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Unique Features and Distribution of the Chicken CD83⁺ Cell

Chris Hansell, Xing Wu Zhu, Heather Brooks, Mark Sheppard, Sam Withanage, Duncan Maskell, and Ian McConnell

The central importance of dendritic cells (DC) in both innate and acquired immunity is well recognized in the mammalian immune system. By contrast DC have yet to be characterized in avian species despite the fact that avian species such as the chicken have a well-developed immune system. CD83 has proven to be an excellent marker for DC in human and murine immune systems. In this study we identify chicken CD83 (chCD83) as the avian equivalent of the human and murine DC marker CD83. We demonstrate for the first time that unlike human and murine CD83, chCD83 is uniquely expressed in the B cell areas of secondary lymphoid organs and in organs with no human or murine equivalent such as the bursa and Harderian gland. Furthermore through multicolor immunofluorescence, we identify chCD83⁺ populations that have unique attributes akin to both DC and follicular DC. These attributes include colocalization with B cell micorenvironments, MHC class II expression, dendritic morphology, and distribution throughout peripheral and lymphoid tissues. The Journal of Immunology, 2007, 179: 5117–5125.

The dendritic cell (DC) population is heterogeneous and uniquely capable of activating naive T cells. DCs are the fundamental bridge between innate, recognition of pathogen-associated molecular patterns and the activation of the acquired immune response. Although present in low numbers, DCs are widely distributed throughout peripheral tissues. In the periphery, DCs sample their environment for pathogen-associated molecular patterns, the detection of which results in the initiation of an “activation” program. This subsequently leads to a down-modulation of sampling functions coupled with the migration of DC to the T cell regions of the proximal secondary lymphoid organs. During migration, DC up-regulate surface molecules, such as MHC class II (MHC-II), CD80, and CD86 (1), required for the interaction with naive T cells thus adopting the so-called “mature” DC phenotype.

The cellular components of the chicken immune system are apparently similar to the immune systems of humans and mice. They have macrophages, heterophils (equivalent to neutrophils), and B and T lymphocytes. Immune responses to model pathogens such as Salmonella result in heterophil and macrophage infiltration (2), and repeated exposure leads to enhanced serum Ab and T cell responses (3). Thus the chicken has the functional components of both innate and acquired immune systems.

In contrast to human and murine systems, avian DC have never been properly defined. Indeed the best evidence for DC in the chicken comes from the demonstration of MHC-II⁺ cells and the identification of Birbeck granules in the chicken epidermis (4, 5). Surface markers that might have been used to identify avian DC have not been easy to exploit for this purpose, as there appears to be little cross-species biological reactivity of specific Ab reagents. Therefore there is a need to generate polyclonal Ab and mAb that can be used to identify and define avian DC.

In human and murine immune systems, a 45-kDa cell surface glycoprotein, CD83, has been identified as a DC marker by the consistent distribution of CD83⁺ cells in the T cell areas of the secondary lymphoid organs (6, 7). It is now evident that CD83 is a marker of mature DC (8). Although the function of CD83 in DC of the peripheral tissues and secondary lymphoid organs is unknown, there is some evidence to suggest that it acts as a costimulatory molecule for T cells (9, 10). In mice CD83 is also essential for the thymic generation of CD4⁺ T cells (11).

In this study we have identified chicken CD83 (chCD83), as the chicken equivalent of mammalian CD83. This marker identifies cells of dendritic morphology throughout the tissues of the chicken. We report the distribution of chCD83⁺ cells in chicken tissues and show that although some aspects of CD83 immunobiology are conserved between species, there are also significant differences, namely the identification of chCD83⁺ populations, which are negative for markers of cell B and T cell lineages but show unique associations with the B cell compartments of the chicken.

