JWPR
Journal of World's Poultry Research
2024, Scienceline Publication
J. World Poult. Res. 14(1): 30-40, 2024
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2024.4
PII: S2322455X2400004-14
ISSN: 2322-455X

Differential Gene Expression Profiling during Avian Immune Organ Development: Insights from Thymus and Bursa of Fabricius Transcriptome Analysis

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Received: November 19, 2023, Revised: December 14, 2023, Accepted: January 16, 2024, Published: March 25, 2024

ABSTRACT

Pluripotent hematopoietic stem cells undergo maturation to regenerate blood cells. T-cell and B-cell maturation from common lymphoid progenitors (CLPs) is regulated by distinct transcription factors and signaling pathways. *Notch* signaling, *GATA3*, and *TCF1* drive T-cell fate, while *EBF1*, *PU.1*, *E2A*, *Pax5*, *TCF1*, and *Foxo1* regulate B-cell differentiation. Alterations in these factors can cause lineage deviations. Gene expression profiling of the chick thymus and bursa reveals dynamic expression patterns of transcription factors, cytokines, and signal molecules. *Notch1* and *Dll1* expression that increase during later stages indicate the ongoing role of *Notch* signaling in T-cell lineage maintenance. The current study aimed to identify differentially expressed genes during the development of the avian immune organs, focusing on the thymus and bursa using 24 Ross 308 avian breed. The mRNA libraries from these organs were analyzed using quantitative Real-time PCR analysis at six time points spanning the embryonic ages (days 15 and 18) and post-hatch age (days 3, 7, 14, and 28). The data for the gene expression indicated significant variations across different stages of immune organ development. Differential gene expression was observed between sorted T and B-cells, with *GATA3*, *CD3e*, *CD4*, and *Ptprc* showing higher expression in the T-cell population, and *Pax5* and *CD81* exhibiting higher expression in the B-cell population. Notably, *ENO1* and *IRF4* showed higher expression in T-cells at E15 and B-cells at E18. The study highlights the importance of regulatory factors and genes in maintaining cellular identity, furthers the understanding of avian immunology, and has the potential for improving poultry health and studying immune-related diseases in humans. These findings pave the way for further research on the role of biochemical components under important disease conditions in avian immunology and their potential applications for poultry health.

Keywords: Avian lymphocyte, B-cell maturation, Gene expression, Regulatory elements, T-cell

INTRODUCTION

Pluripotent hematopoietic stem cells (HSCs) undergo consecutive rounds of biochemical development and maturation during differentiation to regenerate and self-maintain all types of blood cells (Ogawa, 1993). These HSCs give rise to multipotent hematopoietic progenitors (MPPs), which then undergo lineage commitment, resulting in the formation of either common lymphoid progenitors (CLP) or common myeloid progenitors (CMP; Kondo et al., 1997; Akashi et al., 2000). The progression of CLPs toward a specific lymphoid lineage, either T-cell or B-cell, is highly regulated by a network of biochemical transcription factors that have been well established (Naito et al., 2011; Thompson and Zúñiga-Pflücker, 2011; Boller and Grosschedl, 2014; Rothenberg, 2014). As both B-cells and T-cells are initially generated from CLP, they have highly similar receptor structures, progression regulation, and pathways across different stages of development (Borst et al., 1996; Han et al., 2023).

Despite the similarities between T-cell and B-cell maturation, the differentiation and proliferation of precursor cells toward either a mature T-cell or B-cell are coupled to extensive and strictly regulated pathways

across different stages of development (Figure 1). This ensures the expansion of specific cell populations that have passed all the biochemical checkpoints required for expressing a specific repertoire of the properly selected receptor. For instance, in the case of T-cells, several biochemical transcription factors mediate the commitment of progenitor cells toward a T-cell fate (Busslinger, 2004; Rothenberg et al., 2010; Yang et al., 2010; Naito et al., 2011). The *Notch* signaling pathway is a crucial signal for the initiation of the T-cell generation pathway and differentiation in the early stages of T-cell development (Radtke et al., 1999; Sambandam et al., 2005). *GATA3* (a double zinc finger) and *TCF1* (T-cell factor 1) are important biochemical transcription factors required for determining T-cell fate and have been identified as downstream factors of *Notch1* in nearly all stages of early T cell progenitor lineage (Figure 1, Hozumi et al., 2008; Weber et al., 2011).

