolerogenic (14, 15). Therefore, influencing the development of monocyte into DCs and maturation differentiation of DCs can affect DC-mediated immune response. Remoli et al. (16) found that *Mycobacterium tuberculosis* (Mtb) could interfere with monocyte development into DCs by restricting the generation of functional DCs and then inhibiting specific adaptive immunity.

Many factors are involved in the immune evasion of parasites that can survive and develop into a chronic disease after invading the host. Besides some well-known immunoevasion strategies, some new mechanisms have recently been developed. Parasites can produce a variety of regulatory proteins, which can change the host's immune function to interfere with the innate and the acquired immune response produced by the host against the parasite (17). Riganò found that Antigen B of *E.granulsous* interferes with the differentiation of monocytes, thus affecting the maturation of DC and inducing T helper (Th) 2 immune response (18). In our previous study, we also found that some antigens of

E. granulosus can block DC maturation and induce immune tolerance (19). Based on these results, we explored whether some antigens of *E. granulosus* impacted the differentiation of monocytes to DCs and further influenced DC polarization. For this speculation, we observed different effects of *E. granulosus* antigens on the in vitro development of monocytes from the bone marrow over time. These findings will help us understand the immune mechanism induced by the antigen of *E.granulosus*, which can help us resolve unclear aspects in the previous studies.

MATERIALS AND METHODS

Ethics Statement

The study was approved by the Ningxia Medical University Ethics Review Committee (permit number: 2021-N135).

The antigen of Echinococcus granulosus

Eg.ferritin (accession number: DQ678103), Eg.10 (accession number: AY942146), and Eg.mMDH (accession number: AJ621238) proteins were expressed in the prokaryotic expression systems Eg.ferritin/pET28a/BL21, Eg.10/pET28a/BL21, and Eg.mMDH/pET28a/BL21, respectively. Three of these proteins existed as inclusion bodies in the expression system of *Escherichia coli*. They were treated with urea and purified using Ni^{2+}-affinity column chromatography (Roche, Basel, Switzerland) under denatured conditions and then subjected to refolding with a protein refolding solution using the gradient method, followed by 10000 ×*g* centrifugation for 10 min, and the supernatant was collected. Endotoxin was then removed from these proteins using the ToxinEraser™ Endotoxin Removal Kit (GenScript Inc., New Jersey, USA) and measured using a ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (GenScript Inc.) according to the manufacturer's instructions, to ensure that the endotoxin concentrations were <0.01 U/mL and to avoid interference in subsequent experiments. This was followed by filtration with a 0.45-μm film membrane (MILLEX-HP; Millipore, Massachusetts, USA) and quantification using the Bradford protein assay.

Preparation of DCs and Culture

C57BL/6 mice were purchased from the Laboratory Center of Animals of Ningxia Medical University and fed under pathogen-free conditions. Mice aged 6–8 weeks were sacrificed, the two terminal leg bones were opened, and bone marrow cells were obtained by rinsing the medullary cavity with sterile phosphate-buffered saline (PBS). Monocytes from the bone marrow were separated using a Ficoll-Pague PLUS (General Electric Company, Boston, USA) density gradient centrifugation method, resuspended, and counted, whereafter 1×10^6 cells were cultured in complete culture medium containing 10% fetal bovine serum (FBS) (HyClone Laboratories, Inc., Utah, USA), 2 mM glutamine (Sigma-Aldrich, Missouri, USA), 50 μM β-mercaptoethanol (Sigma-Aldrich), 1% penicillin and streptomycin (Gibco Invitrogen, New York, USA), GM-CSF (10 ng/ml), and IL-4 (5 ng /ml) (R&D Systems, Abingdon, UK) in a 24-well culture plate for 7 days. Half of the culture medium was changed on days 3 and 5 and used for GM-CSF and IL-4 cytokines during this period.

Treatment with Antigens

The cells were divided into various groups and exposed to different antigens and culture times. They were cultured with Eg.ferritin (1 μg/ml), Eg.10 (1 μg/ml), or Eg.mMDH (1 μg/ml) on days 0, 3, 5, and 7. The control group was cultured in a 1640 RPMI cell medium with no antigen.

