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Full Length Article

Establishment of Optimal Culturing Method and Biological Activity Analysis of Chicken Bone Marrow Dendritic Cells using Chi-rGM-CSF

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Abstract

Denderitic cells (DCs) play a central role in immune regulation and antigen presentation in animals, but quantitative description of chicken DCs culturing method employing rGM-CSF and optimal biological response is still lacking. Therefore, we designed the study to obtain the chicken recombinant granulocyte-macrophage colony-stimulating factor (chi-rGM-CSF), expressed in *E. coli* BL21 and established a standard concentration unit of chi-rGM-CSF to generate optimal biological respondent denderitic cells from chicken bone marrow. DCs were divided into two groups, (A and B) each was employed two level of chi-rGM-CSF (10 and 20 ng/mL) respectively, co-cultured with interleukin-4 (IL-4, 10 ng/mL), and treated with lipopolysachride (LPS, 1 µg/mL). The results showed that chi-rGM-CSF was effective to induce avian DCs growth. However, no significant difference was observed in cell proliferation, morphology and phenotype between group A and B, but the mRNA expression levels of surface marker genes, MHC-II, CD40, CD80, CD83 and CD86 were up regulated (<0.05) after LPS stimulation. DCs in group B showed significantly higher response to LPS with increased mRNA expression levels of pro-inflammatory cytokines (*INFγ, IL-1β, IL-2* and *IL-6*) and anti-inflammatory cytokine (*IL-10*) as compared with group A. Therefore, it could be concluded that *E. coli* expressed rGM-CSF with concentration unit (20 ng/mL) is more effective to obtain optimal culturing and biological response of avian BMDCs. © 2013 Friends Science Publishers

Keywords: Biological activity; Bone marrow; Chicken; Dendrite cells; rGM-CSF

Introduction

Chicken (*Gallus gallus*) is one of the most abundant species among domesticated animals, which entails short period of time for growth, and extensively used to meet ever increasing food demand. During growth period poultry birds encounter a wide range of pathogens, however most of the avian species and chicken lack of lymph nodes, making them susceptible to infection. Due to lack of lymph nodes, antigen presentation occurs either in the spleen or at local sites of diffused structures (Li *et al*., 2011). Denderitic cells are professional antigen-presenting cells (APC) of the immune system, with unique capability to defend by maturation, capturing and presentation of antigens to initiate primary immune response (Steinman, 1991; Banchereau and Steinman, 1998). DCs progenitors are generated by haematopoietic stem cells (HPSC) in the bone marrow and migrate in non-lymphoid tissues to consequently change into immature DCs (Winzler *et al*., 1997). Immature DCs have capacity to capture antigens by phagocytosis (Austyn, 1998, 1996), macropinocytosis (Granucci *et al*., 1999) and receptor-mediated endocytosis (Sallusto *et al*., 1995; Khan *et al*., 2008). Different microbes and vaccines have induced to promote DC-subset activation, maturation and migration to lymphoid organs for production of inflammatory cytokines in the body (Caux *et al*., 1996). DCs undergo phenotypic and functional changes (such as appearance of projections (dendrites), up regulation of surface markers including CD40, CD80, CD83, CD86, MHC-II and CD11c) during maturation and migration, (Mohamadzadeh *et al*., 2001) and migration to T cell regions of secondary lymphoid tissues such as the local draining lymph node, where naïve T cells are localized (Zhiguang and Pete, 2011). After maturation, DCs become specialized in presenting of collected antigens (Ags) for the initiation of immune responses.

In mammalian, DCs have been generated in vitro in adequate numbers to analyze their biological responses (Caux *et al*., 1992). In a previous study primary DCs were successfully cultured from bone marrow and blood under various conditions, in the presence and/or absence of granulocytes macrophage colony stimulating factors (GM-CSF) or co-culturing interleukin-4 (IL-4) (Inaba *et al*., 1992), where different concentrations of IL-4 and GM-CSF were used for optimal growth and biological response (Lardon *et al*., 1997). However, recent study was carried out to characterize the bone marrow DCs (Zhiguang *et al*., 2010) but, a scientific question addressed to understand rGM-CSF preparation and its standard concentration units to obtain

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optimal culturing and biological response. Therefore, present study was designed to prepare chi-rGM-CSF expressed in *E. coli* BL21 (DE3) and purified. Meanwhile, to establish a standard concentration unit of chi-rGM-CSF for optimal culturing and biological respondent denderitic cells from chicken bone marrow.

