Research Note: Development and characterization of monoclonal antibodies specific for chicken interleukin-7 receptor α (CD127)

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ABSTRACT CD127, also named interleukin-7 receptor (**IL-7R**), is expressed on various cell types including naive and memory T cells, and plays a critical role in the differentiation and activation of T lymphocytes. The availability of poultry-specific immune reagents to identify and measure chicken CD127 response will enhance fundamental and applied research in poultry immunology. Mouse monoclonal antibodies (**MAbs**) against chicken CD127 (**chCD127**) were developed and characterized. More specifically, a 678 bp ectodomain of chCD127 gene was cloned in the pET28a (+) vector and expressed in BL21-AI *E. coli* competent cells. The recombinant chCD127 protein with a size of 30 KDa which was also recognized by a mouse anti-human CD127 MAb (Clone G-11) was used to immunize mice, and 6 new mouse MAbs which specifically detected chicken CD127 were developed and characterized. Availability of these new sets of chCD127-specific MAbs will facilitate the immunological studies on CD127 in poultry, especially in understanding effector and memory T immune cell responses in normal and diseased states.

Key words: chicken, IL-7Ra, CD127, monoclonal antibody, thymocyte proliferation inhibition

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INTRODUCTION

Interleukin-7 (**IL-7**), mainly produced by non-hematopoietic stromal cells in bone marrow, thymus, and spleen, is a member of the gamma chain cytokine family (also referred as IL-2 superfamily) which includes IL-2, IL-4, IL-9, IL-15, and IL-21 (Lin et al., 2017). The IL-7 receptor (**IL-7R**) is composed of 2 chains, the IL-7 receptor-α chain (CD127) and a common-γ chain receptor (CD132) with the latter being shared as the common receptor for IL-2, IL-4, IL-9, IL-15, and IL-21 (Ziegler et al., 1995; Mazzucchelli and Durum, 2007). IL-7, upon interaction with its heterodimeric receptor, triggers the downstream signaling transduction of JAK/STAT, PI3K/KT, and MAPK/Erk pathways via the trans-phosphorylation and activation of JAK1 and JAK3, and the phosphorylation of STAT5A and STAT5B (Reth and Nielsen, 2014; Thiant et al., 2016; Abdelsamed et al., 2018). Given the critical role of IL-7 in these signaling pathways, IL-7 can regulate a broad range of cellular functions including cell survival and development, especially for the T cell survival and homeostasis, as well as B cell proliferation and differentiation (Reth and Nielsen, 2014; Zaunders et al., 2014; Majumdar and Nandi, 2018).

CD127, a type I cytokine receptor (McElroy et al., 2012), is expressed on most leukocytes (Mazzucchelli and Durum, 2007), and has been widely used in investigation of the IL-7-mediated differentiation, maturation, and survival of lymphocytes (Hur et al., 2009). Like other interleukin receptors, mammalian CD127, a glycoprotein consisting of multiple asparagine molecules, can self-associate to generate homodimers that are incapable of signaling (Hamming et al., 2012). Mammalian CD127 is essential for the regulation of naive and memory T-cell homeostasis (Vranjkovic et al., 2007). In humans, mutations of CD127 are associated with type I diabetes, colitis, breast cancer, lymphomas, leukemia, and severe combined immunodeficiency (Shochat et al., 2011; Zenatti et al., 2011; Hamming et al., 2012). Although the physiological roles of CD127 in B and T cell development of humans and mice were studied over 10 years ago (Mazzucchelli and Durum, 2007), little is known about the functional activities of CD127 in chickens (van Haarlem et al., 2009).

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Limited availability of poultry-specific immunoreagents hinders progress in fundamental and applied research in poultry species (Ariaans et al., 2008). Most commercially available immunological reagents for mammalian species have limited cross-reactivity with poultry immune molecules due to the evolutionary divergence. Therefore, there is a significant knowledge gap in understanding poultry CD molecules. In this study, we report the cloning and protein expression of IL-7Ra, mouse MAb development to chCD127, and the development of various immunoassays for specific detection of chIL-7Ra in tissues for fundamental and applied immunological studies.

MATERIALS AND METHODS

Molecular Cloning and Gene Expression of Chicken CD127

A set of primers (forward primer 5' – CGGCTAGCATGGAAAGTGGTTGCACCTCAGC AGATGG – 3' and reverse primer 5' – CCGCTCGAGTCACATGCTGCTATAGCTCTCCATG GA – 3') containing restriction enzyme (Nhe I and Xho I, respectively) sites (underlined) were designed to amplify a flanking 678 bp ectodomain of chicken IL-7R gene from spelenocytes of 3-wk-old chickens (Genbank Accession No.: NM_001080106 XM_423732). The amplified chCD127 was cloned into the pET-28a(+) expression vector, and the recombinant plasmids were transformed into *E. coli* strain BL21-AI cells (Thermo Fisher Scientific, Waltham, MA). Protein expression was induced using 0.25 mM isopropyl-β-D-thiogalactopyranoside (IPTG, Sigma-Aldrich, St Louis, MO).

Purification and Characterization of Recombinant Chicken CD127 Protein

Bacterial Growth and Induction Six flasks of 600 mL of LB media (Research Products International Inc, Mt Prospect, IL) with 100 μg/mL of ampicillin (Sigma-Aldrich) were inoculated with freshly grown *E. coli* transformed with chCD127 plasmid and incubated with shaking for 5 to 6 hours (h) at 225 rpm, 37°C till OD_{590} reaching 0.6. IPTG was added to each flask for a final concentration of 0.25 mM and the bacterial culture was incubated at 37°C with shaking at 225 rpm overnight.

Purification of Chicken CD127 Protein The expressed chCD127 protein was purified by affinity chromatography with the Ni-NTA Agarose column (QIAGEN, San Francisco, CA), and its purity was assessed with a specific mouse anti-chCD127 MAbs by Western Blot analysis. Briefly, bacteria were harvested by centrifugation at 15,000× *g* for 10 min at 4°C, and the cell pellet was resuspended with 1× PBS. Samples were sonicated (Sonication power of 200 W. Pulse duration: 10 s on, 10 s off) for 10 min, centrifugated at 15,000× *g* for 20 min at 4°C, and the pellet was collected and resuspended in 8 M Urea at room temperature for over 5 h. Samples were centrifugated at 15,000× *g* for 30 min at 15°C. Ni-NTA resin in PBS buffer was added to the supernatants, and tubes were incubated on a shaker for 1 h. Samples were then centrifugated at 1,100× *g* for 5 min, and resins were collected and washed with 1× PBS, 15 mL of 0.1 M Tris-HCl pH 7.4 and 15 mL of 0.1 M Tris-HCl pH 8.0, respectively. Recombinant chCD127 protein was eluted from the column with 0.25 M Imidazole in PBS and protein concentration was measured using Bradford Reagent after dialysis against 3 changes of distilled water at 4°C (Sigma-Aldrich). The molecular weight and purity of the purified protein were determined by SDS-PAGE and Western Blot analysis.

Characterization of Chicken CD127 Protein The supernatant (**SP**) and inclusion body (**IB**) samples of chCD127 protein were run on SDS-PAGE and transferred onto PVDF membrane (Trans-Blot Turbo Mini PVDF Transfer Packs, Bio-Rad, Hercules, CA). Western Blot analysis was conducted using anti-His Tag MAb (HIS.H8, MA1-21315, Invitrogen, Waltham, MA) and anti-human IL-7R MAb (G-11, sc-514445, Santa Cruz Biotechnology Inc., Dallas, TX), respectively, to determine the specificity or cross-reactivity of chCD127 protein.