Detection of Surface Marker Expression of DCs Using Flow Cytometry

The cells were collected on day 8 and stained with different antibodies, including CD11c-PE (phycoerythrin), CD80-FITC, CD86-FITC, MHC II-PEcy5 (yanin5-conjugated), and CD40-PEcy5 (eBioscience,

San Diego, USA), for 30 min at 4°C, fixed with 2% paraformaldehyde in PBS buffer, and detected with flow cytometry of Guava EasyCyte plus (MerkMillpore, Darmstadt, Germany). The cells that were more than 1×10^4 were detected three times. The light was avoided throughout. Flow J software was used to analyze the data.

MoDCs, Mature DCs, and iDCs Quantification

The cells were collected in groups on day 8 and stained with antibodies to CD11c. The percentage of the $CD11c^+$ cell population was detected using flow cytometry, and the R1 gate was set according to the control group. Based on the R1 gate, the G1 and G2 gates were set according to the sizes and particles of the cells with side scatter (SSC) and forward scatter (FSC), which can be calculated as the percentage of mDCs and iDCs. The SSC^{High} (G1) represented mDCs, and the SSC^{low} (G2) represented iDCs. These data were analyzed with FlowJ software.

Supernatant Cytokine Determination of DCs

On day 8, the supernatant of DCs in different groups was obtained, and the levels of IL-1β, IL-4, IL-6, IL-10, IL-13, interferon-γ (IFN-γ), TNF-α, IL-12p70, IL-18, IL-23, and IL-27 cytokines were analyzed according to the manufacturer's instructions for the Th1/Th2/Th9/Th22 cytokines 17-Plex Mouse ProcartaPlex™ Panel Assay Kit (Thermo Fisher Scientific Inc, MA, USA) using Luminex xMAP technology. Each sample was repeated twice.

Statistical Analysis

Statistical analysis was performed using SPSS 17.0. We used the two-tail unpaired student t-test to compare the two groups' mean of triplicate values±standard deviation (SEM). P-values<0.05 were used to show any statistical significance.

RESULTS

moDCs Quantification in Monocytes from the Bone Marrow

Monocytes from the bone marrow were stimulated with different antigens on days 0, 3, 5, or 7. On day 8, the culture cells were collected, and the cell population was detected with $CD11c^+$ expression representing moDC. The R1 gate was set according to the percentage

