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Avian dendritic cells: Phenotype and ontogeny in lymphoid organs

Nándor Nagy*, Ildikó Bódi, Imre Oláh

Department of Human Morphology and Developmental Biology, Faculty of Medicine, Semmelweis University, 1094 Budapest, Tuzolto str. 58, Hungary

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ABSTRACT

Dendritic cells (DC) are critically important accessory cells in the innate and adaptive immune systems. Avian DCs were originally identified in primary and secondary lymphoid organs by their typical morphology, displaying long cell processes with cytoplasmic granules. Several subtypes are known. Bursal secretory dendritic cells (BSDC) are elongated cells which express vimentin intermediate filaments, MHC II molecules, macrophage colony-stimulating factor 1 receptor (CSF1R), and produce 74.3+ secretory granules. Avian follicular dendritic cells (FDC) highly resemble BSDC, express the CD83, 74.3 and CSF1R molecules, and present antigen in germinal centers. Thymic dendritic cells (TDC), which express 74.3 and CD83, are concentrated in thymic medulla while interdigitating DC are found in T cell-rich areas of secondary lymphoid organs. Avian Langerhans cells are a specialized 74.3−/MHC II+ cell population found in stratified squamous epithelium and are capable of differentiating into 74.3+ migratory DCs. During organogenesis hematopoietic precursors of DC colonize the developing lymphoid organ primordia prior to immigration of lymphoid precursor cells. This review summarizes our current understanding of the ontogeny, cytoarchitecture, and immunophenotype of avian DC, and offers an antibody panel for the *in vitro* and in vivo identification of these heterogeneous cell types.

1. Introduction

Antigen presenting or accessory cells are comprised principally of dendritic cells (DC) and macrophages. These cells are highly versatile cells capable of endo- and exocytosis, digestion of foreign substances, cytokine production and antigen presentation to naive T cells. These functions occur in both healthy and pathological conditions. Here we review the avian dendritic cells that are present in both central and peripheral lymphoid organs.

The central lymphoid organs in the birds are the bursa of Fabricius and the thymus, which are responsible for the production and maturation of B and T lymphocytes, respectively. During their maturation, T and B cells “learn” to distinguish between self and non-self. The self-reactive lymphocytes are eliminated, while the others work together with the accessory cells in the peripheral lymphoid organs.

Both DCs and macrophages are bone marrow-derived and have a common hematopoietic stem cell (HSC) precursor expressing common leukocyte antigen (CD45+). The fetal liver or bone marrow-derived HSC develops through diverse ontogenic pathways to give rise to heterogeneous DC types with specialized functions (for review see Steinman, 2012; Merad et al., 2013). As a general rule, DC lack lineage specific markers; however, during antigen presentation, they constitutively express major histocompatibility complex (MHC) class II glycoproteins. DC progenitors colonize lymphoid and non-lymphoid organs where they complete their maturation, which is highly influenced by the microenvironment and microbial stimuli. Recent experiments using transgenic mice provide experimental evidence that the vast majority of the Langerhans cells (LC) originate from yolk sac erythro-myeloid progenitors and not from the fetal liver or bone marrow-derived HSCs (Gomez Perdiguero et al., 2015).

Mature DCs are present in lymphoid organs, and their counterpart the Langerhans cells are located in the stratified squamous epithelium. The essential function of these highly ramified cells is to acquire antigens to induce and regulate the immune response throughout the entire lifespan of the vertebrate organism. Thymic dendritic cells (TDC) present in the thymic medulla are responsible for central tolerance. Interdigitating dendritic cells (IDC) in the T cell dependent areas of the lymph node and spleen stimulate naive T cells. Follicular dendritic cells (FDC) are nonphagocytic cells of unclear origin that are located in the germinal centers of B cell follicles and have a crucial role in germinal center responses and B

* Corresponding author. Semmelweis University, 85 Tuzolto str., 1094 Budapest, Hungary.
E-mail address: nagy.nandor@med.semmelweis-univ.hu (N. Nagy).