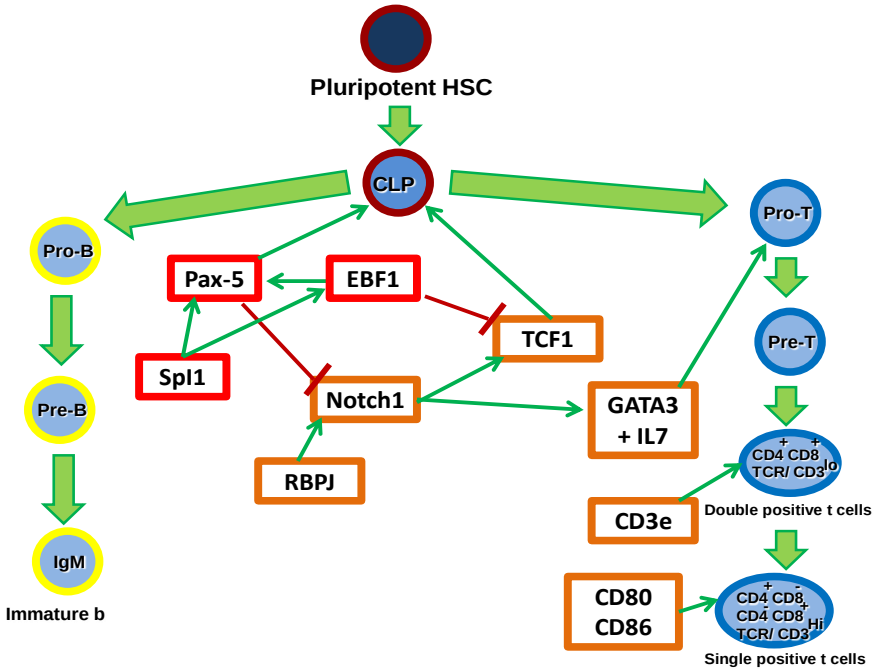

Figure 1. The pluripotent hematopoietic stem cells (HSCs) toward common lymphoid progenitor (CLP) and lymphocyte lineage commitment through the regulation of mRNA networks and pathway

In the case of B-cells, a network of transcription factors, including *EBF1*, *PU.1*, *E2A*, *Pax5*, *TCF1*, and *Foxo1*, regulates the differentiation of progenitor cells into pro-B-cells (Decker et al., 2009; Medvedovic et al., 2011; Boller and Grosschedl, 2014) as well as the rearrangement of immunoglobulin heavy chain loci in B-cells (Liu et al., 2003; Dengler et al., 2008). *Pax5* and *EBF1* play crucial roles in suppressing the T-cell lineage genes *Notch1* and *GATA3*, respectively, which are the key biochemical regulatory factors contributing to T-cell fate commitment (Delogu et al., 2006; Nechanitzky et al., 2013; Delpoux et al., 2021). Conditional knockout of *Pax5* in mature B-cells leads to their conversion into a T-cell-like state, indicating the importance of *Pax5* in maintaining B-cell identity (Cobaleda et al., 2007).

In pro-B-cells, *Pax5* deficiency can also lead to the deviation of B-cell lineage toward alternative lineage commitments, such as myeloid cell, dendritic cell, or even T-cell fates under the influence of other lineage gene signals (Nutt et al., 1999; Rolink et al., 1999; Mikkola et al., 2002). Moreover, *EBF1* is a key mediator of B lymphopoiesis (Lin and Grosschedl, 1995; Zandi et al., 2008). It has been shown to rescue B-cell fate commitment even in the absence of *E2A* or *Pax5* in knockout mice (Seet et al., 2004; Zandi et al., 2012). *EBF1* can also restore the activity of the Pu.1 transcription factor in Pu.1-deficient multipotent progenitor cells, further confirming its importance in the differentiation of progenitor cells into B-cells (Medina et al., 2004). Several studies have shown that a positive feedback loop exists between *Pax5* and *EBF1*, revealing the importance of *Pax5* in maintaining *EBF1* levels for the proper regulation of B-cell commitment of progenitor cells (Roessler et al., 2007; Decker et al., 2009; Haniuda et al., 2020).

A combination of transcription factor networks orchestrates gene expression in different types of cells to maintain their identity throughout the sequential signals of activation and repression. Consequently, determining an extensive view of gene expression will aid the elucidation of different genes' biochemical mechanisms and levels during immune cell development. Biochemical gene expression profiling was conducted to characterize avian immune lymphocyte development using the two primary immune tissues (the thymus and bursa of Fabricius) across different stages of chick development.

The current study aimed to comprehensively investigate the regulatory mechanisms and biochemical pathways governing the differentiation and maturation of pluripotent HSCs into various blood cell lineages and their subsequent commitment to T-cell and B-cell lineages. Through a profile analysis of the network of transcription factors involved in this process, to enhance the understanding of the molecular events that underlie avian immune cell development.

MATERIALS AND METHODS

Ethical approval

This study was performed and carried out following the Faculty of Agriculture, Cairo University, Committee of Animal and Poultry Production welfare treatment, and complied with relevant legislation of the Ministry of Agriculture in Egypt on animal ethics and welfare (Decree No. 27 (1967).

Animals

A total of 24 Ross 308 broiler chickens were used in this study from February to March 2020. All experiments were carried out following the Faculty of Agriculture Cairo University's intuitional approved protocols. The thymus and bursa of Fabricius were collected from the sacrifice of four chickens at each of the six crucial time points during the development of T- and B-cells maturation across two embryonic ages (days 15 and 18) and four post-hatches days (days 3, 7, 14, and 28), snap frozen in liquid nitrogen, and stored at -80°C until further analysis. T- and B-cells were further isolated from six Ross 308 embryos at 15 and 18 days of embryonic development to validate the gene expression in the sorted cells. The thymus and bursa of Fabricius were collected at two-time intervals, three at 15 embryonic days (E15) and the other three at 18 embryonic days (E18). Both the thymus and bursa of Fabricius were directly used for cell isolation to further assess the gene expression in the sorted cells compared to the whole organs for the Ross 308 avian breed thymus and burse.

RNA isolation and mRNA library construction

Total RNA was isolated from the 24 thymus and 24 bursa of Fabricius samples. The 6 (3 E15 and 3 E18) sorted B-cell, and the 6 (3 E15 and 3 E18) sorted T-cells samples were processed using TRI Reagent® RNA Isolation Reagent (Sigma, St. Louis, MO, USA) according to the manufacturer's instructions, then subjected to a DNase treatment using a TURBO DNA-free™ Kit (Life Technologies, USA). RNA quality was assessed with an RNA 6000 Nano Assay kit and the Model 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). An RNA integrity number (RIN) of greater than 9.0 was observed for all samples.