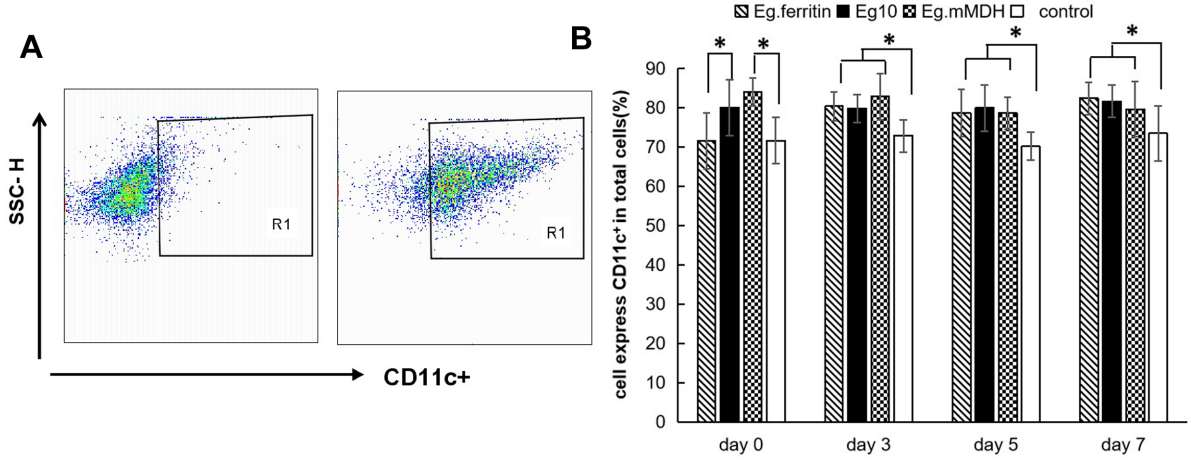

Fig. 1. The percentage of moDCs in total cells in different groups. A) R1 gate was set according to $CD11c^+$ expression population in the control group, $CD11c^+$ expression represented moDC. B) Cells of different groups with Eg.ferritin, Eg.10, or Eg.mMDH at different times were collected on day 8 and stained with CD11c-PE antibody and the percentage of moDCs in different groups was determined and compared with each other. Data were collected for 10,000 cells. (*p<0.05). Control group of cells was cultured with no antigen. Data were presented as means±SEM of three independent experiments. Monocyte-derived DCs (moDCs)

of moDCs in the control group (Fig. 1A). The results indicated that the three antigens (Eg.ferritin, Eg.10, and Eg.mMDH) promoted the development of mononuclear cells into DCs. The percentage of moDCs in total cells reached about 80% in most groups, which was significantly higher than in the control group (p<0.05), and there were no distinct changes among these groups, except for Eg.ferritin. On day 0, Eg.ferritin had no significant effect on the development of monocytes to DCs, which was the same as the control group, and on day 3, Eg.ferritin promoted moDCs up to 80.0% (Fig. 1B).

Mature and Immature DCs Quantification in MoDCs

Based on the R1 gate, the CD11c^{+} cell population was divided into G1 and G2 groups according to the size and particles in the cells with SSC and FSC. The SSC reflects cell irregularity, which may correlate with the outgrowth of the dendrites of DCs. The SSC^{high} (G1) cells showed more dendrites on the cells representing mDCs, whereas SSC^{low} (G2) cells represented iDCs. The transition percentage of SSC^{low} to SSC^{high} assists in determining dendrite change. Eg.ferritin exposed to monocytes in different periods indicated a

