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Chicken bone marrow-derived dendritic cells maturation in response to infectious bursal disease virus

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ABSTRACT

Infectious bursal disease virus (IBDV) is highly contagious disease which easily lead to immunosuppression and a decreased response to vaccinations in young chicken. Since dendritic cells (DCs) are crucial to induce immunity and their maturation and functions are influenced by microbial and environmental stimuli, we investigated the effects of inactivated IBDV and IBDV on chicken DC activation and maturation. Chicken bone marrow-derived dendritic cells (chBM-DCs) cultured in complete medium (including recombinant chicken: granulocyte-macrophage colony-stimulating factor and interleukin 4) expressed high levels of MHC-II and the putative CD11c. After LPS or virus stimulation, chBM-DCs displayed the typical morphology of DCs. In addition, stimulation by LPS or viruses significantly elevated chBM-DCs surface expression levels of CD40 and CD86 molecules, as well as the ability to induce T-cell proliferative response, compared to the non-stimulated chBM-DCs. Interestingly, inactive IBDV showed stronger ability to up-regulate expression levels of CD40 and CD86 molecules and stimulate naive T cells proliferation than live IBDV. These results revealed that live viruses infection impaired DC maturation and functions, probably explaining why chickens infected with IBDV fails to trigger an effective specific immune response or develop immune memory.

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1. Introduction

Infectious bursal disease virus (IBDV), a member of the Birnaviridae family whose genome consists of two segments of double stranded RNA, causes an acute, highly contagious disease in young chickens (Jacobs and Damania, 2012; Rautenschlein et al., 2002). The disease has an important economic impact on the poultry industry worldwide because it is associated with a high mortality and immunosuppression in recovered chickens, which leads both to a variety of secondary infections and a decreased response to vaccinations (Roh et al., 2006). Immune responses are readily measurable, which are effective for the control of IBDV in chickens. Dendritic cells (DCs) are professional antigen-presenting cells (APCs) with the unique ability to induce both innate immune responses and a highly specific acquired immunity (Banchereau and Steinman, 1998).

Avian DCs were first reported in the cecal tonsils, the secondary lymphoid organs located in the intestinal mucosa (Olah and Glick, 1979). Since then, three major subsets of DCs (inter-digitating DCs, follicular DCs, and epidermal DCs) also were identified in chicken lymphoid tissues or epidermis (Del Cacho et al., 2009; Igyártó et al., 2006). Immature DCs become mature when they sense pathogen-associated molecular patterns released from pathogens through a limited number of pattern recognition receptors (PRRs). Several classes of PRRs expressed on

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DCs, including Toll-like receptors (TLRs) and cytoplasmic receptors, can recognize distinct microbial components and directly initiate signal pathways, ultimately resulting in the activation of immunity (Akira et al., 2006).

In recent years, with the emergence of new chicken DCs markers, it has become increasingly possible to culture chicken DCs *in vitro* (Fu et al., 2014; Wu et al., 2010). There is currently no knowledge about the nature of virus interactions with chicken bone marrow-derived dendritic cells (chBM-DCs). Therefore, in this study we decided to investigate the activation and maturation of chBM-DCs by stimulation with IBDV and inactivated-IBDV in an attempt to understand the involvement of chBM-DCs in the immune suppressive diseases caused by IBDV infection.

2. Materials and methods

2.1. Chickens and viruses

Specific pathogen-free (SPF) ROSS 308 chickens (4–6-week-old) were obtained from Jiangsu Academy of Agricultural Sciences (Nanjing, China), and maintained at an animal facility under pathogen free conditions. A tissue culture infectious dose 50 of $10^{-6.38}$/0.1 ml of the IBDV strain (B87; Jiangsu Academy of Agricultural Sciences, China) was used. Virus was heat-inactivated at 56 °C for 30 min.