Animals One-day-old Ross 708 broiler chicks (Longenecker's Hatchery, Elizabethtown, PA) were housed in Petersime starter brooder units and the 14-day-old chickens were then transferred into large hanging cages. All chicken studies were approved by the Institutional Animal Care and Use Committee at the Beltsville Agricultural Research Center.

Immunization of BALB/c Mice With Purified Chicken CD127 Protein and Development of Anti-chCD127 Monoclonal Antibodies The development of monoclonal antibodies was performed by GenScript Biotech Inc. (Piscataway, NJ). Briefly, 6 BALB/c mice were immunized 3 times with purified chCD127 protein to develop hybridomas secreting MAbs against chCD127. Mouse sera were collected, screened, and characterized by ELISA and Flow Cytometry. Monoclonal antibodies were developed by fusing the spleen cells from the selected mice with SP2/0 cells (Segers et al., 1990). Selected mouse hybridoma cells secreting MAbs against chCD127 were subcloned by limited dilutions and their supernatants were used for further screening and characterization. Twenty-two clones of new anti-CD127 MAbs binding to recombinant chCD127 in ELISA were selected for initial characterization. Two clones (13C9 and 3B8) were used to detect subpopulations of peripheral blood and spleen lymphocytes from 3-wk-old chickens by flow cytometric analysis, whereas 6 clones (1G5, 2A6, 3B8, 9A8, 13C9, and 14B1) were used to block IL7-induced thymocyte proliferation.

Screening of Chicken CD127-Specific Monoclonal Antibodies Mouse sera and hybridoma supernatants containing chCD127 antibodies were screened by indirect ELISA as follows: CD127 antigen (1 μg/mL in 1× PBS) was added to 96-well ELISA plates and incubated at 37°C for 2 h or at 4°C overnight; Plates were washed once and blocked with 200 μL of Blocking Buffer (Thermofisher, Waltham, MA) and incubated at 37°C for 1 h.

After plate washing, 100 μL of mouse antisera or hybridoma culture supernatants were added to each well of the plate for incubation at 37°C for 1 h. Plates were washed 4 times, and 100 μL of diluted HRP-conjugated goat anti-mouse IgG (whole molecule) antibody (Sigma-Aldrich) was added to each well, and incubated at 37°C for 30 min. Plates were washed four times, 100 μL of TMB Reagent (GenScript Biotech) was added to each well and incubated at room temperature for 15 to 20 min; Finally, 100 μL of Stop Solution (2N H_2SO_4) was added to the wells and OD_{450} value was read using a microplate reader (ELx-800, BioteK, Winooski, VT).

Purification and Labeling of Chicken CD127 Monoclonal Antibodies Protein G Agarose (Pierce, Thermo Scientific Inc., Rockford, IL) column containing 1 mL of beads capable of binding 10 to 20 mg antibody was used to pass 1 Liter of hybridoma supernatant. Columns were washed with 100 mM Tris-HCl pH 8.0. The pH of cell culture supernatant was adjusted by adding 1/10 volume of 1.0M Tris-HCl pH 8.0, and passed through the protein G column at a speed of 2 mL/min. Columns were washed with at least 10 column volumes of 100 mM Tris-HCl and then with 10 mM Tris-HCl. Then columns were eluted with 50 mM glycine (pH 3.0), and eluted fractions were collected into 1.5 mL Eppendorf tubes containing 100 μL of 1 M Tris-HCl for immediate neutralization of antibody solution. Bradford reagent was used to monitor eluted protein and SDS-PAGE was used to check the purity of purified antibody. Six out of 22 clones were selected and tested further for their binding capabilities with recombinant chCD127 protein in Western Blot analysis, and the unrelated chicken Granzyme A protein expressed on the same vector was used as a negative control.

Purified chicken CD127 MAbs were labeled with Fluorescein isothiocyanate (**FITC**) Protein Labeling Kit following the manufacturer's instruction (Invitrogen, Eugene, OR). Labeled chicken CD127 MAbs were purified, and their protein concentrations were measured using Bradford Reagent (Sigma-Aldrich).

Immunoglobulin Isotyping of Chicken CD127 Monoclonal Antibodies Immunoglobulin isotyping of chCD127 MAbs was conducted by using a mouse Immunoglobulin Isotyping ELISA Kit (Ready-SET-Go! Kit, Affymetrix eBioscience, Waltham, MA). ELISA plates were coated with 100 μL/well of capture antibody in Coating Buffer and incubated at 4°C overnight. Plates were washed twice and blocked at RT for 2 h. Plates were washed twice and 50 μL of Assay Buffer A (1X) added to all wells. Then 50 μL/well of samples, 50 μL/well of positive control and 50 μL/well of negative control (cell culture medium) were added for incubation at room temperature (**RT**) for 1 h. After plate washing, 100 μL/well of diluted Detection Antibody was added and incubated at RT for 1 h. A 100 μL of Substrate Solution was added to each well after washing the plate. After incubation at room temperature for approximately 15 min, 100 μL of Stop Solution was added to each well. Plates were read at 450 nm and 570 nm, respectively, and the values of 570 nm were subtracted from those of 450 nm for final data analysis.

Expression of Chicken CD127 Protein on Different Tissues To investigate chCD127 expression in chicken lymphoid and non-lymphoid organs, tissue samples were collected from 3-wk-old naïve chickens. Tissue samples (500 mg) were homogenized in 5 mL of cold RIPA buffer (Thermo Fisher Scientific, Waltham, MA) supplied with 10 μL/mL of Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific) on the ice. After centrifugation at 10,000× g for 30 min at 4°C, the extracts of tissues were collected and then loaded onto protein gels (5 μg/lane) for further Western blot analysis, as stated above. Protein samples prepared from different tissues (thymus, spleen, muscle, cecal tonsil, bone marrow, bursa, heart, liver, kidney, jejunum, lung, and brain) were run on SDS-PAGE and transferred onto PVDF membrane (Trans-Blot Turbo Mini PVDF Transfer Packs, Bio-Rad). Western Blot analysis were conducted using purified chCD127 MAbs to detect the expression of chCD127 protein on chicken tissues.

Expression of Chicken CD127 Protein on Leukocytes From Blood and Spleen CD127 protein expression was examined on peripheral blood mononuclear cells (**PBMC**) and splenocytes from broiler chickens at 3 wk of age. PMBC and splenocytes were isolated from chicken blood and spleen, respectively, using Ficoll-Paque Plus (GE Life Sciences Inc., Uppsala, Sweden). Cells were collected and washed in PBS twice at 1,500× g for 10 min. About 1 million cells were suspended in 50 μL of PBS buffer, and stained with 1 μL of Fixable Viability Stain 780 to differentiate live cells from dead cells (BD Biosciences, Franklin Lakes, NJ) for 10 min at 4°C. The cells were washed once and resuspended in 50 μL PBS/1% BSA buffer, followed by addition of 50 μL of 1:50 diluted CD panels to stain for 30 to 45 min at room temperature: Panel 1 for CD4 and CD8a staining: CD3-PB, CD4-PE, CD8a-AF700, CD127(3B8)-FITC or mouse IgG2b-FITC; Panel 2 for B cell staining: Bu1-PE, CD127 (3B8)-FITC or mouse IgG2b-FITC; Panel 3 for monocyte staining: KUL01-PE, CD127(3B8)-FITC or mouse IgG2b-FITC. Except for the CD127 antibodies which were labeled in the lab with FITC conjugation kit according to manufacturer instruction (Thermo Fisher Scientific Inc, Frederick, MD), all other CD reagents specific for chickens were purchased commercially (Sothern Biotech Inc, Birmingham, AL). After staining, the cells were washed once, resuspended in 50 μL PBS/1% BSA buffer, and subjected to analysis in flow cytometry. Cells were acquired on a CytoFlex Flow Cytometer (Beckman Coulter, Brea, CA) and analyzed. Approximately 50,000 cells were evaluated on flow cytometry.