Materials and Methods

Cloning of Chicken GM-CSF and Construction of Expression Vector

E. coli BL21 DE3 was used to clone chicken *GM-CSF* gene and to express proteins from pET-28a (+) constructs (Novagen) the method was followed according to (Inumaru and Takamatsu, 1995). Briefly, chicken spleen cDNA was used for PCR amplification that induced with phytohemagglutinin (PHA) (Sigma-Aldrich). The primers 5PcGM and 3PcGM were used (Table 1a) obtained from chicken *GM-CSF* coding sequence available on GenBank (Accession: NM_001007078). The PCR cycling protocol was 94°C for 3 min pre denaturation, 35 cycles of 25s at 95°C for denaturation, 30 s at 60°C for annealing, and 30s at 72°C for extension. *NdeI/XhoI* (TaKaRa) was applied for the digestion of PCR product and cloning performed on pET28a (+) restriction sites (Novagen), and *pET-GM-CSF* plasmid was transformed into the *Escherichia coli* BL21 (DE3) using standard method.

Chicken rGM-CSF Protein Expression and Purification

Inoculation of recombinant bacteria was done in Luria Bertain (LB) broth in addition of antibiotic (50 µg/mL kanamycin). Culturing agitation at 180 rpm and expression of protein was tempted by 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 6 h at 37°C. Recombinant protein insertion bodies were purified following the method of (Wu *et al.,* 2012). In brief, bacteria were harvested and re-suspended into lysis buffer (20 mL, 50 mM Tris-HCl (pH 8.0), 100 mM NaCl, and 1 mM EDTA). The DNA and endotoxins were removed according to the method of (Cavallaro *et al*., 2011).

SDS-PAGE and Western Blotting

Sample buffers and recombinant protein were mixed with (1:1) ratio. Latter were applied to a gel for 10 min. 15% acrylamide gel was used to perform electrophoresis as per standard method. Coomassie blue staining was used to detect the proteins.

Separated proteins were transferred by semidry electro blotter (GE). Moreover was blocked using skimmed milk (5%) and further dissolved in TBST (0.05% Tween-20 in TBS). The sheet was incubated with chicken anti-His antibody and blocking solvent. Finally, washed with TBST for 10 min and step repeated with blocking solvent and horseradish peroxidase-labeled rabbit anti-mouse IgG (1:5000 dilutions), than washed thrice with TBS. The staining was perfomed to develop the color using tetramethyl benzidine and peroxidase substrate.

Isolation of Progenitor Cells from Chicken Bone Marrow

Chinese cross breed chicken (Sanghuang) was kept under standard hygienic conditions of Animal Center at Institute of Medical Science, Zhejiang University, Hangzhou, P.R. China. The femur was collected and surrounding muscle tissues were detached aseptically. Bones were flushed into petri dishes and transferred into sterilized tubes and centrifuged at 1400 xg for 5 min. Supernatant was removed and ammonium tri-chloride 0.25% (Sigma-Aldrich) was added and again centrifuged at 1300 xg for 20 min. The supernatant was discarded and PBS was added and centrifuged at 1400 xg for 5 min, subsequently, complete medium was added to homogenize the cells. Cell counting was performed by suspending in PBS and trypan blue solution at the ratio of 50:50.

Growth and Maturation of Dendrite Cells (DCs)

Growth of chicken bone marrow DCs were maintained by using warm RPMI-1640 media throughout the growth and maturation period for 7 days at 41°C, 5% CO_2. Diverse dilutions of chicken recombinant GM-CSF were used to optimize the growth conditions (data not shown). The DCs cells were divided in to two groups, group A and B was employed two level of recombinant GM-CSF (rGM-CSF) (10 ng/mL and 20 ng/mL), respectively co-cultured with chicken IL-4 at 10 ng/mL (Kingfisher Biotech, USA). Then maturation was induced using lipopolysachride (LPS) at 1 µg/mL (Sigma-Aldrich) on 7^{th} day. Non induced maturation group was used as a control group (CK).

Cell Yield Evaluation

Cultured cells were washed once and an aliquot volume of trypan blue solution was mixed at 1:1 (Sigma-Aldrich). Trypan blue negative cells counted under a compound microscope in a Neubauer counting chamber were considered as viable and positive cells as dead in both groups, respectively.

Morphological Examination

Effects of recombinant chicken GM-CSF and IL-4 collectively and individually on cell segregation were recorded by observing cell morphology, clustering, none clustering and maturation of cell. The cells cultured were photographed at 6^{th} and 7^{th} day of culture by inverted microscope.