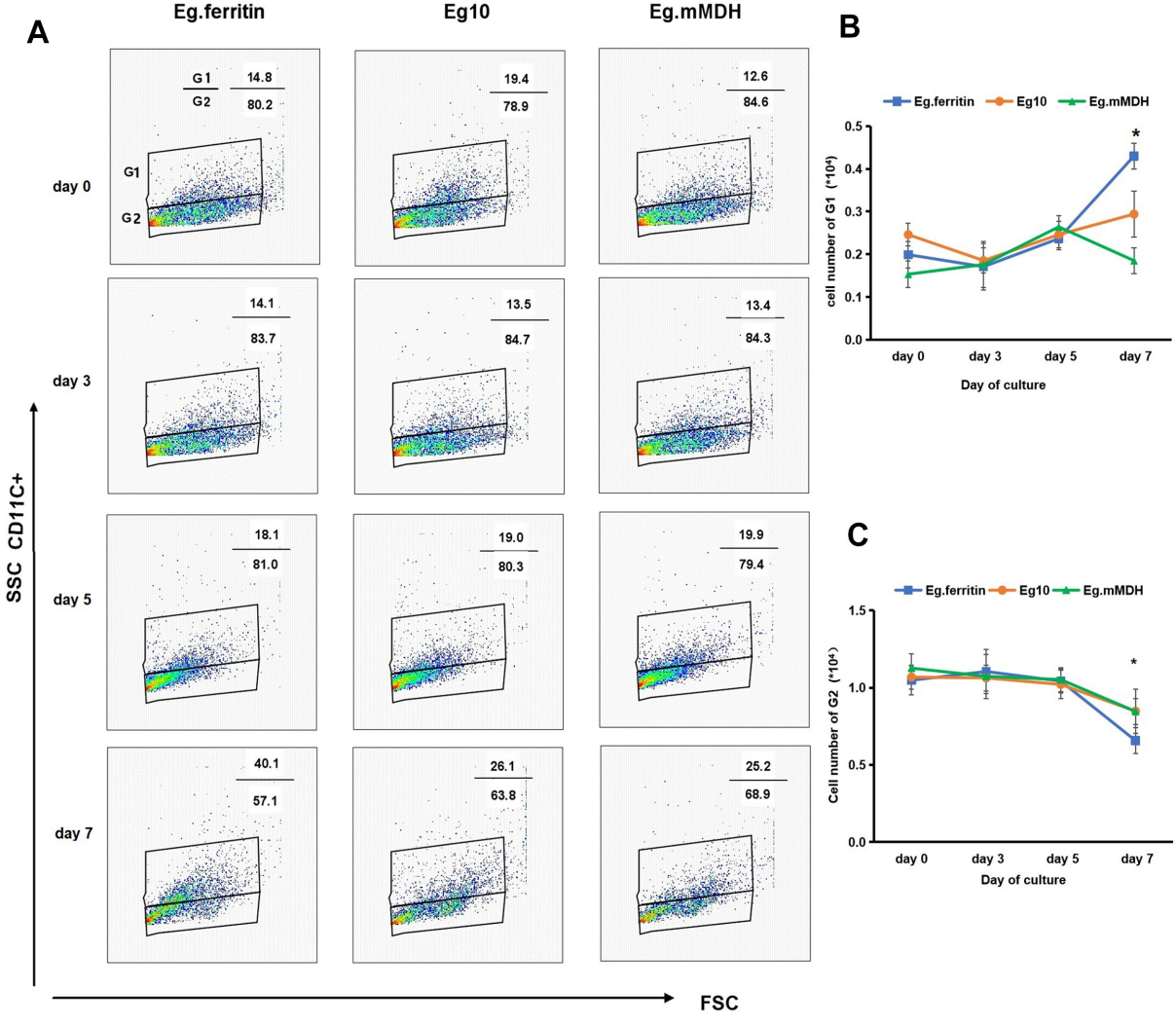

Fig. 2. Comparison of moDCs differentiation in different antigens. The CD11c^{+} cell population was divided into two subsets G1 and G2, according to Side Scater (SSC ^{high} and SSC ^{low}). G1 was the SSC ^{high} subset representing mDCs and G2 was SSC ^{low} subset consisted of iDCs. A) The percentage of mDCs and iDCs in moDCs were analyzed using flow cytometry. B) Cell number of mDCs (G1) were compared in each group. C) The number of iDCs (G2) were compared in each group. Data were presented as means±SEM of three independent experiments. Monocyte-derived DCs (moDCs), mature DCs (mDCs), immature DCs (iDCs)

sharp increase in the percentage of mDCs from 14.8% to 40.1%, and the percentage of iDCs decreased remarkably from 80.2% to 57.1%. Interestingly, the Eg.10 antigen improved moDCs maturation on day 0 and reached 19.4%, higher than the other antigen groups on day 0. However, following day 3, the percentage of mDCs decreased to a similar level as the Eg.ferritin and Eg.mMDH groups. On days 5 and 7, small amounts of mDCs were produced. For Eg.mMDH antigen exposure on days 0, 3, 5, and 7, the subset of mDCs increased slightly to 25.2% on day 7 (Fig. 2A). All three antigens enhanced moDCs to a small number of mDCs on day 0, but there was no significant difference between them on days 3 or 5. On day 7, there were distinct differences in the percentage of mDCs and iDCs induced by different antigens (p<0.05) (Figs. 2B and C).

Surface Marker Expression in DCs of Different Groups

Table 1 indicates the three different antigens that stimulated moDCs on day 0; there were no significant distinctions for surface marker expression between them, except for CD80 and CD40 of the Eg.ferritin group, which were lower than those in the other groups (p<0.05). However, on day 3 with Eg10 plus the surface marker MHC II, CD80, CD40, and CD86 all decreased remarkably more than in other groups (p<0.05). On day 5, moDCs were stimulated by Eg.ferritin and expressed higher MHC II and CD86 than in the other groups (p<0.05). On day 7, moDCs of the Eg.ferritin group showed MHC II and CD80 expression that continued to increase from day 5 (p<0.05). However, the CD86 and CD40 decreased significantly in the Eg.mMDH group on day 7 compared with day 3. For Eg.10 antigen, the expression increased on day 5 (except for MHC II), and the other surface markers had no distinct change on days 3, 5, and 7.