Semi-quantitative real-time PCR analysis for the selected mRNAs

First-strand complementary DNA (cDNA) was synthesized from 1 µg of DNaseI-treated total RNA from 24 thymus samples and 24 bursa of Fabricius samples as well as 12 samples of sorted T- and B-cells samples (6 samples from each cell type) using a Miscript II RT kit (Qiagen) following the manufacturer's instructions. Real-time PCR reactions were carried out in duplicate using the primer sets in Table 1 (Ye et al., 2012).

Table 1. Semi-quantitative real-time PCR primers for mRNA expression generated using Primer-BLAST developed at NCBI for thymus and bursa of Fabricius of broiler chickens

Gene Name	Primer Sequence
GATA3-F	TCTACTACAAGCTGCACAATA
GATA3-R	TCTCATTTTGAGACCGTAAG
Pax5-F	TCCTGTTTCCTTCCACCGAC
Pax5-R	AGCTGCCTGGAGATGTCGCA
ENO1-F	CGGAGCGGTGTTCAAGATGT
ENO1-R	CCCAAGTACAGGTCAGCCAG
Cd3e-F	ATCCACCCCATAGCCCTTCT
Cd3e-R	TGAAACGGCACCAGCAAATG
Cxcr4-F	CCCTTGCGTTCTTCCATTGC
Cxcr4-R	AACCACTTGTCCACAGGACC
Cd4-F	TGGAACCTGGATGTGTCACG
Cd4-R	AACATGAGCTTCCTCCACGG
IRF4-F	ATCCCCCTACCTGGAAGACC
IRF4-R	CGGGGCAAATTCTCTCCAGT
Ptprc-F	CTTCTCTGCTGGAGGCGAAA
Ptprc-R	CAAAGGTGGAGACCACTCCC
Cd81-F	AGCTGGAATCTGGGGGTTTG
Cd81-R	TCCATCTCCTCGGGACACAT

For mRNA expression analysis, each reaction contained 10 ng of cDNA, 500 nM of the forward primer, and 1× iQ SYBR green supermix (Bio-Rad, Hercules, CA, USA). PCR was performed using the cycling conditions of 95°C for 15 minutes followed by 40 cycles of (95°C for 10 seconds and 60°C for 20 seconds) using a MyiQ Real-Time PCR detection System (Bio-Rad, USA). Melting curve analysis was assessed to determine amplification specificity for each mRNA. Normalization was conducted using the expression of the GAPDH housekeeping gene (Huitorel and Pantaloni. 1985). The threshold cycle (Ct) values were transformed to a relative expression in arbitrary units by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Isolation of T- and B-cells

T and B lymphocytes were purified from the whole thymus and bursa of Fabricius, respectively, from three embryos at two embryonic ages (E15 and E18). Mouse anti-chicken CD3 and mouse anti-chicken Bu-1 antibodies (Southern Biotech, USA) were used for sorting T-cells and B-cells, respectively. Tissues were mechanically disrupted, passed through a 70µm nylon mesh cell strainer (BD Biosciences, USA), and washed three times with phosphate-buffered saline (PBS). The isolated cells were mixed with 1µg/ml anti-CD3-conjugated phycoerythrin (PE) and anti-Bu1a-conjugated Fluorescein Isothiocyanate (FITC), respectively, and incubated for 15 minutes at room temperature. An Easysep positive selection kit (Stemcell Technologies, USA) was used to isolate T-cells and B-cells according to the manufacturer's protocol. Briefly, 100µl/ml of the Easysep fluorescein isothiocyanate (FITC) or phycoerythrin (PE) positive selection cocktail was added to antibody-bound cells and incubated for 15 minutes at room temperature. Then, 50 µl/ml of the magnetic nanoparticles' beads were mixed with the cells and incubated for an additional 10 minutes. The tube was placed on the Easysep magnet for 5 minutes. Negative cells were aspirated, and the remaining cells were washed three times with a washing buffer and resuspended in a resuspension buffer (Stemcell Technologies, USA) with 1% FBS. A group of unsorted cells was treated with 1µg/ml of the isotype negative control IgG1 conjugated with either FITC or PE to set the gates accordingly.

Flow cytometric analysis

Phenotyping of the CD3+ or Bu1a+ cells was detected and sorted using a Beckman Coulter flow cytometry machine (USA), and Cell Lab Quanta SC analysis was utilized to assess the sorted cell population using an electronic volume of 2.60 and 7.20 side scatter value.

Statistical analysis

Relative fold change in expression and significant differences across development stages and between tissues were determined using Student's t-test (SPSS version 26, USA).