Extraction of RNA and cDNA Synthesis

Total RNA was extracted from DCs using TRIzol (TaKaRa) and purified using RNeasy® MinElute™ (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

Table 1: (a) Oligonucleotides sequences used in this study and sequence bold and underline were restriction sites; (b) Real-time quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) primers sequences used for gene expression

Gene name	Sequence (5'-3') F: Forward R: Reverse	Accession numbers
5PcGM	TTTCATATGACCACAACATACTCCTGCTG	NM001007078
3PcGM	GGGCTCGAGTTAGATGCAGTCTTTCTC	NM001007078
(b)		
β -Actin	F"GAGAAATTGTGCGTGACATCA R"CCTGAACCTCTCATTGCCA	JN639846.1
MHCII alpha	F-GGGGTTTACGACAGCGTCTATT; R-TTCCGGGTCCCACATCCT	NM001001762.1
CD40	F-AACGCAACGCACAACACTG; R-GTCCCTTTCACCTTCACCACA	EF554723.1
CD80	F-CAGCAAGCCGAACATAGAAAGA; R-AGCAAACTGGTGGACCTGAGAA	XM418929.3
CD83	F-GCCTACACTCTACTCTTCACCCTG; R-TATTCTGTCGCCAACTCC	EF554724.1
CD86	F-TGTAGGGATTGTGGATGAGGG; R-CGCTGGAAGAGCAGGAAAGAT	JN639846.1
IFN-γ	F- GTGAAGAAGGTGAAAGATATCATGGA; R- GCTTTGCGCTGGATTCTCA	NM205149.1
IL-1β	F –GCTCTACATGTCGTGTGTGATGAG; R- TGTCGATGTCCCGCATGA	FJ537863.1
IL-2	F- TTGGAAAATATCAAGAACAAGATTCATC; R- TCCCAGGTAACACTGCAGAGTTT	NM204153.1
IL-6	F –GCTCGCCGGCTTCGA; R- GTAGGTCTGAAAGGCGAACAG	HM179640.1
IL-10	F- CATGCTGCTGGGCCTGAA; R- CGTCTCCTTGATCTGCTTGATG	NM001004414.2

The amount of total RNA was quantified by Nano Drop (Thermo Scientific). RNA was reverse transcribed using StrataScript first-strand synthesis system (TaKaRa) according to the manufacturer's recommendations. Briefly, 1 µg of RNA was combined with 10× first strand buffer, 1.0 µL of oligo (dT) primer and RNase-free water upto 6 µL, mixed well and incubated at 70°C for10 min. 5×M-MLV Buffer (2 µL), 1 µL of dNTP mix (10 mM), RNase Inhibitor 0.5 µL (40 U/µL), TRase M-MLV 2 µL (200 U/µL) and RNase-free water to total volume 20 µL. The mixture was incubated at 42°C for 1 h, and the reaction stopped by heating at 70°C for 15 min.

Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)

Oligonucleotide primers for chicken DCs surface marker, cytokines and β-actin were designed based upon sequences available from public databases (Table 1b). Real-time qRT-PCR was performed using an ABI 7500 Real-time Detection System (Applied Biosystems, USA). A total of 20 µL volume was used to carry out amplification, containing 10 mL of 2× SYBR Green I real-time PCR Master Mix (Takara), diluted cDNA 1 µL, and 1.6 µL of mixed primers. qRT-PCR followed, denaturing at 95°C for 1 min, stepped by 40 cycles of 95°C for 15 s and 55°C for 60 s. amplification products dissociation analysis was performed at the end of each PCR. The baseline was automatically adjusted by the software and data were analyzed with software (ABI- 7500) with. The method of (Livak and Schmittgen, 2001), comparative CT value was used to determine fold-changes in gene expression, calculated as $2^{-\Delta\Delta Ct}$.

Bone Marrow Cells, Developmental and Morphological Changes

Chicken bone marrow obtained cells and their developmental and morphological changes (Fig. 1) during growth, are drawn by flow diagram.

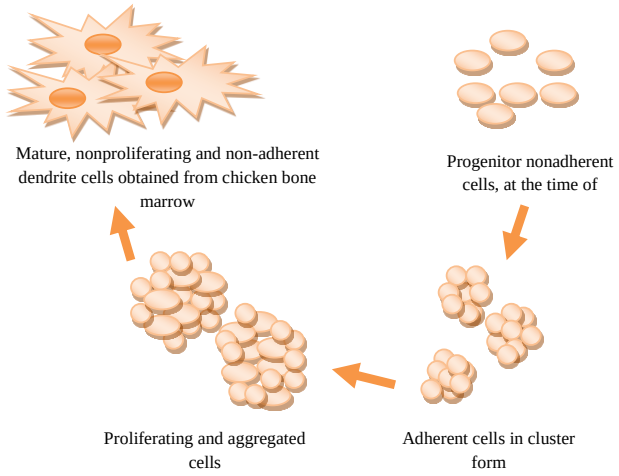

Fig. 1: Diagram of the proposed pathway of chicken bone marrow dendritic cell development, cultured with rGM-CSF and IL-4, proginator cells starts to grow at the beginning and convert in to cluster form. They become adherent and continue proliferation and appear in colonies. Stimulation decreases their progressive increase in numbers and appears non proliferating and non-adherent with dendrites

Statistical Analysis

Data were analyzed using SPSS 16.0 for Windows (SPSS Inc., Chicago, USA) and presented as means standard deviation (± S.D). Student t-test was used to calculate differences among groups and ($P < 0.05$) considered to be significant.