IL-7-Induced Proliferation of Chicken Thymic Lymphocytes by Chicken CD127 MAbs In order to test whether chicken IL-7-induced thymocyte proliferation can be inhibited by chCD127 MAbs, a neutralization experiment with the chCD127 MAbs was conducted in a method described elsewhere (Bucy et al., 1990; Shen et al., 1994). Briefly, chicken thymocytes were prepared from 3-wk-old chickens and seeded into 96-well plates with 1×10^6 cells/well. Increasing amounts (4 ng−1,325 ng/well) of chCD127 MAbs were added to wells, and

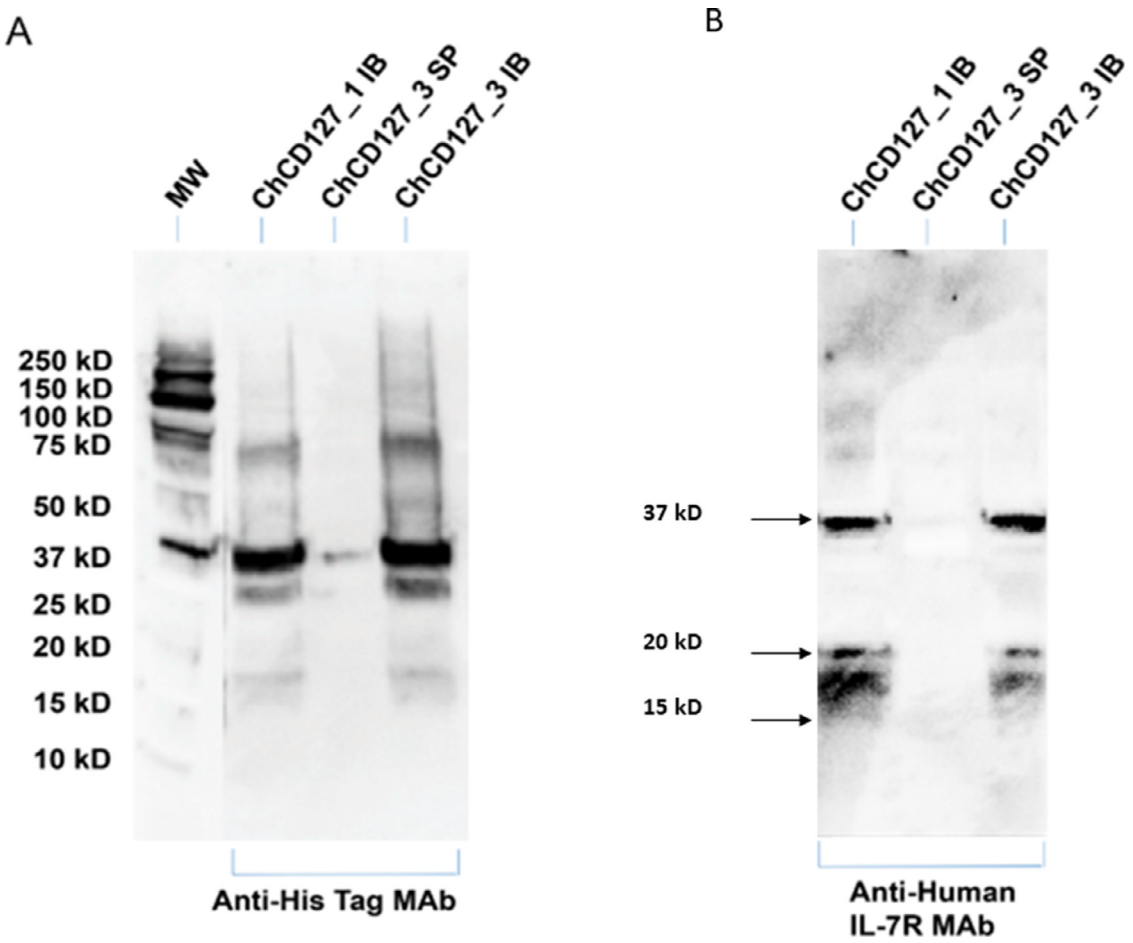

Figure 1. Expression and characterization of recombinant chicken CD127 protein. *E. coli* carrying chCD127 gene was induced with IPTG. Protein samples were processed and separated into different portions, such as portions of supernatant (SP) and inclusion body (IB). Expression of ChCD127 protein was detected and reactivity was confirmed in the portion of inclusion body (IB) but not in the supernatant (SP) by Western Blot analysis using anti-His Tag MAb (A) and anti-human IL-7R MAb (B).

cultured at 41°C for at least 2 h. Chicken IL-7 protein produced in CHO cells (Genscript Biotech) was then added to each well at a concentration of 5 ng/well. Phytohemagglutinin (**PHA**, 10 μg/mL) and Bovine serum albumin (**BSA**, 10 μg/mL) were included as positive and negative controls, respectively. Cells were incubated at 41°C for 72 h and lymphoproliferation was monitored by using CCK8 reagent (Cell Counting Kit − 8, Sigma-Aldrich) and OD_{540} reading was used to determine the inhibitory activity of anti-chCD127 MAbs.

Statistics The OD_{540} values in lymphoproliferation inhibition assay were analyzed using the One-way ANOVA. MAb treatment-group values were compared with that in IL-7 positive control (without addition of any antibodies). Differences were considered statistically significant at $P \leq 0.05$ (*) and $P \leq 0.001$ (***). All the data were expressed as mean ± SD for each treatment in the lymphoproliferation assay.

RESULTS

Molecular Cloning and Gene Expression of Extracellular Region of Chicken IL-7R

A 678 bp extracellular region of chicken IL-7R gene was amplified by PCR with a set of primers containing engineered restriction enzyme (Nhe I and Xho I) sites. After transformation, four positive clones (1, 3, 4, and 8) were identified by colony PCR and by restriction enzyme digestion with Nhe I and Xho I of mini-prep DNA. *E. coli* carrying chCD127 gene was induced in a time- and dose-dependent manner with IPTG (data not shown). The recombinant CD127 protein present in the inclusion body was detected using Western Blot analysis by anti-His Tag MAb (Figure 1A), and also by cross-reactive anti-human CD127 MAb (Figure 1B).