2.2. Culture of chBM-DCs

ChBM-DCs were generated from 4 to 6-week-old inbred line ROSS 308 chickens as previously described (Wu et al., 2010; Liang et al., 2013). Briefly, Chicken bone marrow precursors were obtained from femurs and tibias and cultured at a final concentration of 2×10^6 cells/ml in six-well plates in 3 ml the culture medium containing RPMI-1640 (GIBICO, USA), 10% fetal bovine serum (FBS) (Wisent, CAN), 50 ng/ml recombinant chicken GM-CSF (Abcam, USA) (ab119158), 10 ng/ml recombin-ant chicken IL-4 (Kingfisher, USA) (RP0110C-025), 1 U/ml penicillin and 1 μg/ml streptomycin, for 7 days at 37 °C, 5% CO_2. Half of the medium was replaced with fresh, prewarmed complete medium at day 2 and day 4 to remove non-adherent cells (such as dead cells and granulocytes). Immature chBM-DC aggregates started to grow from day 4. On day 6, LPS (200 ng/ml, Sigma-Aldrich) IBDV (10 μl/ml) or inactivated-IBDV (10 μl/ml) was used to stimulate immature chBM-DC for 24 h. At day 7 of culture, all cells were harvested by gentle pipetting and centrifugal separation.

2.3. Observation of morphology

Effects of recombinant chicken GM-CSF and IL-4 on cell differentiation were recorded by observing cell morphology, clustering and cell growth at day 4 and day 7. The stimulatory effects of LPS and IBDV on cells maturation were observed with a digital camera on an inverted microscope on day 7.

2.4. Flow cytometry analysis

The harvested cells were washed one with 0.01 M phosphate buffered saline (PBS). The un-stimulated chBM-DCs (0.5×10^6 cells/ml) were incubated with 0.05 mg/ml of PE-conjugated mouse anti-human CD11c antibody (eBioscience, USA) (Fu et al., 2014) or 0.5 mg/ml of Fluorescein isothiocyanate (FITC)-labeled mouse anti-chicken MHC-II antibody (Abcam, USA) for 20 min at 25 °C. In addition, the stimulated chBM-DCs were incubated with 25 μg/ml of mouse anti-chicken CD40 or CD86 antibody (Abd, UK) for 20 min at 25 °C. After being washed, the cells were then stained with PE-conjugated Goat anti-mouse IgG second antibody diluted 1:5000 (MultiScience, China) for 15 min at 25 °C. After the final wash with PBS, the levels of fluorescence from DCs were determined by a FACSCalibur (BD Bioscience, Cowley, UK).

2.5. T-cell proliferation assays

To determine the T-cell stimulatory capacity of chBM-DCs, allogeneic mixed lymphocyte reaction (MLR) of DCs was assayed as previously described (Wu and Kaiser, 2011). Briefly, after cultured with the complete medium for 6 days, the DCs were stimulated with viruse or LPS, respectively, for 24 h before allogeneic MLR. Allogeneic T lymphocytes were isolated from spleen of 4–6 week-old chickens and purified on nylon wood column. T lymphocytes were added to 96-well round-bottomed cell culture plates and 1×10^4 DCs were added, giving a ration of T cells: DCs of 1:1, 10:1, 100:1, in a culture volume of 100 μl. Control cultures contained T cells or DCs cells only. All experiments were performed at least in triplicate. The cells were cultured for two additional days in 5% CO_2 at 37 °C, and then T cells proliferation was determined by cell counting kit-8 (CCK-8) assay (Beyotime, Jiangsu, China) according to the manufacture's instruction. Cells in 96-well plate were added with 10 μl CCK-8 solution, and incubated for 2 h at 37 °C. Absorbances of each well were read at 450 nm using an automated ELISA reader (Bio-Tech instruments, USA). The Stimulation Index was calculated as formula: $SI = (OD_{sample} - OD_{DCs\ only})/(OD_{T\ cells\ only} - OD_{blank\ control})$.

2.6. Statistical analysis

All date analysis was performed one-way ANOVA analysis in SPSS 17.0. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. The morphologies of non-stimulated cells and viruses-stimulated cells when cultured in the presence of GM-CSF and IL-4

Cells were cultured in the presence of GM-CSF and IL-4 for 4 day; many cell aggregates were seen under an inverted light microscope (Fig. 1A). After viruses stimulation on day 6, these cells aggregates grew and became bigger and floating or loosely adherent (Fig. 1B). In addition, cells stimulated by viruses showed dendrites (Fig. 1C)

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