RESULTS

Functional and pathway analysis for the selected mRNAs

To provide a further assessment of the potential roles of these differentially expressed mRNAs across development in the avian immune organs, functional analysis using the DAVID 6.7 algorithm (Huang et al., 2008) was performed (Table 2). CD81, PrKcd, ENO1, IFNAR1, and IRF4 were identified as potential genes involved in B-cell proliferation, while CD4, CD3e, Cxcr4, and IL2ra were identified as T-cell differentiation and proliferation genes. Following a study by Franceschini et al. (2013), a group of these genes known to be involved in the development, proliferation, and/or differentiation of either T-cells or B-cells was selected for further analysis using the STRING v9.1 database (Figure 6). This analysis revealed a direct interaction between *EBF1* and *Pax5*, with both being actively regulated by PU.1 (SPI1), a member of the ETS family of transcription factors. SPI1 could also repress GATA3 expression. In turn, IRF4 could directly affect the expression of SPI1. Cxcr4, Ptprc, CD44, and CD3e were found to interact with CD4 which can activate their expression.

Table 2. mRNA functional annotation (DAVID) of the selected gene expressed in thymus and bursa of Fabricius development of broiler chickens

Functional classification	Gene symbol	Gene description
B-Cell Proliferation	CD81	Target Of Antiproliferative Antibody
	Prkcd	Protein kinase C, delta
	ENO1	Enolase 1, Alpha
	IFNAR1	interferon (alpha, beta, and omega) receptor 1
	SOCS3	Suppressor Of Cytokine Signaling 3
	IRF-4	Interferon Regulatory Factor 4
T-Cell Differentiation	CD4	membrane glycoprotein of T lymphocytes
	Nos2	Nitric Oxide Synthase 2, Inducible
	CD3e	CD3e molecule, epsilon (CD3-TCR complex)
T-Cell Proliferation	Cxcr4	Chemokine (C-X-C motif) receptor 4
	Il2ra	Interleukin 2 Receptor, Alpha

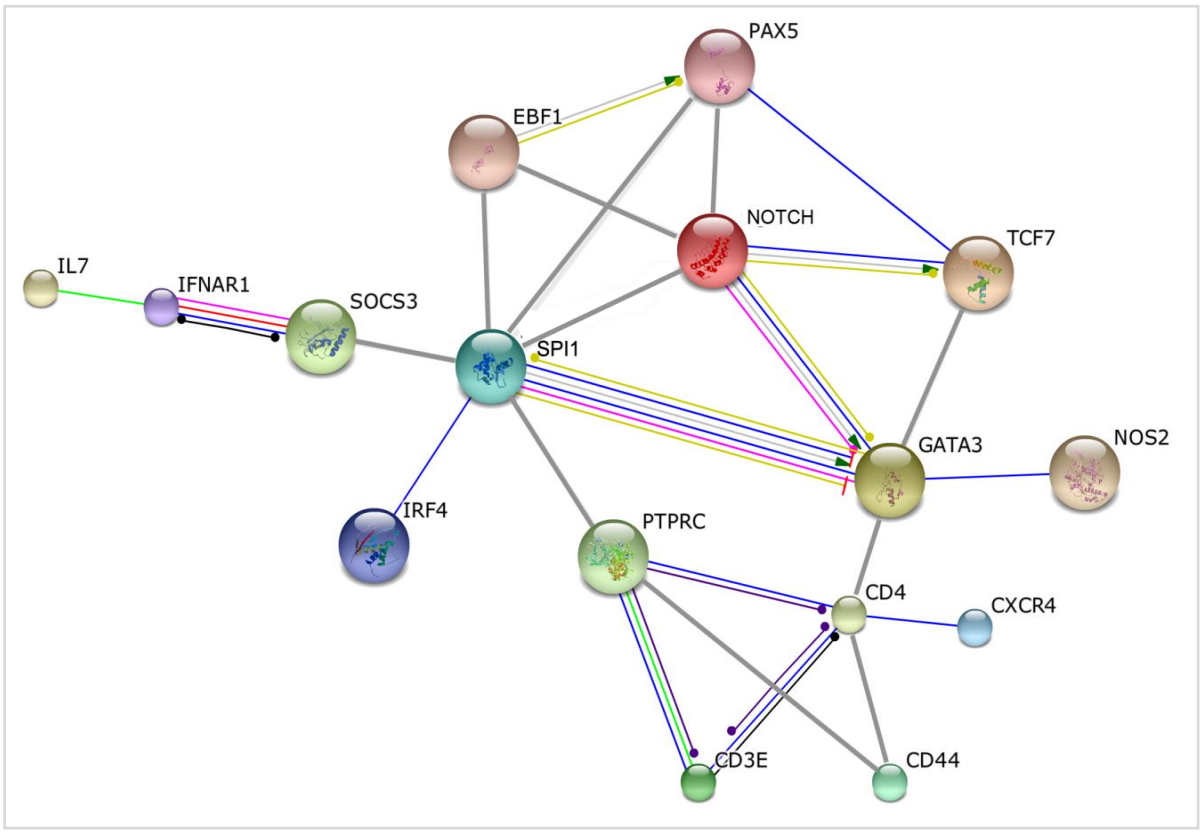

Figure 2. Pathway analysis of genes influencing the differentiation and proliferation of T-cells and B-cells in broiler chickens

Sorting B-cells and T-cells

T-cells and B-cells were purified from the thymus and bursa of Fabricius, respectively, using the EasySep system (Stemcell Technologies, USA) for subsequent gene expression analysis. In the sorted B cells, approximately 81.27% and 83.58% of the B-cell population expressing the Bu-1a surface marker were successfully recovered from the E15 and E18 in the bursa of Fabricius tissues, respectively (Figure 3). Similarly, approximately 77.45% and 74.36% of the cells expressing *CD3e* surface marker were successfully harvested from the E15 and E18 in thymus tissue, respectively (Figure 4).