Results

Cloning, Expression and Purification of Chicken GM-CSF

A product of 435 bp was amplified using polymerized chain reaction for mature region of chi- *GM-CSF* gene (Fig. 2A),

and further inveterated by DNA sequencing. The sequence analysis manifested 100% similarity to the sequence of NCBI accession NM_001007078.

SDS-PAGE and Western blot results showed in (Fig. 2B and 2C) indicated that the Chi-rGM-CSF was appeared about 18 kD, which was reliable with the theory molecular weight calculated by the Compute pI/Mw program of Bioinformatics Resource Portal ExPASy of Swiss Institute of Bioinformatics (http://web.expasy.org/compute_pi/). And finally, we obtained purified protein chi-rGM-CSF at concentration of 298 µg/mL.

Cell Culture Yield

Non adherent cells were removed from the supernatant before collection of cells, on the 2nd, 4th and 6th day, and cell yield revealed significantly improved in both groups A and B (Fig. 3). There was no significant growth difference between group A and B groups during 6th to 7th day.

Morphological Examination of Cultured BMDCs

Cells obtained from bone marrow were cultured at 1 x 10^6/mL in the presence of chi-rGM-CSF at the concentration of 10 and 20 ng/mL in two different groups, co-cultured with 10 ng/mL *IL-4*. The growth was observed under confocal microscope on day 6th (Fig. 4), cells in group a (supplemented with rGM-CSF only) showed growth but appeared in clusters rather than colonies, whereas cells in group b (supplemented with IL-4 only) contained few dark color floating dead cells and light color viable cells, and, a few cells in group c (containing merely the medium) were survived until 6th day, but cells in group d(supplemented with rGM-CSF and IL-4) aggregated and most of them appeared as colonies, and clustering structures. Moreover, proper growth of cells was noticed using rGM-CSF at 10 ng/mL with two different magnifications (Fig. 4, group e and g), and 20 ng/mL rGM-CSF in group f and h. It was interesting that no significant difference was observed in growth, clustering and colony sizes between groups A and B (10 and 20 ng/mL).

Phenotype of Immature and Mature BMDCs

Chicken bone marrow cells showed their clustering and colonization at 6th day, and on the 7th day their maturation response was observed stimulated with 1 µg/mL LPS. Phenotype analysis revealed (Fig. 5) that after stimulation with LPS for 3 h, many cells remained immature, although few cells with veils appeared as a sign of maturation, and few colonies could be observed at the same time. Cell colonies converted into single round shape with a high yield of mature cells at 6th h, and cells appeared highly dendritic, and few individual round cells were observed and no colony appeared at 12th h.

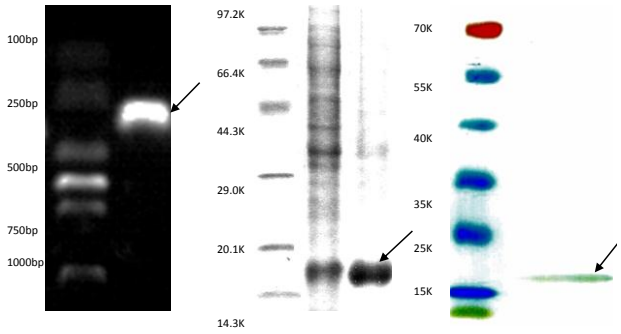

Fig. 2: Cloning, expression and purification of chicken rGM-CSF, A: DNA electrophoresis of PCR (from left Lane1: DNA marker, lane2: rGM-CSF), B: SDS-PAGE of protein expression, C: Western blot analysis of expressed protein (from left Lane1: protein marker, lane2chi- rGM-CSF, Lane3 Western bloting)

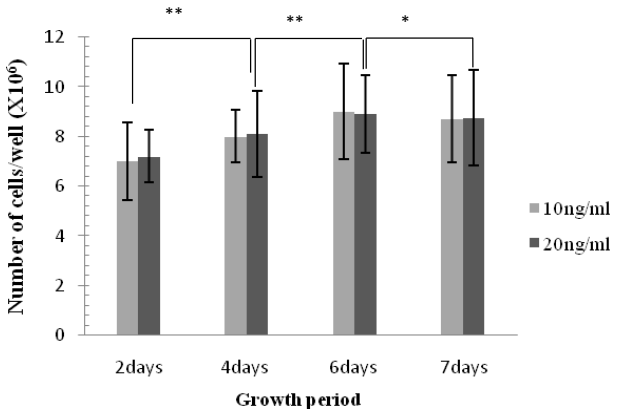

Fig. 3: Showed growth yield, after 48 h intervals at 10 and 20 ng/mL rGM-CSF, till maturation of chicken bone marrow generated denderitic cells, statistical difference in between group,* non-significant and, **(<0.05)