Production and Screening of Monoclonal Antibodies Against Chicken CD127 Protein

Six BALB/c mice were immunized with purified chicken CD127 protein. After immunizations, mouse sera were collected and screened for reactivity with 30 kDa of chCD127 protein expressed in *E. coli*. Twenty-two clones of hybridomas secreting chCD127 MAbs were selected and tested in indirect ELISA, and all were reactive to recombinant chCD127 protein (Figure 2). Six clones (1G5, 2A6, 3B8, 9A8, 13C9, and 14B1) were further selected based on their high binding activity in ELISA (Figure 2) and on

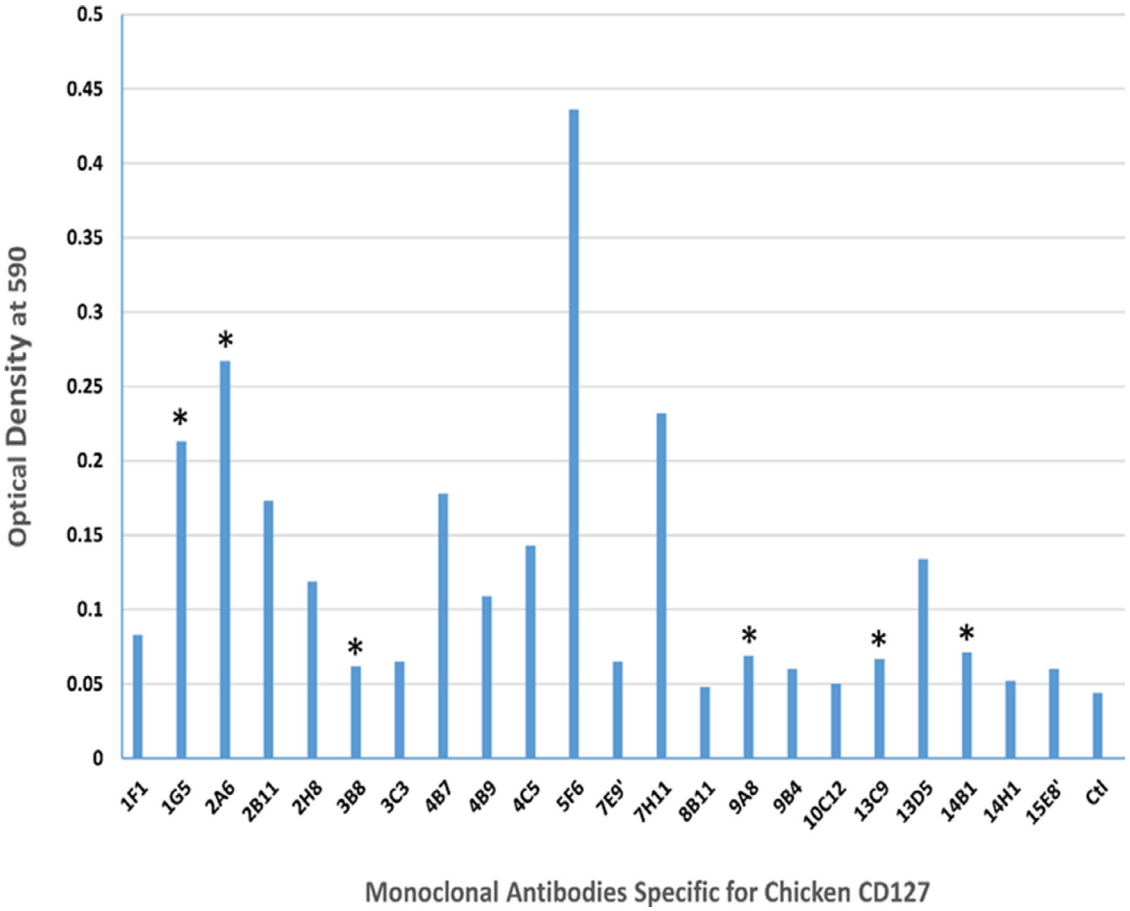

Figure 2. Screening of anti-chCD127 antibodies from chCD127 MAb hybridoma supernatants. Supernatants from ChCD127 MAb hybridoma were collected, and twenty-two clones of hybridomas secreting chCD127 MAbs were tested in indirect ELISA, and all reactive to recombinant chCD127 protein. Six clones (1G5, 2A6, 3B8, 9A8, 13C9, and 14B1) were selected based on their high binding activity in ELISA. Culture Medium was used as a control (ctl).

their binding capability to PBMC and spleen lymphocytes by flow cytometric analyses (data not shown), and for further specificity evaluation in Western Blot analysis.

Immunoglobulin Isotyping of Anti-chCD127 Monoclonal Antibodies

Immunoglobulin isotyping of antibodies which detect chCD127 protein showed that 6 anti-chCD127 MAbs belong to 3 kinds of isotypes, IgG1 (13C9), IgG_{2a} (1G5 and 9A8), and IgG_{2b} (2A6, 3B8, and 14B1) (Table 1).

Table 1. Immunoglobulin isotyping of mouse anti-chicken CD127 monoclonal antibodies.

Clone name	Isotype
1G5	IgG2a, kappa
2A6	IgG2b, kappa
3B8	IgG2b, kappa
9A8	IgG2a, kappa
13C9	IgG1, kappa
14B1	IgG2b, kappa

Specific Interaction of Anti-CD127 Monoclonal Antibodies With Recombinant Chicken CD127 Protein

Six clones (1G5, 2A6, 3B8, 9A8, 13C9, and 14B1) which showed reactivity with chCD127 protein in ELISA were further evaluated by Western Blot analysis using chCD127 protein and unrelated chicken Granzyme A protein expressed in the same vector as a control. Specificities of these MAbs were validated with their specific binding capability to chCD127, but not to an unrelated chicken Granzyme A protein (Figure 3A).

Expression of Chicken CD127 on Different Tissues of Naïve Chicken

Crude tissue extracts from different tissues (thymus, spleen, muscle, cecal tonsil, bone marrow, bursa, heart, liver, kidney, jejunum, lung, and brain) of naïve chicken were prepared and incubated with chCD127 Mab 3B8 in Western blot analysis (Figure 3B). The native chCD127 protein was detected at the high level in tissues like the