Differences in gene expression in purified T-cell and B-cell populations

The expression of a selected set of differentially expressed genes was further assessed in sorted T-cells and B-cells, using bio rad real-time PCR (USA, RT-PCR) analysis (Figure 5). The expression of *GATA3*, *CD3e*, *CD4*, and *Ptprc* was high in the T-cell population at both E15 and E18, compared to B-cells at the same time points. Both *Pax5* and *CD81* were expressed more in B-cells, compared to T-cells. *ENO1* and *IRF4* were expressed more with a ~2 fold and a ~5 fold increase, respectively, at E15 in T-cells compared to B-cells, while at E18, their expression increased with ~3 and ~7 fold changes in B-cells, compared to T-cells. The semi-quantitative real-time PCR analysis was conducted. Ross 308 broiler chickens were used to study the thymus and the bursa of Fabricius across two embryonic ages (E15 and E18) and four post-hatch ages (D3, D7, D14, and D28). The results of functional gene category annotations revealed dynamic expression patterns of crucial biochemical transcription factors, cytokines, chemokines, cell surface markers, and signal molecules during chicken thymus and bursa of Fabricius development, reflecting the dynamic changes occurring in cell populations. Notably, the expression of the *Notch1* receptor and its ligand Delta-like1 (*Dll1*) showed an increasing trend in both tissues at later stages, suggesting the continuous requirement of the *Notch* signaling pathway for maintaining the T-cell lineage during chick development. The observed changes in the expression of various biochemical genes during different stages of avian immune development will provide insights into the underlying molecular processes and regulatory networks involved in immune cell maturation and differentiation.

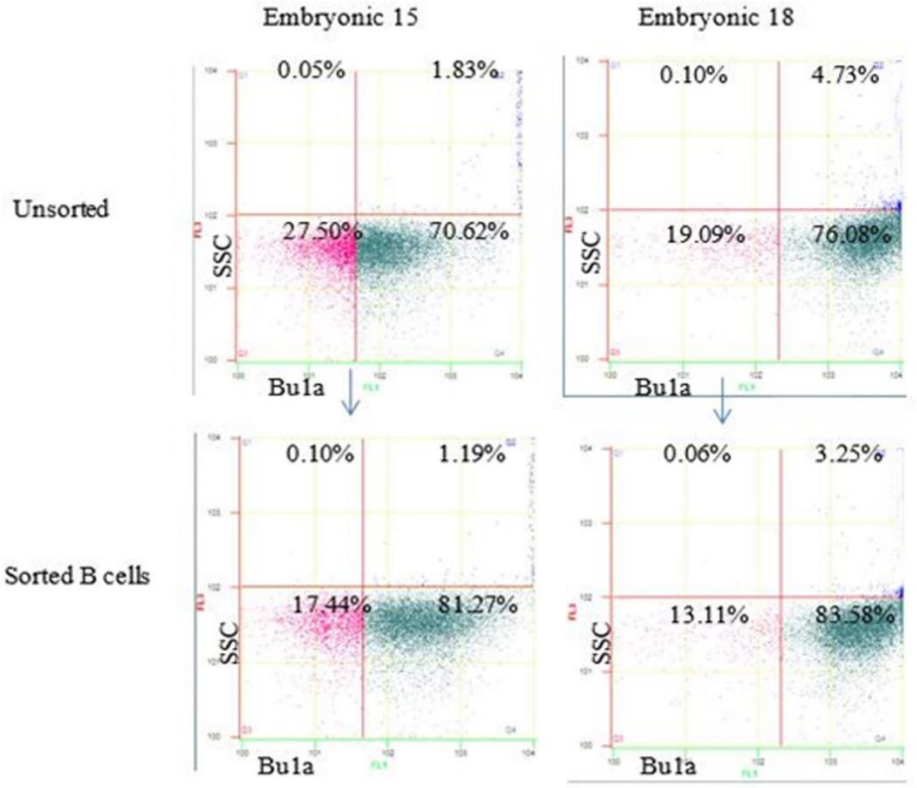

Figure 3. Flow cytometry analysis of sorted B-cells from whole bursa of Fabricius of broiler chickens using stem cell technology, magnetic beads and Bu-1a conjugated FITC antibody at two embryonic stages (E15 and E18)