The mRNA Expression Level of Surface Markers Genes

Compared to CK cells, all stimulated cells showed higher mRNA expression of surface markers genes at all the time except for *MHC class II* and *CD 86* at 12th (Fig. 6). The mRNA expression level in group B cells (20 ng/mL) for MHC II at 6th and 12th h, *CD40* at 3rd, 6th and 12thh, *CD80* at 12th h, *CD83* at 6th and 12th h and *CD 86* at 3rd and 6th h was significantly higher than group A (10 ng/mL), respectively. Thus, cells in group B revealed better response to LPS.

The mRNA Expression Level of Inflammatory and Anti Inflammatory Cytokines Genes

Current findings showed (Fig. 7) that, low mRNA expression level of cytokines genes could be detected in non stimulated bone marrow dendritic cells (BMDC) in CK, whereas LPS could stimulate cells to produce higher mRNA expression level in both groups A and B (10 and 20 ng/mL).

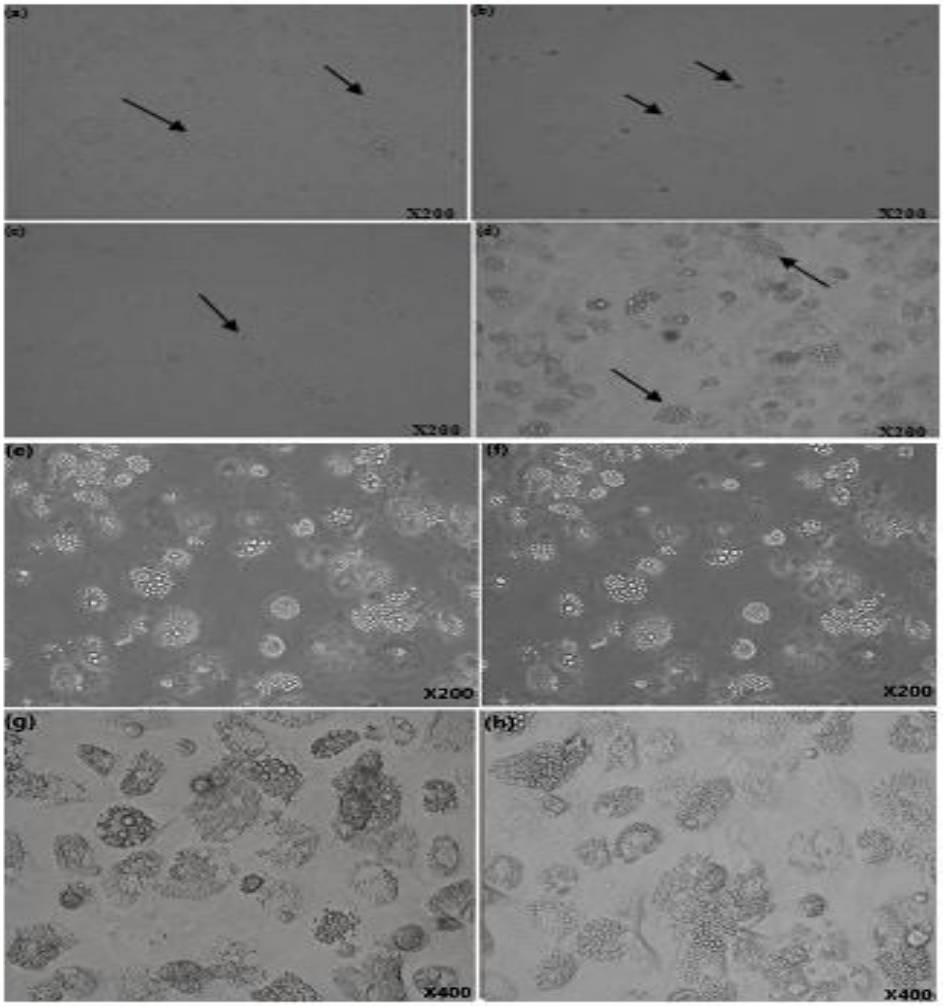

Fig. 4: Morphological view of chicken bone marrow cells cultured for 6 days, (a) Individually chicken recombinant granulocyte macrophage colony-stimulating factor, (b) interleukin-4, (c) medium, (d) combination of rGM-CSF and IL-4 in medium, and aggregation of cells at day 6th of culturing visualized in (e, g) at (10 ng/mL) x200 and x400 magnifications. Clustering of cells and colonies at 20 ng/mL shown in (f, h) at x200 and x400 magnifications, respectively. The data are representative of at least five independent experiments in which dendritic cells (DCs) were cultured