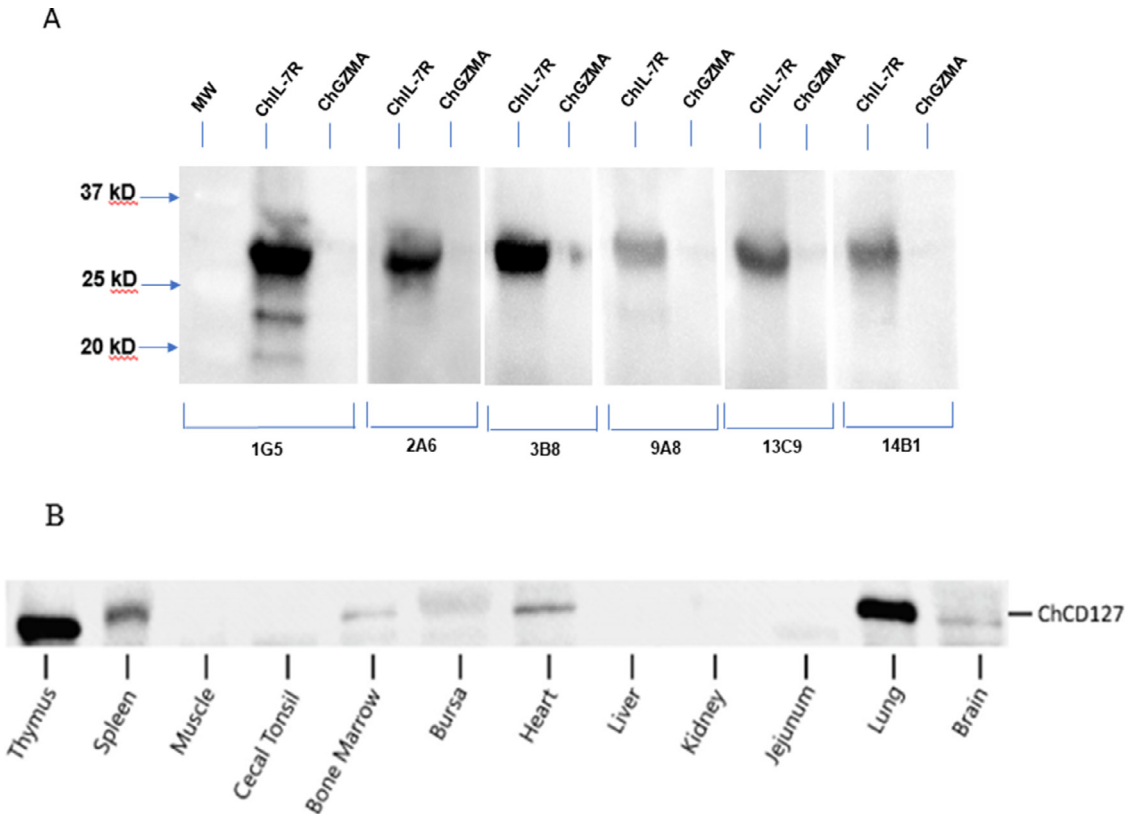

Figure 3. Specific interaction of chCD127 MAbs with chCD127 protein. Specificity of generated ChCD127 MAbs was confirmed by Western Blot analysis using expressed ChCD127 protein and no interaction of these MAbs with unrelated ChGZMA protein was detected (A). Expression of ChCD127 protein was identified in different tissues of naïve chicken (B). Protein samples from different tissues of naïve chicken were prepared, and chCD127 protein was detected by Western blot analysis using purified chCD127 Mab 3B8. Natural chCD127 protein was found to be highly expressed in tissues like thymus, lungs, and spleen, and moderately expressed in heart, brain, bursa, and bone marrow, but no chCD127 protein was detected in muscle, liver, kidney, cecal tonsil and jejunum (B).

thymus, lungs, and spleens, but not expressed in muscle, livers, kidneys, cecal tonsils, and jejunum. Moderate level of CD127 was detected in the crude tissue extracts from heart, brain, bursa, and bone marrow.

Expression of Chicken CD127 Protein on Leukocytes From Blood and Spleen

CD127 protein expression was examined by flow cytometry on PBMC and splenocytes from broiler chickens at 3 wk of age. From 6 different hybridoma clones that we tested, 2 clones (13C9 and 3B8) reacted positively with the PBMCs as well as in spleen lymphocytes. In more details, chCD127 Mab 3B8 strongly reacted to protein expressed on T cells in peripheral blood: 29.15% $CD4^+CD127^+$, 6.64% $CD8^+CD127^+$ (Figure 4A) and in spleen: 10.35% $CD4^+CD127^+$, 6.03% $CD8^+CD127^+$ (Figure 4B), and reacted to protein expressed on B cells in peripheral blood (15.69% Bul^+CD127^+) (Figure 4C) and spleen (4.73% Bul^+CD127^+) (Figure 4D), as well as on monocytes in peripheral blood (52.97% $KUL01^+CD127^+$) (Figure 4E) and spleen (61.43% $KUL01^+CD127^+$) (Figure 4F), by flow cytometry.

Neutralization of IL-7-Induced Thymocyte Proliferation by Anti-chCD127 Monoclonal Antibodies

To test whether chicken thymocyte proliferation induced by chicken IL-7 can be inhibited by anti-chCD127 MAbs, different dilutions of 6 anti-chCD127 MAbs were added to thymocyte proliferation culture. All clones could inhibit lymphocyte proliferation. As shown in Figures 5A−5D, all clones inhibited lymphocyte proliferation, but 4 out of 6 chCD127-specific MAbs showed neutralization capability in a dose-dependent manner (Clones 3B8, 2A6, 9A8, and 14B1).

DISCUSSION

IL-7-induced survival and proliferation of T cells involve Jak/Stat pathways (Swainson et al., 2007), which are triggered following IL-7 binding with IL-7R (Suzuki et al., 2000). T cell survival is maintained by IL-7 which inhibits the mitochondrial apoptotic pathway and regulates the expression of Bcl-2 family of molecules, particularly Bcl-2 and Mcl-1 (Opferman et al., 2003; Dzhagalov et al., 2008).