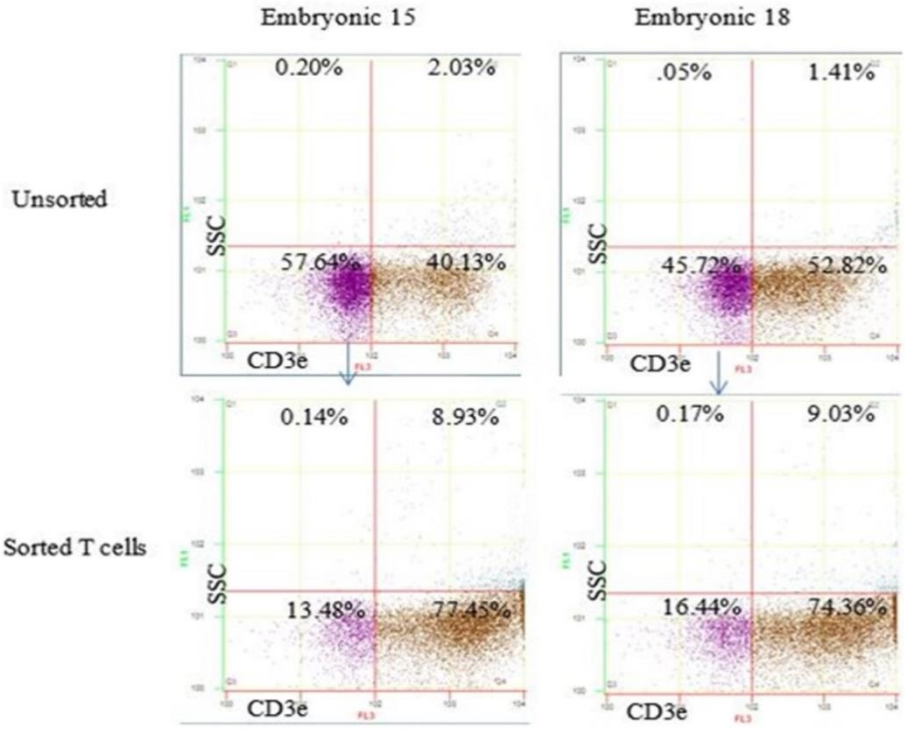

Figure 4. Flow cytometry analysis of sorted T-cells from the whole thymus of broiler chickens using stem cell technology magnetic beads and CD3 conjugated PE antibody at two embryonic stages (E15 and E18)

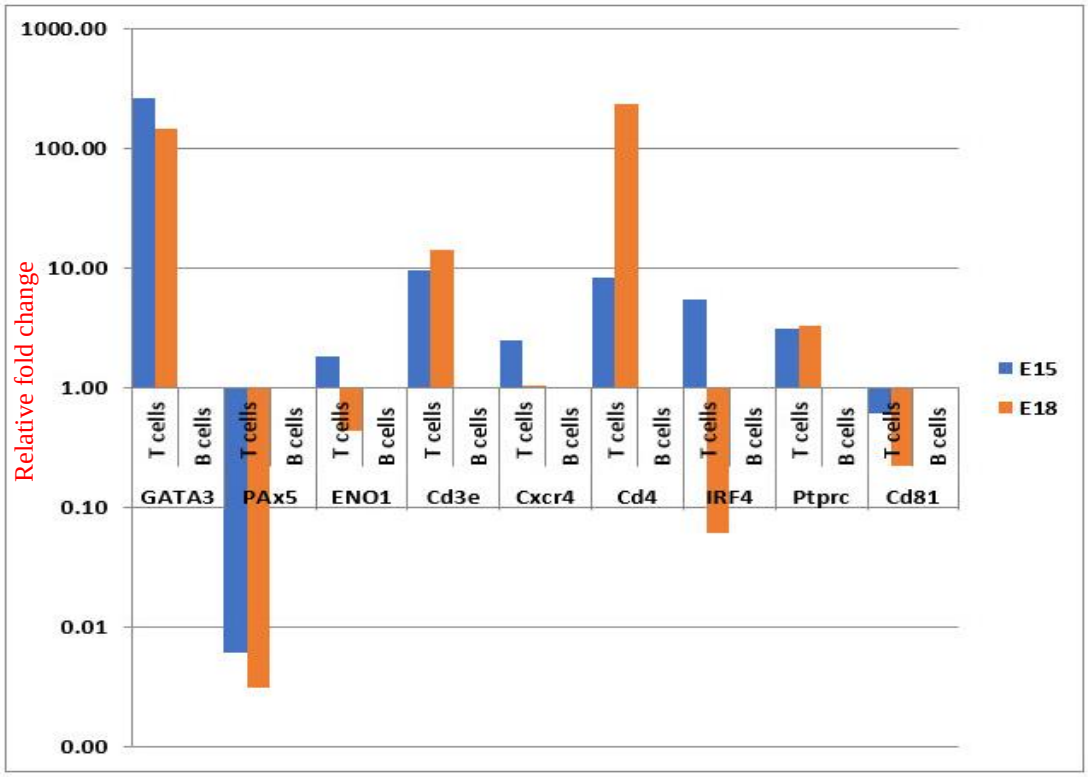

Figure 5. Relative fold change in expression of *GATA3, Pax5, ENO1, CD3e, Cxcr4, CD4, IRF4, Ptprc*, and *CD81* in sorted T-cells from thymus of broiler chickens relative to their expression in sorted B-cells lymphocytes from bursa of Fabricius of broiler chickens at two embryonic stages (E15 and E18) using quantitative real-time PCR

DISCUSSION

Semi-quantitative real-time PCR analysis was used to identify global expression patterns across developmental stages in the key immune organs of avian, the thymus, and the bursa of Fabricius, which represent major repertoires of T- and B-cells, respectively. Changes in gene expression during lymphocyte and immune organ development were profiled from embryonic stages (E15 and E18) to post-hatched stages (D3, D7, D14, and D28). *CD3e* and *GATA3* **(**Figure 6**)** were highly expressed in both thymus and sorted T-cells (Figure 5), compared to the bursa of Fabricius and sorted B-cells. Other genes, such as *Pax5* and *EBF1* (
Figure 7), were highly expressed in the bursa of Fabricius and sorted B-cells (
Figure 5), compared to their expression in the thymus or sorted T-cells. T-cells development is influenced by *Notch* signaling, which acts as a positive regulator of *GATA-3* and *TCF-1* expression to enhance T-cell proliferation and survival (Sambandam et al., 2005; Hozumi et al., 2008). *GATA-3* expression levels significantly decreased by D28 of development, compared to E15 (Figure 6). *GATA-3* exerts a dose-dependent effect on T-cell lineages, as high levels of *GATA-3* expression can suppress T-cell differentiation (Scripture-Adams et al., 2014). This suppression occurs by inhibiting *IL7r* and *TCH-1*, especially when *Notch* signaling is blocked (Rothenberg and Scripture-Adams, 2008; Scripture-Adams et al., 2014). *GATA-3* can also alter precursor cell fate, leading to mast cell differentiation (Taghon et al., 2007).