The highest mRNA expression level for *IL-1b gene* at 12th h, for *IL-2* and *IL-6* at 3h and for *INFγ* and anti inflammatory cytokine IL-10 at 6th h were observed. In addition, cells in group B showed significantly higher response to LPS with higher mRNA expression levels of inflammatory and anti inflammatory cytokines genes as compared with group A.

Discussion

During growth of denderitic cells, *rGM-CSF* is used as a growth factor of DCs, either cultured from blood or bone marrow (Inaba *et al*., 1992). *GM-CSF* is a effective cytokine and is capable of inducing proliferation, differentiation and establishment of immune cells. It is facilitating in development of both cellular and humoral immunity. Therefore, in recent years studies on the use of GM-CSF have attracted considerable attention (Warren and Weiner, 2000; Parmiani *et al.*, 2007). *GM-CSF* not only promotes the growth of dendritic cells but also support in their survival, and induces BMDCs differentiation to mobile, reversibly adherent cells with long ramified projections (Markowicz and Engleman, 1990). Our findings revealed that *rGM-CSF* expressed in *E. coli* was biologically functional in the maintenance and differentiation of DCs. The results are consistent with (Wu *et al*., 2012) who reported that rGM-CSF/SS protein expressed in *E.coli* could function as an adjuvant to enhance immunity against somatostatin peptide epitope and promote growth of immunized animals.

The morphology of BMDCs observed in co-cultured medium, containing growth factor *GM-CSF* and *IL-4*, confocal light microscope on 6th and 7th day. The cells were appeared in cluster of colonies and large in size at 6th day. In the present study LPS was used as a DCs stimulator. Mammalian immature DCs can also be induced to mature *in*

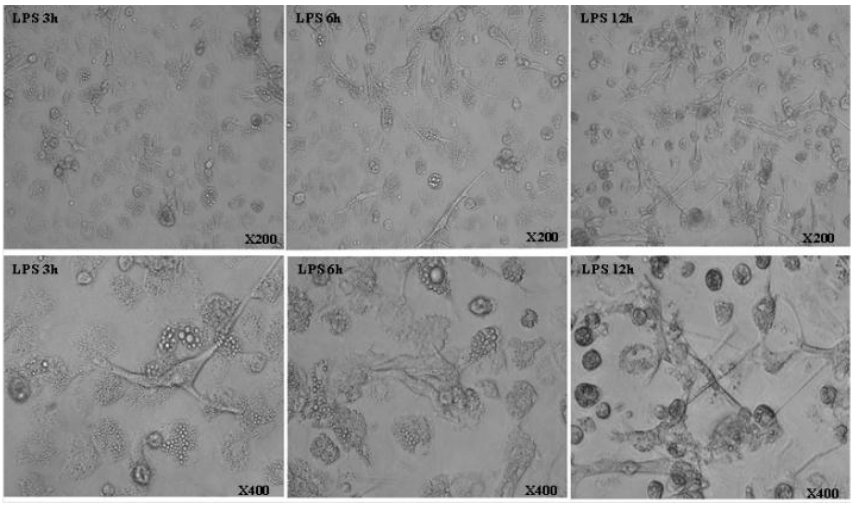

Fig. 5: Phenotype of immature and mature BMDCs stimulated with LPS for 3 h, 6 and 12 h at day 7. Aggregated structure changed, and DCs appeared as mature cells, magnification at x 200, and x400 used with same stimulation to visualize mature dendrite cells. The data are representative of at least five independent experiments in which dendritic cells (DCs) were cultured