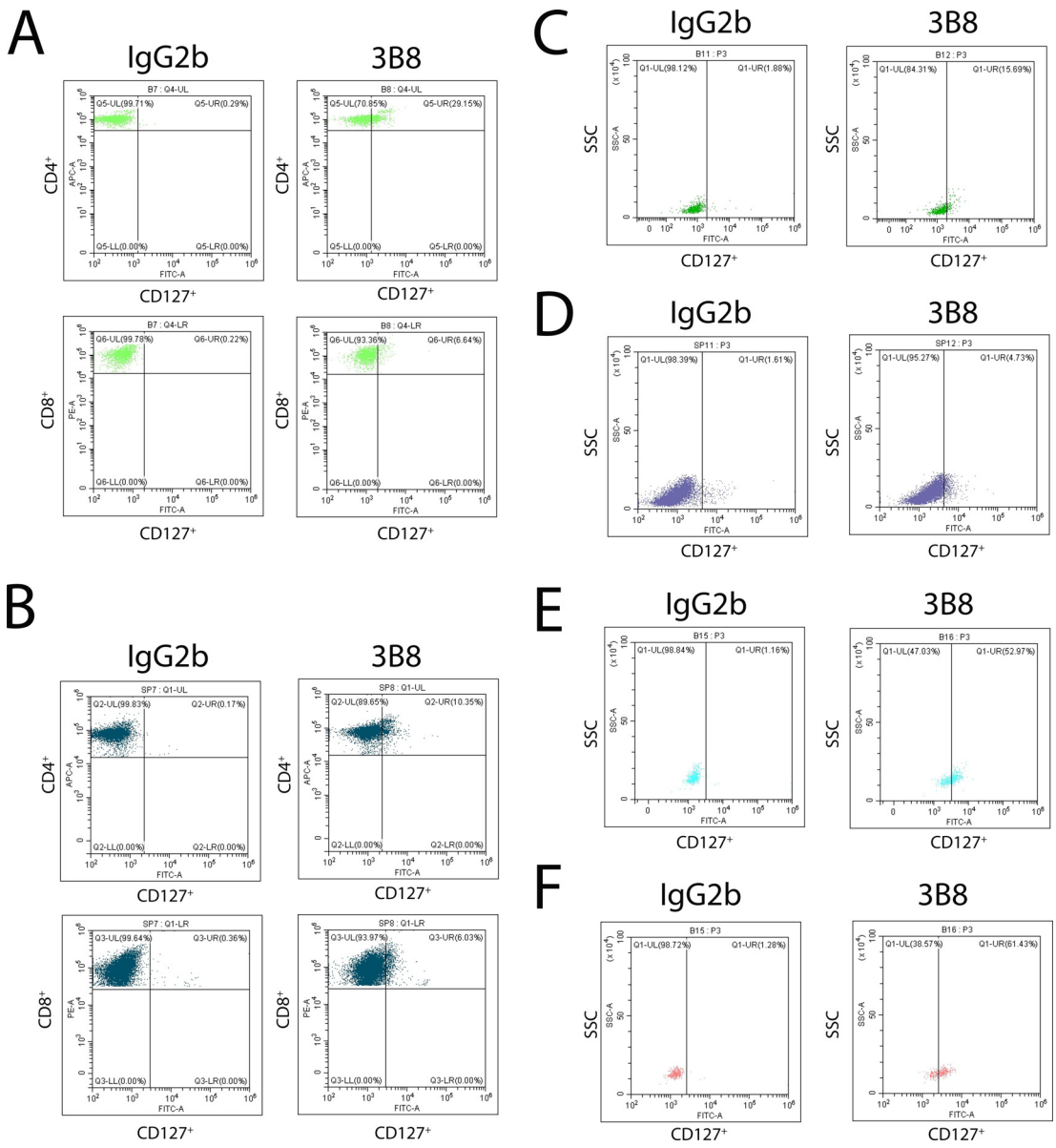

Figure 4. Reaction of chCD127 protein expressed on chicken peripheral mononuclear cells (PBMC) and splenocytes of naïve chicken with chCD127 Mab 3B8. PMBC and single splenocyte were isolated from chicken blood and spleen of broiler chickens at 3 weeks of age and analyzed by flow cytometry using ChCD127 MAbs. ChCD127 protein was found to be expressed on T cells in peripheral blood ($CD4^+CD127^+$, $CD8^+CD127^+$) (A) and spleen ($CD4^+CD127^+$, $CD8^+CD127^+$) (B); it was also identified on B cells in peripheral blood (Bul^+CD127^+) (C); B cells in the spleen (Bul^+CD127^+) (D). In addition, ChCD127 protein was detected on monocytes in peripheral blood ($KUL01^+CD127^+$) (E) as well as in spleen ($KUL01^+CD127^+$) (F).]

CD127 is involved in a signaling pathway to maintain T cell metabolism by regulating glycolysis (Jacobs et al., 2010). However, a better understanding of interaction among IL-7 and the contribution of the Y449 residue of CD127 on GLUT1 expression and glucose uptake is needed (Carrette and Surh, 2012). T cell sensitivity to IL-7 is dictated by the expression level of CD127. Due to the limiting amount of IL-7 availability *in vivo*, the consumption of IL-7 is tightly controlled through IL-7-induced downregulation of CD127 (Park et al., 2004). Upon IL-7 stimulation, CD127 expression on cell surface is downregulated. CD127 regulation occurs at both the transcriptional level as well as the post-translational level (Henriques et al., 2010; Carrette and Surh, 2012). IL-7 signaling can tune the T cells receptor (TCR) sensitivity of $CD8^+$ T cells through the transcriptional regulation of coreceptor CD8 (Park et al., 2007). IL-7 treatment *in vivo* increases CD8 expression on T cells, whereas CD8 expression is decreased in IL-7 deficient mice. Because the expression of CD4 coreceptor is not under IL-7 stimulation, these mechanisms may apply only to CD8, but not CD4 T cells (Park et al., 2007).

IL-7 is an anti-apoptotic cytokine and a crucial survival factor for T cells which is necessary for T cell proliferation, development and homeostasis (Jameson, 2002; Khaled and Durum, 2002), although it is also involved in B cell development. IL-7-mediated homeostasis and antigen-induced selection are the two major processes that govern T cell proliferation and survival (Koenen et al. 2013). The size of the peripheral pool is maintained by homeostasis