The functional and pathway analysis (Table 1 and Figure 6) suggest that interaction between T- and B-cell lineage genes may help maintain specific cell fates. Pu.1 (SPI1) is a known member of the ETS family of transcription factors and has a dose-dependent synergistic effect on B and T lymphocytes binding from CLPs (Anderson et al., 2002; Back et al., 2005; Nutt et al., 2005; Carotta et al., 2014). The B-cell maturation requires high levels of PU.1 (SPI1) to maintain B-cell development, whereas, in T-cells, PU.1 (SPI1) expression increases *GATA-3* levels during the early stages of T-cell development to maintain the CLPs commitment to T-cell fate (Rothenberg and Scripture-Adams, 2008; Real and Rothenberg, 2013). *EBF1* and *Pax5*, two key regulators of B-cell development, are critical for mediating and

maintaining B-cell fate under restricted conditions via *IL-7R* signaling during the early stages of CLP commitment (Åhsberg et al., 2013). A positive feedback interaction between *EBF1* and *Pax5* (Figure 7) blocks B-cell lineage differentiation and development and maintains B-cell identity (Decker et al., 2009; Lin et al., 2010; Mansson et al., 2012).

In the present study, the expression of *EBF1* and *Pax5* (Figure 7) gradually increased, reaching their highest expression in the bursa of Fabricius (B-lymphocyte repertoire) at D28 and D14, respectively. The role of *Pax5* in the commitment of hematopoietic cells-to B-cell fate is elucidated in a study using *Pax5*-deficient pro B-cells, which cannot fully commit to B-cells. However, these cells can maintain the same level of B-lineage gene expression if they are kept in a rich media with *IL-7* (Kikuchi et al., 2008). These pro-B-cells with knocked-out *Pax5* can switch to myeloid cell and dendritic cell fates upon removal of *IL-7* (Dias et al., 2005; Kikuchi et al., 2008) and if they are introduced to Notch signals they can deviate toward a T-cell fate (Nutt et al., 1999; Rolink et al., 1999). These studies illustrate the effect of *Pax5* in repressing *Notch* signaling to shut down T-cell lineage genes and maintain B-cell fates (Souabni et al., 2002). Furthermore, knockout *Pax5* in B-cell precursors can inhibit T-cell development completely in the presence of activated *EBF1* (Pongubala et al., 2008). While *EBF1-deficient* pro-B-cells with normal expression of *Pax5* can shift toward the T-cell pathway (Banerjee et al., 2013; Nechanitzky et al., 2013; Turner et al., 2020).

A study showed that *EBF1* blocks T-cell lineage genes by directly binding to *TCF-1* as well as two sites upstream of the transcriptional start site of *GATA-3*, leading to the repression of those genes critical in the determination of T-cell fate (Banerjee et al., 2013). Together, these studies indicate the vital roles of both *EBF1* and *Pax5* in determining B-cell precursor fate by acting as negative regulators for T-cell lineage genes to prevent the progenitor cells from deviating toward the T-cell pathway (Nutt et al., 1999; Rolink et al., 1999; Zandi et al., 2008; Nechanitzky et al., 2013).In this study, the expression of *EBF1* was significantly higher at E15 (Figure 7) in the thymus, compared to the other five stages of development (E18, D3, D7, D14, and D28), suggesting it may also contribute to adjusting the level of *GATA-3* in the thymus.

The interaction between the network of transcription factors outlined above contributes to determining immune cell fate. However, the diverse combinations of transcription factors and their dose-dependent effect leading to the activation or repression of certain cell types make it more important to profile their expression during different stages of development in immune tissues and in purified immune cell populations (Figure 5). More genome-wide studies need to be conducted to clarify these factors' functional mechanisms and their involvement during development. Moreover, the expression profile data can reveal the global effect of these factors and determine the relationship between them and other external signals. These results enrich the understanding of the expression of different key regulatory elements across the developmental stages in two of the primary avian immune organs (thymus and bursa of Fabricius) as well as sorted T- and B-cells. The findings further the understanding of the general gene expression profiles during vertebrate immune development and reveal a clearer picture of cell fate commitments during avian immune cell development.