Supernatant Cytokines of Different DCs Groups

On day 8, the supernatant of each group was collected and assayed for levels of

Table 1. The expression of surface markers on dendritic cells in different groups

		MHCII	CD80	CD40	CD86
Day 0	Eg.ferritin	68.06±4.67	47.08±3.95	17.99±1.31	47.98±3.62
	Eg.10	70.59±5.32#	54.16±2.47*#	26.15±1.09*#	45.28±2.74
	Eg.mMDH	62.99±3.37	42.9±1.77	20.83±2.543	45.18±3.12
Day 3	Eg.ferritin	68.14±3.74$	52.42±2.32$	25.43±1.61$	50.46±3.29$
	Eg.10	57.44±4.11	48.36±2.05	12.31±0.39	35.9±2.00
	Eg.mMDH	70.08±5.67&	53.4±2.02&	21.23±1.40&	47.34±3.24&
Day 5	Eg.ferritin	73.74±6.12▲@	52.45±3.75▲	18±0.81▲	53.5±4.37▲@
	Eg.10	64.66±3.28	45.46±2.58	10.67±1.33	35.24±1.51
	Eg.mMDH	65.25±8.69	48.26±3.52	15.98±1.02■	41.58±2.76■
Day 7	Eg.ferritin	79.77±4.95●♦	58.57±2.22●♦	15.5±2.44♦	52.01±2.57●♦
	Eg.10	65.66±7.06	43.78±2.62	11.46±0.38	37.35±3.02
	Eg.mMDH	69.87±5.19	47.97±4.07	8.4±0.77	39.61±1.18

Monocytes were exposed to Eg.ferritin (1 µg/ml) or Eg.10 (1 µg/ml), or Eg.mMDH (1 µg/ml) on day 0, day 3, day 5, and day 7 respectively. On day 8, the cells were collected and stained with different antibodies, then detected with flow cytometry. The P values as determined by Student's paired t-test for comparisons and the data are means± SEM for three independent experiments. Eg.10 compared with Eg.ferritin significantly indicated by "*" on day 0 p<0.05; Eg.10 compared with Eg.mMDH significantly indicated by "#" on day 0 p<0.05; Eg.ferritin compared with Eg.10 significantly indicated by "$" on day 3 p<0.05; Eg.mMDH compared with Eg.10 significantly indicated by "&" on day 3 p<0.05; Eg.ferritin compared with Eg.10 significantly indicated by "▲" on day 5 p<0.05; Eg.ferritin compared with Eg mMDH significantly indicated by "@" on day 5 p<0.05; Eg.mMDH compared with Eg.10 significantly indicated by "■" on day 5 p<0.05; Eg.ferritin compared with Eg.10 significantly indicated by "●" on day 7 p<0.05; Eg.ferritin compared with Eg.mMDH significantly indicated by "♦" on day 7 p<0.05

important cytokines such as Th1 and Th2. The results showed that regarding Th1 cytokines, the levels of IFN-γ, TNF-α, IL-12p70, Th1 co-factors IL-23 and IL-27 in the Eg.10 group were higher on day 0 but decreased dramatically on day 3 (p<0.001). However, for the Eg.mMDH groups, Th1 cytokines decreased remarkably only on day 7, except for IL-27, which increased more than on other days and became higher than Eg.ferritin and Eg.10 on day 7 (p<0.01). In addition, the level of TNF-α increased on day 5 but decreased by day 7. For the Eg.ferritin groups, most Th1 cytokines increased remarkably from day 3 and maintained high levels on day 5 and day 7; although some cytokines slightly decreased on day 5, there was no distinct difference from day 7. In contrast, the level of IL-27 decreased sharply on day 5 and continued falling to day 7 (p<0.05) (Fig. 3A). Regarding