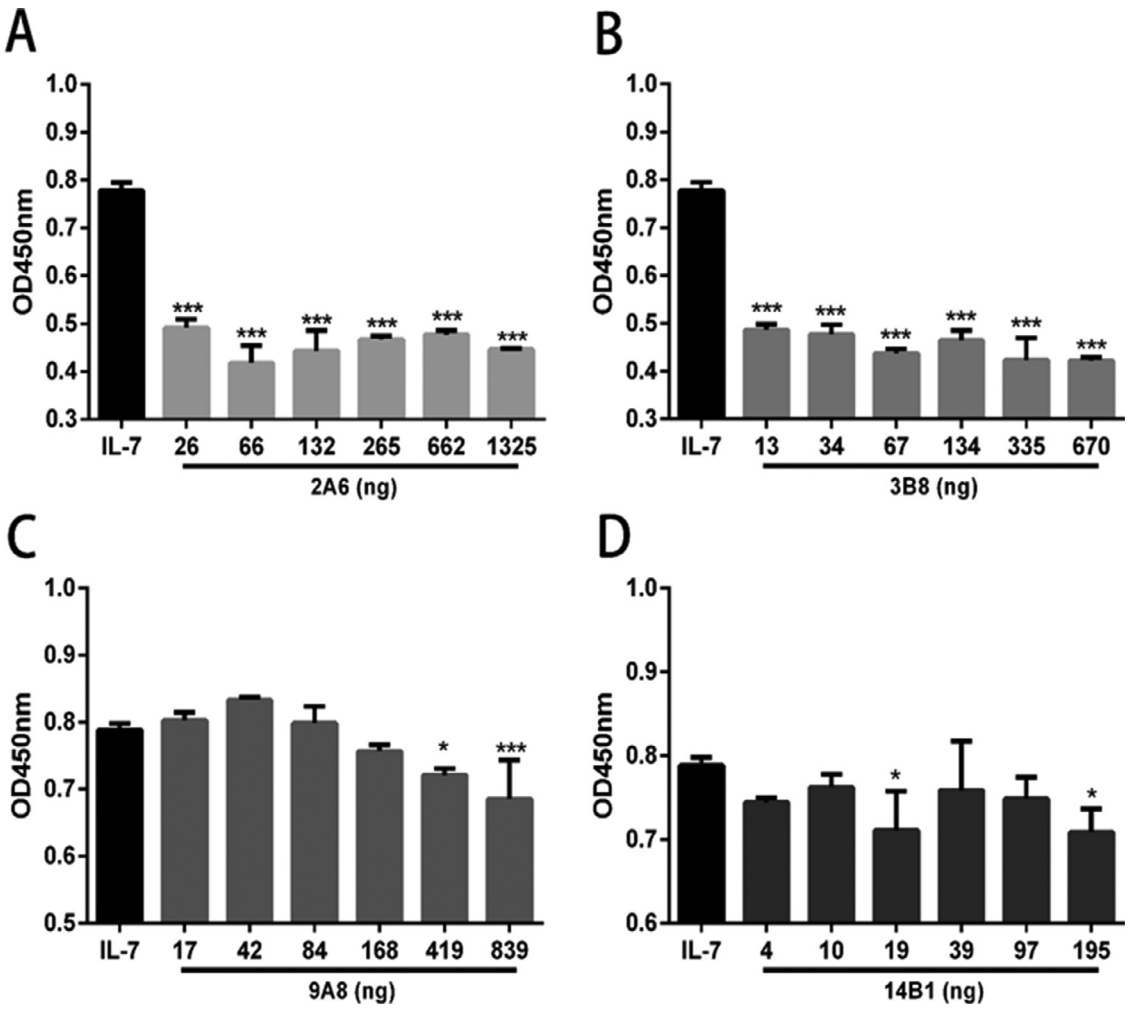

Figure 5. Neutralization of IL-7 induced thymocyte proliferation by anti-chCD127 monoclonal antibodies. In order to test whether chicken IL-7-induced thymocyte proliferation can be inhibited by chCD127 MAbs, chicken thymic lymphocytes were prepared from 3-wk-old chickens, and a neutralization experiment with the chCD127 MAbs was conducted. The data indicated that IL-7-driven chicken thymocyte proliferation was significantly inhibited at a dose-dependent manner by each of anti-chCD127 MAbs added, and proteins expressed on chicken thymic lymphocytes could be neutralized by each of the chicken CD127 MAbs generated (A−D). MAb treatment-group values were compared with that in IL-7 positive control (without addition of any antibodies). Differences were considered statistically significant at $P \leq 0.05$ (*) and $P \leq 0.001$ (***). All the data were expressed as mean ± SD for each treatment in the lymphoproliferation assay.

(Stockinger et al., 2004; Almeida et al., 2005), whereas several rounds of T cell proliferation, differentiation, and cell death are influenced by antigen-receptor activation in response to pathogens (Koenen et al., 2013).

Expression of chIL-7Rα protein was previously reported to be on PBMCs and splenocytes using a monoclonal antibody, 8F10E11, which was mostly expressed on CD8α^{int} cells in peripheral blood lymphocytes (van Haarlem et al., 2009) Interestingly, these cells rarely co-expressed CD8β, indicating that the protein may not be expressed on the surface of these cells, although IL-7Rα mRNA was expressed on CD8β^{+} cells. Furthermore, splenic CD8β^{+} cells did not express chIL-7Rα following stimulation (van Haarlem et al., 2009). In our study, native IL-7Rα protein expression was also - highly detected in the thymus, lung, and spleen, moderately in the brain, heart, bone marrow, and bursa. The high expression levels of IL-7R2 in the lung tissue may be possibly related to the presence of enriched alveolar macrophages in the lungs.

The lack of specific immune reagents available for avian immunology research hinders applied research on host-pathogen immunobiology and vaccine development in poultry (Kaiser et al., 2005). The availability of new CD127-specific MAbs that we described in this paper will help fill the knowledge gap between mammalian and chicken immunology. Like chicken IL-6 and TNF-a (Staeheli et al., 2001; Giansanti et al., 2006), the mouse anti-human CD127 antibody (G-11, sc-514445, Santa Cruz Biotechnology Inc) was found to cross-react with a chicken CD127-like molecule in this study. However, the cross-reactivity between human CD127 and chicken CD127 may be limited. The human CD127 immunogen of the first 215 amino acid deduced protein sequences shared less than 35% homology with the chicken counterpart. Therefore, such cross-reactive antibodies have a limited application for poultry immunology research. Besides, it needs a further test on its specificity to determine if such an anti-human antibody may be expanded to other avian species. We developed mouse anti-avian

CD127 for the characterization of chicken CD127 to better study the interaction between CD127 and IL-7. Furthermore, anti-chicken CD127 mAb could be used for other avian species.

Interestingly, the anti-chCD127 antibodies that showed a strong signal in indirect ELISA did not bind chicken leukocyte antigen well in the Flow Cytometry assay. That may result from the differences in spatial conformation between the prokaryotic recombinant protein used for immunization and the eukaryotic form expressed on chicken leukocytes. Two other clones of MAbs specific for chCD127 (Clones 13C9 and 3B8) were reactive to chicken leukocytes in the Flow Cytometry assay. With the availability of these new MAbs, detailed analysis of the function and relationship of naïve, effector T cells, effector memory, and central memory T cells will be possible

In this study, the results of IL-7-induced lymphocyte proliferation assay demonstrated the functionalities of our MAbs specific for chCD127. Four MAbs (2A6, 3B6, 9A8, 14B1) were able to neutralize CD127 on the thymocytes, and blocked the interaction of IL-7 with CD127, therefore, inhibiting the lymphocyte proliferation.

CONCLUSIONS

In this study, we described the cloning and expression of the chicken CD127 protein, development and characterization of anti-chCD127 monoclonal antibodies. Chicken CD127 protein was expressed in *E. coli* and used to immunize mice to develop mouse hybridomas which secret MAbs specifically binding to chCD127. Six hybridoma cell lines were selected based on their high binding characteristic in ELISA. Anti-chCD127 Mabs were further characterized according to their isotypes, tissue specificity and their abilities to neutralize IL-7-induced thymic lymphocyte proliferation. The IL-7R expression patterns in immune tissues were confirmed by FACS analyses. Together, these results demonstrate that new sets of mouse Mabs detecting chCD127 protein have been produced. They will be critical immune tools for fundamental and applied studies in poultry immunology, especially for studies in effector and memory T cell typing and vaccine development for infectious diseases of poultry. The availability of new monoclonal antibodies specific for molecules of Clusters of Differentiation in poultry may benefit avian immunological research to promote animal health and food animal production.

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Ethics approval and consent to participate: The tissue sampling in the animal study was approved in advance by The Institutional Animal Care and Use Committee of the Beltsville Agriculture Research Center.

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

Abdelsamed, Hossam A., Caitlin C. Zebley, and Ben. Youngblood. 2018. Epigenetic maintenance of acquired gene expression programs during memory CD8 T cell homeostasis. Front. Immunol. 9:6.

Almeida, A. R., B. Rocha, A. A. Freitas, and C. Tanchot. 2005. Homeostasis of T cell numbers: from thymus production to peripheral compartmentalization and the indexation of regulatory T cells. Semin. Immunol. 17:239–249.

Ariaans, M. P., P. M. van de Haar, J. W. Lowenthal, W. van Eden, E. J. Hensen, and L. Vervelde. 2008. ELISPOT and intracellular cytokine staining: novel assays for quantifying T cell responses in the chicken. Dev. Comp. Immunol. 32:1398–1404.

Bucy, R. P., C. H. Chen, and M. D. Cooper. 1990. Ontogeny of T cell receptors in the chicken thymus. J. Immunol. 144:1161–1168.

Carrette, F., and C. D. Surh. 2012. IL-7 signaling and CD127 receptor regulation in the control of T cell homeostasis. Semin. Immunol. 24:209–217.

Dzhagalov, I., A. Dunkle, and Y. W. He. 2008. The anti-apoptotic Bcl-2 family member Mcl-1 promotes T lymphocyte survival at multiple stages. J. Immunol. 181:521–528.

Giansanti, F., M. F. Girardi, and D. Botti. 2006. Avian cytokines - an overview. Curr. Pharm. Des. 12:3083–3099.

Hamming, Ole J., Lishan. Kang, Anders. Svensson, Jesper L. Karlsen, Henrik. Rahbek-Nielsen, Søren R. Paludan, Siv A. Hjorth, Kent. Bondensgaard, and Rune. Hartmann. 2012. Crystal structure of interleukin-21 receptor (IL-21R) bound to IL-21 reveals that sugar chain interacting with WSXWS motif is integral part of IL-21R. J. Biol. Chem. 287:9454–9460.