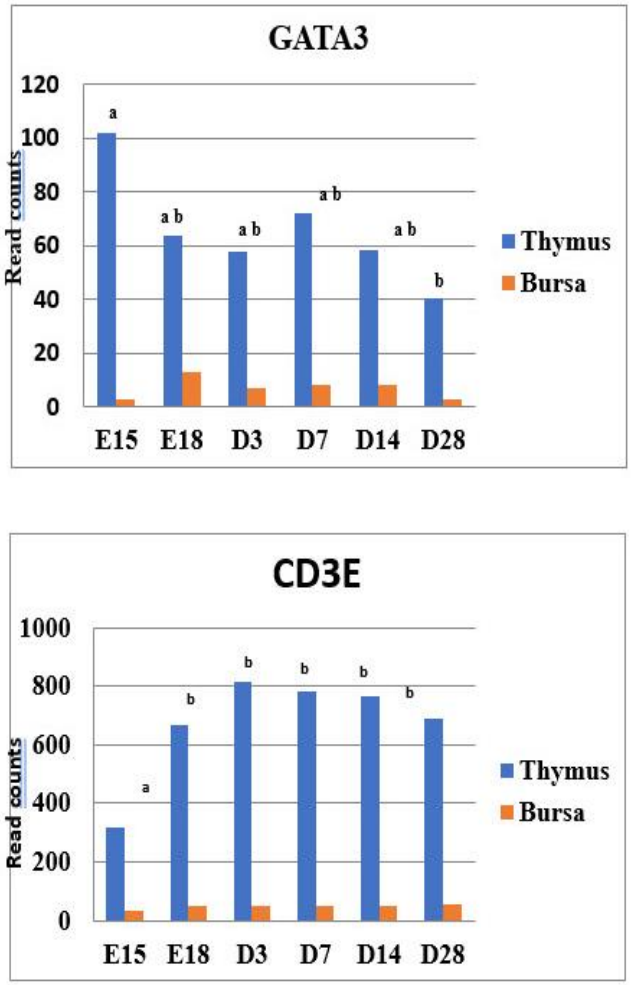

Figure 6. Expression of T-cell lineage genes; GATA3, CD3E identified in the bursa of Fabricius and thymus of broiler chickens throughout development, during the

embryonic stage (E15 and E 18) and the post-hatch days (D3, D7, D14, and D28).

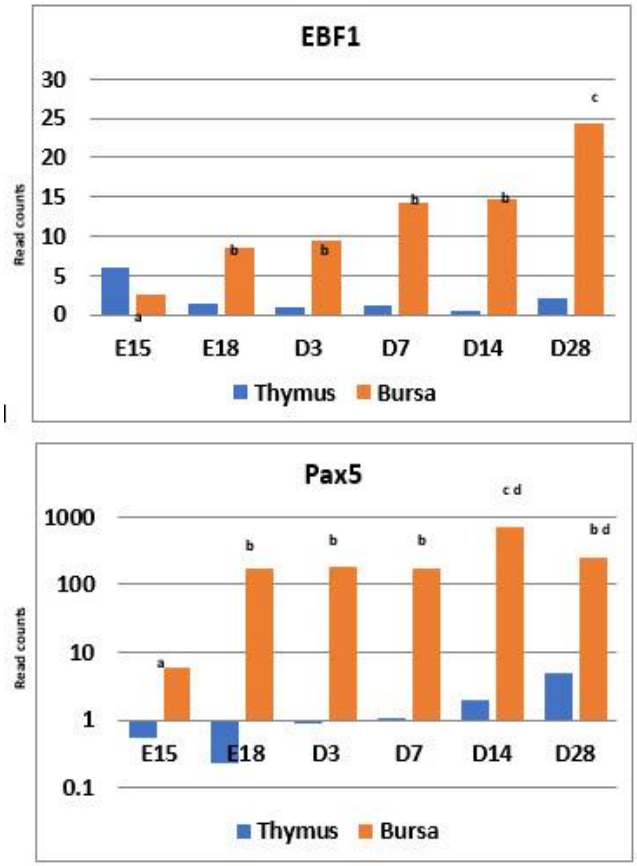

Figure 7. Expression of B-cell lineage genes; *Pax5* and *EBF1* identified in the bursa of Fabricius and thymus of broiler chickens throughout development, during the embryonic stage (E15 and E 18) and the post-hatch days (D3, D7, D14, and D28).

CONCLUSION

The results indicated that regulatory elements (*EBF1, Pax5, GATA3,* and *TCF1*) play critical roles in the development of the avian immune system, exhibiting dynamic expression patterns throughout the maturation stages. These conserved functions suggest their importance in controlling the T- and B-cell development across species. The results also highlighted the role of regulatory elements in avian lymphocyte maturation and lineage coupling and their importance in biochemical regulation. This knowledge has implications for the study of human immune-related diseases and the development of therapeutic strategies. Further research efforts will improve the understanding of the mechanisms by which these factors control lineage linkage in chickens and interact with other transcriptional regulators. Further studies are required to understand the transcriptional regulatory networks and post-transcriptional regulation of these genes to build a solid understanding of the lymphocyte developmental programs in birds.

DECLARATIONS

Funding

This research was funded and supported by GOHARZ Group and National Poultry Company 15192018.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available in the supplementary material file submitted and from the corresponding author on reasonable request.

Authors' contributions

The data curation was conducted by M. N. Goher. and M. A. El-Menawey while the statistical analysis was carried out by F. K. R. Stino, and M. N. Goher. The investigation was carried out by M. N. Goher, Reda E. A. Moghaieb, F. K. R. Stino. The methodology was performed by M. N. Goher, Reda E. A. Moghaieb, M. A. El-Menawey, and F. K. R. Stino. The project administration and validation were carried out by F. K. R. Stino and M. N. Goher. The whole visualization and writing were drafted by M. N. Goher. The writing, review, and editing of the manuscript were done by M. N. Goher, Reda E. A. Moghaieb, M. A. El-Menawey, and F. K. R. Stino. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare that there is no conflict of interest in the outcome of this research.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) were checked by the authors before the submission.

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