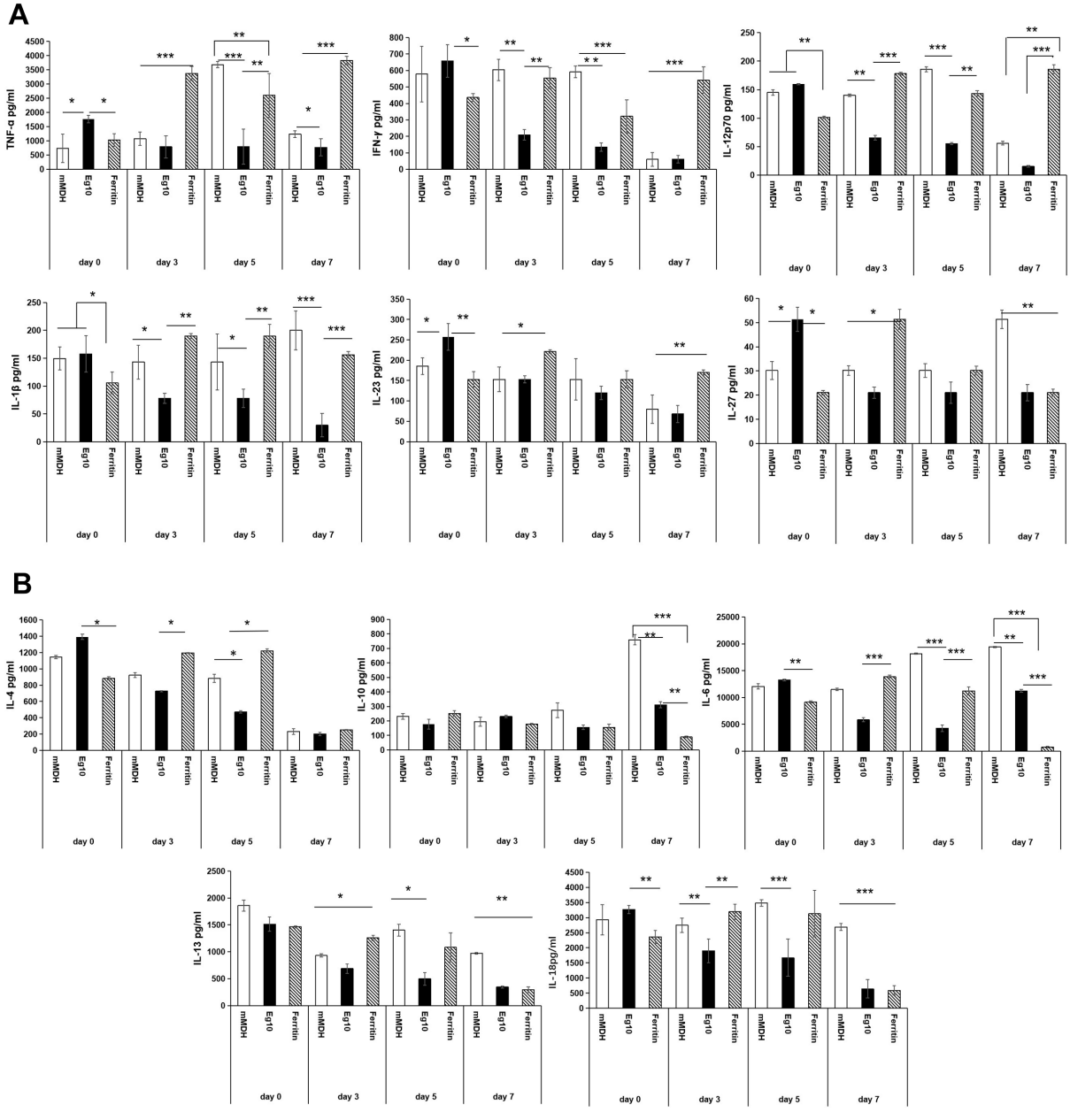

Fig. 3. The content of cytokines in supernatant of different groups. The supernatants were collected from each group on day 8. Th1 and Th2 type cytokines were measured using Luminex xMAP technology. A) Th1 cytokines expression in different groups. B) Th2 cytokines expression in different groups. The data were presented as means ± SEM of three independent experiments (*p<0.05, **p<0.01, ***p<0.001). T help type 1(Th1), T help type 2 (Th2)