Henriques, C. M., J. Rino, R. J. Nibbs, G. J. Graham, and J. T. Barata. 2010. IL-7 induces rapid clathrin-mediated internalization and JAK3-dependent degradation of IL-7Ralpha in T cells. Blood 115:3269–3277.

Hur, Y. J., J. G. Kim, K. Y. Whang, and Y. B. Kim. 2009. Molecular cloning and expression analysis of CD127 in pig. Anim. Biotechnol. 20:40–45.

Jacobs, S. R., R. D. Michalek, and J. C. Rathmell. 2010. IL-7 is essential for homeostatic control of T cell metabolism in vivo. J. Immunol. 184:3461–3469.

Jameson, S. C. 2002. Maintaining the norm: T-cell homeostasis. Nat Rev Immunol 2:547–556.

Kaiser, P., T. Y. Poh, L. Rothwell, S. Avery, S. Balu, U. S. Pathania, S. Hughes, M. Goodchild, S. Morrell, M. Watson, N. Bumstead, J. Kaufman, and J. R. Young. 2005. A genomic analysis of chicken cytokines and chemokines. J. Interferon. Cytokine Res. 25:467–484.

Khaled, A. R., and S. K. Durum. 2002. Lymphocide: cytokines and the control of lymphoid homeostasis. Nat. Rev. Immunol. 2:817–830.

Koenen, P., S. Heinzel, E. M. Carrington, L. Happo, W. S. Alexander, J. G. Zhang, M. J. Herold, C. L. Scott, A. M. Lew, A. Strasser, and P. D. Hodgkin. 2013. Mutually exclusive regulation of T cell survival by IL-7R and antigen receptor-induced signals. Nat. Commun. 4:1735.

Lin, Jack., Ziwen. Zhu, Huaping. Xiao, Mark R. Wakefield, Vivi A. Ding, Qian. Bai, and Yujiang. Fang. 2017. The role of IL-7 in immunity and cancer. Anticancer Res. 37:963–967.

Majumdar, S., and D. Nandi. 2018. Thymic atrophy: experimental studies and therapeutic interventions. Scand. J. Immunol. 87:4–14.

Mazzucchelli, R., and S. K. Durum. 2007. Interleukin-7 receptor expression: intelligent design. Nat. Rev. Immunol. 7:144–154.

McElroy, C. A., P. J. Holland, P. Zhao, J. M. Lim, L. Wells, E. Eisenstein, and S. T. Walsh. 2012. Structural reorganization of the interleukin-7 signaling complex. Proc. Natl. Acad. Sci. U. S. A. 109:2503–2508.

Opferman, J. T., A. Letai, C. Beard, M. D. Sorcinelli, C. C. Ong, and S. J. Korsmeyer. 2003. Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1′. Nature 426:671–676.

Park, J. H., S. Adoro, P. J. Lucas, S. D. Sarafova, A. S. Alag, L. L. Doan, B. Erman, X. Liu, W. Ellmeier, R. Bosselut, L. Feigenbaum, and A. Singer. 2007. 'Coreceptor tuning': cytokine signals transcriptionally tailor CD8 coreceptor expression to the self-specificity of the TCR. Nat. Immunol. 8:1049–1059.

Park, J. H., Q. Yu, B. Erman, J. S. Appelbaum, D. Montoya-Durango, H. L. Grimes, and A. Singer. 2004. Suppression of IL7Ralpha transcription by IL-7 and other prosurvival cytokines: a novel mechanism for maximizing IL-7-dependent T cell survival. Immunity 21:289–302.

Reth, M., and P. Nielsen. 2014. Signaling circuits in early B-cell development. Adv. Immunol. 122:129–175.

Segers, R. P., A. De Nijs, P. J. van Kooten, W. Gaastra, and B. A. van der Zeijst. 1990. The use of immunodeficient male (CBA/N x BALB/c) F1 mice to produce monoclonal antibodies directed to proteins of Leptospira interrogans rather than to immunodominant lipopolysaccharides. Hybridoma 9:275–283.

Shen, G. X., X. L. Wang, H. F. Zhu, Y. Zhang, and J. F. Shao. 1994. Experimental study on anti-tumor effect of splenocytes induced by anti-CD3 McAb, PHA and IL-2. J. Tongji Med. Univ. 14:12–15.

Shochat, Chen., Noa. Tal, Obul R. Bandapalli, Chiara. Palmi, Ithamar. Ganmore, Geertruy te. Kronnie, Gunnar. Cario, Giovanni. Cazzaniga, Andreas E. Kulozik, and Martin. Stanulla. 2011. Gain-of-function mutations in interleukin-7 receptor-α (IL7R) in childhood acute lymphoblastic leukemias. J. Exp. Med. 208:901–908.

Staeheli, P., F. Puehler, K. Schneider, T. W. Gobel, and B. Kaspers. 2001. Cytokines of birds: conserved functions—a largely different look. J. Interferon Cytokine Res. 21:993–1010.

Stockinger, B., G. Kassiotis, and C. Bourgeois. 2004. Homeostasis and T cell regulation. Curr. Opin. Immunol. 16:775–779.

Suzuki, K., T. Oida, H. Hamada, O. Hitotsumatsu, M. Watanabe, T. Hibi, H. Yamamoto, E. Kubota, S. Kaminogawa, and H. Ishikawa. 2000. Gut cryptopatches: direct evidence of extrathymic anatomical sites for intestinal T lymphopoiesis. Immunity 13:691–702.

Swainson, L., S. Kinet, C. Mongellaz, M. Sourisseau, T. Henriques, and N. Taylor. 2007. IL-7-induced proliferation of recent thymic emigrants requires activation of the PI3K pathway. Blood 109:1034–1042.

Thiant, Stéphanie., Moutuaata M. Moutuou, Dominique. Leboeuf, and Martin. Guimond. 2016. Homeostatic cytokines in immune reconstitution and graft-versus-host disease. Cytokine 82:24–32.

van Haarlem, D. A., P. J. van Kooten, L. Rothwell, P. Kaiser, and L. Vervelde. 2009. Characterisation and expression analysis of the chicken interleukin-7 receptor alpha chain. Dev. Comp. Immunol 33:1018–1026.

Vranjkovic, A., A. M. Crawley, K. Gee, A. Kumar, and J. B. Angel. 2007. IL-7 decreases IL-7 receptor alpha (CD127) expression and induces the shedding of CD127 by human CD8+ T cells. Int. Immunol. 19:1329–1339.

Zaunders, John J., Yves. Lévy, and Nabila. Seddiki. 2014. Exploiting differential expression of the IL-7 receptor on memory T cells to modulate immune responses. Cytokine Growth Factor Rev. 25:391–401.

Zenatti, Priscila P., Daniel. Ribeiro, Wenqing. Li, Linda. Zuurbier, Milene C. Silva, Maddalena. Paganin, Julia. Tritapoe, Julie A. Hixon, André B. Silveira, and Bruno A. Cardoso. 2011. Oncogenic IL7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia. Nat. Genet. 43:932.

Ziegler, S. E., K. K. Morella, D. Anderson, N. Kumaki, W. J. Leonard, D. Cosman, and H. Baumann. 1995. Reconstitution of a functional interleukin (IL)-7 receptor demonstrates that the IL-2 receptor gamma chain is required for IL-7 signal transduction. Eur. J. Immunol. 25:399–404.