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cell selection (Aguzzi et al., 2014).

Similar to mammals, different subtypes of DCs have been identified in birds. In chicken a possible DC was first described by light- and electron microscope in the bursa of Fabricius (Olah and Glick, 1978; Olah et al., 1979) and in the cecal tonsil's germinal center (Olah and Glick, 1979). Based on their highly elongated shape with short dendrite-like processes and presence of secretory granules in the cytoplasm, the cell was named the bursal secretory dendritic cells (BSDC; Olah and Glick, 1978; for review see Olah and Nagy, 2013). One of the first molecular markers found to distinguish avian DC from other cells of lympho-myeloid lineage was the vimentin intermediate filament recognized by the monoclonal antibody (mAb) 3B4. BSDC, located in the bursa follicles, express vimentin (Olah et al., 1992a), similar to the FDC in the germinal center of the peripheral lymphoid organs (Olah and Glick, 1994, 1995; Nagy and Olah, 2007) and ATP-ase positive LC of the skin and esophagus (Nagy et al., 2005a; Igyarto et al., 2006). Recently we have identified the presence of dendritic cells in chicken thymus by transmission electron microscope and by CVI-ChNL-74.3 mAb (Bodi et al., 2015). This antibody also recognizes the vimentin+ and MHC class II+ BSDC, FDC and IDC in the chicken lymphoid organs, representing a convenient tool for identifying chicken DCs (Jeurissen et al., 1992; Nagy et al., 2004a; Nagy and Olah, 2007; Olah and Nagy, 2013). Despite the extensive use of 74.3 as a means of establishing DC identity, the protein recognized by 74.3 mAb has been unknown.

For identification of avian DC several other immunohistochemical markers have been reported, such as Trk neurotrophin receptor-like proteins (Ciriaco et al., 1996); S100 antigen (Gallego et al., 1996), NIC2 antigen (Nagy et al., 2001; 2004b), ovoinhibitor (Moore et al., 2004), growth hormone (Luna et al., 2008); CD83 (Hansell et al., 2007; Lee et al., 2012; Staines et al., 2003), DEC205 (Staines et al., 2013), and colony stimulating factor 1 receptor (CSF1R; Garcia-Morales et al., 2014). Growth hormone immunoreactivity was primarily observed in lymphocytes, epithelial cells and BSDC (Luna et al., 2008). S100 is a calcium binding protein expressed by the DC of the chicken immune system in addition to the supporting cells of the nervous tissue (enteric glia, pituitary stellate cells), melanocytes, adipocytes and eosinophilic granulocytes. NIC2 mAb was generated against guinea fowl (*Numida meleagris*) bursa cell suspension, and recognizes a DC specific cytoplasmic antigen (Nagy et al., 2001). Our immunostainings confirm the locations described for DC and indicate that NIC2 protein can be considered a selective cell marker for the identification of the quail (*Coturnix coturnix*) and guinea fowl DC (Nagy et al., 2001, 2004a,b). However, NIC2 mAb is less useful to identify the chicken DC because B lymphocytes also express NIC2 at low levels on their cell surface. Another limitation of this antibody, similarly to 74.3 mAb, is the lack of molecular characterization of the antigens recognized by this reagent.