Th2 cytokines detection results, we found that for the Eg.ferritin groups, the levels of IL-4, IL-10, IL-6, and IL-13 did not significantly change on days 0, 3, and 5, but decreased on day 7. For the Eg.mMDH groups, the level of IL-4 decreased on day 7, and the levels of IL-6 and IL-10 on days 5 and 7 were higher than in other groups at the same time ($p<0.05$). For the Eg.10 groups, the levels of IL-4 and IL-13 gradually reduced with shorter days in which the cells were exposed to Eg.10, the level of IL-10 elevated on day 7, and the level of IL-6 fluctuated remarkably and decreased on day 3, but rose again on day 7. For the IL-18, on day 7, Eg.ferritin and Eg.10 group levels decreased and were lower than in those of the Eg.mMDH group in the same period (Fig. 3B).

DISCUSSION

Our previous studies found that mice immunized with Eg.ferritin produced 85.6% protection against protoscolex and can stimulate DC maturation to express higher levels of MCH-II and co-stimulatory molecules (20). Furthermore, Eg.mMDH and Eg.10 were identified as candidate vaccines because of their importance in the vital activities of *E. granulosus*; however, the mice immunized with Eg.mMDH or Eg.10 displayed promotion of protoscolex infection and more severe infection through the production of iDCs to induce immune tolerance (19). We recognized the need for more research to determine whether monocytes are blocked from development to DCs or that the reduction in the number of DCs affects the number of mature DCs and thus produces immune tolerance. First, we measured the number of DCs in different groups exposed to various antigens at different times. Monocytes were induced with GM-CSF and IL-4 to develop into DCs in vitro; under this situation, $CD11c^+$ was an indicator of the overall DC population. The detection of the percentage of cells with $CD11c^+$ in total cells reflected whether Eg.ferritin, Eg.10, or Eg.mMDH can promote or block monocyte development to DCs. The three antigens were unable to block monocyte development in DCs but promoted more monocyte development to DCs. The percentages of iDCs and mDCs were then detected in different groups according to the SSC^{low} and SSC^{high} clusters based on the $CD11c^+$ cell population through flow cytometry. The results indicated that from day 3, with different antigens, there was a small amount of mDC produced, until day 5, there were more mDCs produced. This could be due to the fact that the number of DCs transformed from monocytes was too low to be sensitive to antigens on day 3. On day 7, there were many mDCs differentiated from moDCs stimulated by Eg.ferritin, which was similar to Wang (21), as they found that LPS stimulated a few of mDCs on day 3; whereas on days 6 and 9 more mDCs were stimulated. However, Eg.10 and Eg.mMDH showed a weak ability to induce DC maturation, compared with Eg.ferritin. This was likely due to high levels of IL-10 and IL-6 in Eg.10 and Eg.mMDH groups, which, as immune suppression factors, can inhibit DC maturation (8, 22). On day 3, the percentage of mDCs in the Eg.10 group decreased slightly, and the superficial marker expression assay on day 3 showed that cells stimulated with Eg.10 expressed lower MHC II and costimulatory molecules. This result explains why the proportion of mDC in Eg.10 treated cells decreased on day 3, but the cause was not clear.

Mature DCs are based on phenotypic and functional levels of maturation. Phenotypic level maturation relies on DC upregulation of MHC II molecules and CD80 and CD86 surface maturation ligands. Functional maturity is demonstrated by the ability of stimulated DC to secrete the cytokines to polarize native T cell-induced immune responses (13, 23). In this study, we detected the levels of IL-4, IL-6, IL-13, IL-18, IL-10, IL-1β, TNF-α, IFN-γ, IL-12p70, IL-23, and IL-27 in groups subjected to different