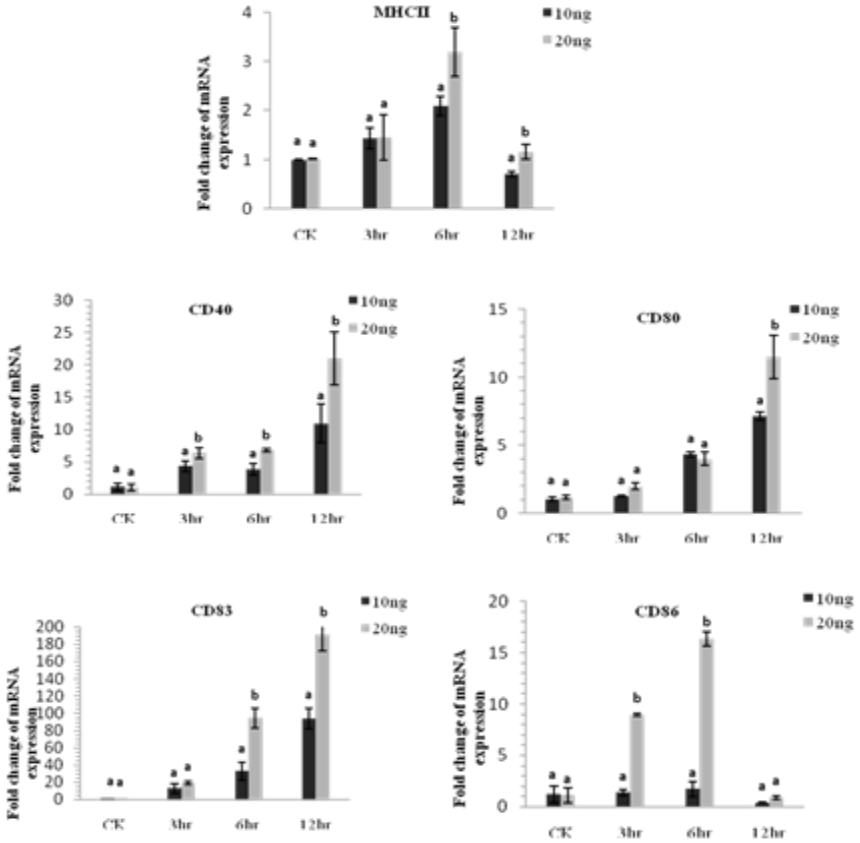

Fig. 6: mRNA expression profiles of MHC class II, CD40, CD80, CD83 and CD86 in non-stimulated chicken bone marrow generated denderitic cells at different time hrs using rGM-CSF (10 ng and 20 ng/mL). Data are expressed as fold change in expression of mRNA levels in surface marker, compared in between two different concentration of rGM-CSF added in medium for growth and biological response of chBMDC, (Comparative CT method was used to determine fold-changes in gene expression, calculated as$2^{\Delta\Delta Ct}$), a, b levels statistically non-significant and significantly different from stimulated two different groups (A and B) in chBMDC respectively; ($P < 0.05$)