In recent years a number of antibodies have been generated against purified proteins to identify chicken CD83 as the avian homologue of the human and murine DC marker CD83. In mammals, CD83 is a surface glycoprotein belonging to immunoglobulin superfamily predominantly expressed on DC and found to a lesser extent on activated lymphocytes and macrophages. Hansell et al. (2007) reported generation of sheep anti-CD83-specific polyclonal serum and mouse monoclonal anti-CD83 antibody against the extracellular domain of chicken CD83 protein. Immunohistochemistry, using a CD83 polyclonal antibody, identified immunoreactive cells with dendritic morphology in the medulla of bursal follicles, dendritic-like cells surrounding the splenic ellipsoids (ellipsoid-associated cells) and in germinal centers of spleen and cecal tonsil. Furthermore, CD83 expression was observed on the interstitial cells of liver and lung, and on the epithelial lining of the Harderian gland (Hansell et al., 2007). Lee et al. (2012) reported a similar staining pattern with two mAbs, chCD83-159 and chCD83-227. MAb chCD83-159 recognizes a 53 kDa protein expressed by a chicken macrophage cell line and stains the dendritic cells inside the germinal centers. In addition, CD83 positive DC were also found in the cortical and medullary areas of the bursa follicles. Furthermore, Staines et al. (2013) searched chicken EST databases using human and mouse peptide sequences and generated another CD83 specific antibody (clone: IAH F890:GE8). Interestingly, this CD83 mAb strongly stained cells with dendritic phenotype found in small aggregates in the medullary region of the thymus and within the T cells of the spleen, but not within the bursal follicles and germinal centers. The diverse staining pattern obtained with different CD83 specific antibodies can be explained by the presence of alternate glycosylation forms of its ectodomain. Antibody generated against DEC205 recognizes a C-type lectin receptor on the surface of chicken DC (Staines et al., 2013). We have found that this antibody specifically recognizes thymus cortical epithelial cells (Bodi et al., 2015), while in the bursa of Fabricius, in addition to immunoreactive surface epithelium, DEC205+ cells with ramified morphology were uniformly distributed in the cortex and medulla. Recently, Garcia-Morales et al. (2014) produced a mAb against chicken CSF1R, clone ROS-AV170, which recognizes the chicken homologue of human CSF1R, also known as CD115 on chicken macrophage progenitors, monocytes, microglia and DC. We find anti-CSF1R mAb yields strong membrane-bound immunoreaction on the cell surface of the BSDC, FDC and the tissue macrophages on the lymphoid organs. Double immunofluorescence staining with vimentin clearly shows colocalization of vimentin with CSF1R, providing a valuable tool for identification of chicken DC (Figs. 1, 3 and 4). 8F2 mAb has been applied by several groups to selectively stain chicken DC, NK cells and macrophages. Recently, 8F2 mAb was used to distinguish isolated splenic DC-like cells from KUL01+ macrophages (Quéré et al., 2013). Of note, this antibody is referred to as putative CD11c even though 8F2 mAb reacts with a still uncharacterized cell surface heterodimer protein (Kaspers and Kaiser, 2014). In our experience, 8F2 mAb strongly stains the BSDC. Surface expression of CD11c, MHC II, DEC205, CD80 antigens and the absence of phagosomal acidification led to the identification of a novel lung-specific DC population located in the interstitial tissues of the parabronchial wall (de Geus et al., 2012).

In addition to the limited number of biochemically characterized DC-specific antibodies (Table 1), several genes expressed by the isolated avian DC-type cells have been recently cloned. These include chicken orthologues of dendritic cell-lysosomal associated membrane protein (DC-LAMP; CD208), Toll-like receptors (TLR4 and TLR15), CC chemokine receptors 6 and 7 (CCR6 and CCR7), receptor activator of NF-κB ligand (RANKL) and its signaling receptor, cloned from chicken bone marrow-derived DC (Wu et al., 2010a, 2011; Wu and Kaiser, 2011; Sutton et al., 2015). As yet, there are no avian-specific antibodies available against these newly identified DC signaling molecules, precluding tissue expression studies of these proteins.

In conclusion, these studies generated puzzling data regarding avian DC and no study attempted to summarize the various avian DC subtypes recognized by immunohistochemical markers. Elucidating an adequate DC-specific marker panel is essential for improving our understanding of their role in the avian immune response. In this review we discuss the *in situ* distribution and phenotype of avian DC subtypes, and summarize their ontogeny to provide an overall picture of what we currently know about the nature of this complex cell network in the avian lymphoid organs.