antigens. Overall, these cytokines did not change significantly in the groups on days 0, 3, and 5, while the cytokines secreted by DCs showed distinct changes on day 7, except for IL-4. The level of IL-4 was significantly higher on days 0, 3, and 5 in all groups than in the level on day 7, and the possible reason was the addition of IL-4 to promote the development of monocytes to DCs on days 0, 3, and 5. On day 7, no more IL-4 was supplied; therefore, the level was low and did not differ for various antigens. Similar results were reported by other studies, showing that IL-4 is rarely detected in a secreted form because DC can secrete IL-4, and IL-4 receptors are exposed on DC, so the secreted IL-4 binds to the IL-4 receptor and activates the DC to form the autocrine pathway (24). Apart from IL-4 enhancing DC's ability to induce Th2 cytokines and inhibit the expression of MHC II, IL-13 has a similar function (25). Therefore, the level of IL-13 was higher in the Eg.mMDH group and lower in the Eg.ferritin group on day 7, indicating that IL-13 and IL-4 regulated DC maturation together. It is well known that IL-6 has pleiotropic effects on cell growth, differentiation, survival, and migration during immune responses. It blocks DC differentiation by increasing the number of iDCs and decreasing the number of mDCs (8). In the Eg.ferritin group on day 7, the level of IL-6 was much lower than in Eg.10 and Eg.mMDH groups. This was consistent with the previous results involving mDCs and iDCs detection in various groups on day 7 mentioned above. Interestingly, IL-18 is a member of the IL-1 family of pro-inflammatory cytokines produced by DC, which promotes T cell differentiation to Th1 cells (26). The most important biological activity of IL-18 is to induce immune cells to secrete IFN-γ, but the conflicting results in our study showed that the level of IL-18 was extremely low in the Eg.ferritin group and high in the Eg.mMDH group on day 7. IL-18 derived from iDCs may promote T cell conversion to Treg cells, inducing tolerant immunity, as described by Oertli et al. (27). It could be explained by our previous finding in which DCs exposed to Eg.mMDH enhanced Treg cell proliferation (19). In addition, the higher levels of IL-10 in the Eg.mMDH and Eg.10 groups on day 7 was another important factor in helping DCs induce Th2 immune response, which was in line with the study by Rachele Rigano (18).

DCs are an important source of pro-inflammatory cytokines and chemokines. IL-12p70 is a hall-mark cytokine involved in the initiation of the adaptive Th1 immune responses; IL-23 belongs to the IL-12 cytokine family, and has been reported that it could synergize with IL-12 in promoting the production of cytokines by DC themselves (28). Our results showed that the secretions of IL-23 and IL-12p70 increased dramatically in Eg.ferritin groups DCs compared with the DCs in Eg.mMDH and Eg.10 on day 7, and IL-23 is involved in the initiation and sustainment of Th17 differentiation. Although IL-27 is a newly discovered heterodimeric cytokine belonging to IL-12 family, it is also a pleiotropic cytokine, with some opposite effects. We found that IL-27 decreased from day 3 to day 7 in Eg.ferritin groups and increased in Eg.mMDH on day 7. It may play an immune tolerance role by regulating the Treg cell population and inducing IL-10-producing Treg cells (29).

From what we discussed above, exposure of monocytes to antigens of Eg.ferritin, Eg.10, and Eg.mMDH at different times cannot block monocyte development to DCs and instead promote more DCs than in the control group. Monocytes were cultured for 7 days to develop into DCs (previously established as the optimal time), and moDC was sensitive to the stimulation of external antigens. The premature action of antigens on monocytes did not affect the development of monocytes into DC and had little influence on the differentiation of DC. Eg.ferritin increased a greater number of moDCs toward maturation to DCs on day 7. According to MHC II molecule detection, costimulatory molecules and cytokines of DCs in different groups

showed that Eg.ferritin stimulated DCs in a fully mature state and secreted higher levels of Th1 type cytokines to induce the Th1 immune response. Eg.mMDH and Eg.10 were low-immunogenic antigens, had a weak effect on DC maturation, and expressed low levels of costimulatory molecules. They also secreted higher levels of Th2 and lower levels of Th1 cytokines to make DCs take on a semi-mature state, inducing immune tolerance. This study further elaborated that Eg.10 and Eg.mMDH induced immune tolerance, not because they prevented monocyte development to DCs, resulting in a decrease in the number of DCs and reduced numbers of mDCs to induce immune tolerance.

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CONFLICT OF INTEREST

None declared.

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