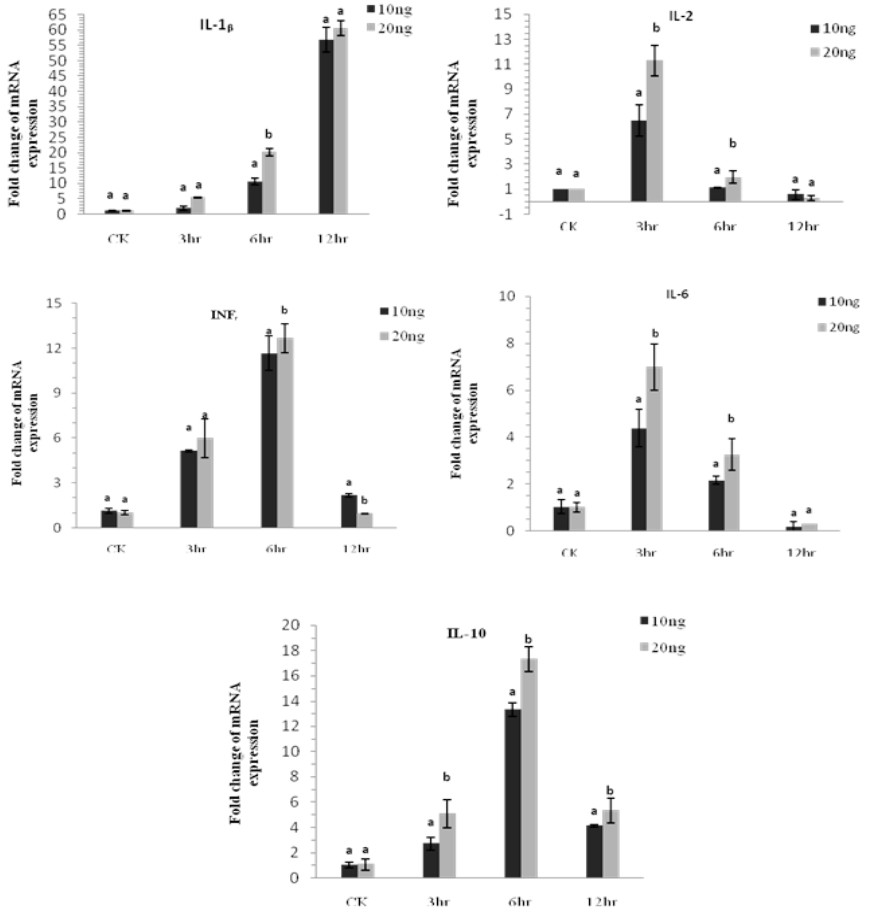

Fig. 7: mRNA expression profiles of inflammatory and anti-inflammatory cytokines genes of chicken bone marrow generated denderitic cells at different times (h) using rGM-CSF (10 and 20 ng/mL). Data are expressed as fold change in cytokine mRNA levels compared between two groups treated with different concentration of rGM-CSF and stimulated with LPS (Comparative CT method was used to determine fold- changes in gene expression, calculated as $2^{\Delta\Delta Ct}$), a, b levels statistically non-significant and significantly different from stimulated two different groups (A and B) in BMDCs, respectively ($P < 0.05$)

vitro with LPS, which is known as strong inducers of DCs maturation (Wurtzen *et al*., 2001; Bai *et al*., 2002). The cells appeared as an adherent with long-branched projections, typical shape of DCs could be seen in (Fig. 5), and where some cells formed larger cell mass with duration of time. Guolong *et al.* (2011) reported that with the prolongation of time, most cells' spindle state appeared retraction phenomenon; DCs could be seen in the culture medium. These phenotypic characteristics have also been observed previously in porcine Mo-DC (Carlos *et al*., 2001), human and mouse hematopoietic progenitor-derived DCs (Thomas *et al*., 1993), all of which have similitude in morphological characteristics in chicken bone marrow derived DCs.

In the previous study (Depaz *et al*., 2003) reported that DCs express high levels of co-stimulatory molecules after stimulation, such as *MHC-II, CD40, CD80, CD83 and CD86*, that can play an important role in functional maturation. The cell surface markers, *MHC class II, CD40, CD80, CD83*, and *CD86* typically expressed on DCs in mammalian species (Banchereau and Steinman, 1998). Cell surface marker mRNA expressions levels (Fig. 6) of *MHC class II, CD40, CD80, CD83*, and *CD86* of immature chicken DCs were found moderate as compared to mature chicken DCs. The results showed significant change in response of MHC-II, *CD40, CD80, CD83* and *CD86* in both groups A and B. The *rGM-CSF* (20 ng/mL) at different time durations had shown highly significant response in group B as compared to group A (10 ng/mL). These results coincide with the results of (Lutz *et al*., 1999), who reported that, immature bone marrow-derived DCs of mammalians showed only moderate mRNA expressions of *MHC class II* molecules. Conversely, mature BMDCs expressed high expression levels of *MHC class II, CD40, CD80* and *CD86* (Lutz *et al*., 2000). Surface marker *CD83* is one of the prime surface marker to determine fully mature DCs (Zhou and Tedder, 1995) and human DCs also express high expression of *CD83* after stimulation (Cao *et al*., 2005). The findings of earlier researcher's demonstrated different CD markers to

confirm maturation of denderitic cells, we applied similar approach to determine chicken DCs, and found resemblance with previous reports of mammalian DCs.

Avian species (chicken) is lacking lymph nodes, therefore antigen presenting dendritic cells play a key role in avian specie's innate and adaptive immunity (Li *et al*., 2010). In the present study, inflammatory cytokines *INF*$_{\gamma}$, *IL-1*$_{\beta}$, *IL-2*, *IL-6* and anti inflammatory cytokine *IL-10*, showed high mRNA expression levels after maturation of denderitic cells. The report manifested that, in mammalians, denderitic cells rapidly produce inflammatory and anti inflammatory cytokines in response to combat the bacteria (Granucci *et al*., 2001). Inflammatory cytokine mRNA expression levles increased at early time points after Gram-negative bacteria stimulation that might be associated with the initiation of immune responses (Granucci *et al*., 2003). The chBM-DCs could be stimulated to mature phenotype and cytokine expression by LPS, as demonstrated by increased Th_1 and Th_2 cytokines expression levels (Zhiguang *et al*., 2010). Our findings showed, chi-rGM-CSF expressed in *E. coli*, was effective to obtain optimal cell culturing, and concentration of chi-rGM-CSF 20 ng/mL induced optimal biological activity and gene expression levels in chicken DCs. Moreover, chi-rGM-CSF 20 ng/mL might be considered as standard unit to obtain optimal response of chicken BMDCs.

In conclusion, for the first time we have cloned GM-CSF gene in *E. coli* and prepared applicable rGM-CSF. Moreover, standard concentration unit has been established to obtain optimal culturing and biological response via maturation and cytokine response to LPS. Future studies will explore mechanism involved in antigen presentation and role of chicken BMDCs in immunity development.

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