Materials and Methods

Experimental animals

One-day-old Salmonella serovar Typhimurium specific pathogen-free Rhode Island Red chickens were obtained from the Poultry Production Unit at the Institute for Animal Health (Berks, U.K.). Birds were reared in specific pathogen-free conditions at 30°C and allowed ad libitum access to water and a vegetable protein-based diet (Special Diet Services). The outbred broiler chickens were fed a similar heat-treated commercial whole grain broiler starter ration comprising 22% crude vegetable protein. Birds taken at, 3, 7, and 14 days posthatch were used to provide the specific pathogen-free tissues for subsequent studies. Tissues designated “normal tissues” were taken from 2-wk-old outbred broiler chickens obtained locally, and these chickens provided tissues for immunohistochemistry and RNA isolations. Where comparisons between normal and specific pathogen-free chickens were made, birds were age-matched.
Identification of chCD83 sequences

Five chicken EST clone sequences (603771265F1, 603771889F1, pgpln.pkb14.j17, pgpln.pkb022.d7, and pat.pk0054.c3.f), with a high degree of peptide identity to human and murine CD83, were identified using two public domain EST databases (www.chick.umist.ac.uk and www.chicken.uel.ac.uk). A consensus sequence was formed.

All sequence homologies were calculated using Blast2-WU. Structural predictions were made using the InterPro scan program. Glycosylation patterns were predicted using NetGlyc1.0 and protein alignments were made using the ClustalW program.

Quantitative RT-PCR

Purified RNA was eluted in 50 μl of RNase-free water and stored at −70°C until use. cDNA for RT-PCR was generated using SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen Life Technologies) and supplied oligo(dT) primers. Subsequent PCR was performed with the following primers: forward 5’-TATGGCGGATCCTAGCTGCTTC AGCAGCTCCTACATC-3’ and reverse 5’-ATATCGCTGGATAGTGAATGGT AATAGAATAGATACC-3’.

TaqMan primers and probes for specific amplification of CD83 and 28 S RNA were designed using the Primer Express software program (PE Applied Biosystems) and were as follows: CD83 probe, (FAM)−TATGGCGGATCCTAGCTGCTTC AGCAGCTCCTACATC(TAMRA); CD83 forward and reverse GAGACGGTAACATGCTGAGGCAG and reverse GACGACGGATCCATGGCTTCAGCAGCCTA; 28 S probe (FAM)-AGGACCCTGCTGACGCTCCCAA-(TAMRA); 28 S forward and reverse GGCGAAGCAGAGGAAACT and reverse GACGACGGATCCATGGCTTCAGCAGCCTA. RT-PCR was performed using the Reverse Transcriptase qPCR MasterMix kit (Eurogentec). Amplification for specific products was performed using the ABI PRISM 7700 detection system (PE Applied Biosystems) with the following cycle profile: 50°C for 2 min, 96°C for 5 min, 60°C for 30 min, and 95°C for 5 min, followed by 40 cycles of 94°C for 20 s and 95°C for 1 min. Amplification was based on the increased fluorescence detected by the ABI PRISM 7700 sequence detection system due to hydrolysis of the target-specific probes by the 5’ nuclease activity of the Taq nuclease enzyme during PCR amplification. Primers for 28 S rRNA (-forward 5’-TCTAGACTCAGTCTTGTATTTTTTCAG-3’; reverse 5’-ATATCGCTGGATAGTGAATGGT AATAGAATAGATACC-3’).

ETISA

Chicken serum was bought from Sigma-Aldrich. To concentrate chicken serum, peptide DRIAWKVLDVESRHPKGL from the chCD83 extracellular domain of CD83 peptide was identified using the Bio-Toolkit 300 software. The sequence corresponding to the extracellular portion of CD83 (aa 14–152) was ligated into the eukaryotic expression vector pcDNA3.1 (Invitrogen Life Technologies) and the prokaryotic expression vector pGEX-6P-1 (GE Healthcare) containing the GST tag. COS-1 cells was transfected with the pcDNA3.1 construct using Fugene 6 (Roche) following the manufacturer’s instructions. Successfully transfected cells were selected using DMEM (Sigma-Aldrich) containing 10% FCS, 1% glutamine, and 1% penicillin/streptomycin and 800 μg/ml G418 (Sigma-Aldrich) in a humidified atmosphere of 5% CO₂ and air. Cells surviving the initial selection were maintained in medium containing 400 μg/ml G418.

Expression of chCD83

Chicken serum was bought from Sigma-Aldrich. To concentrate chicken serum, peptide DRIAWKVLDVESRHPKGL from the chCD83 extracellular domain of CD83 peptide was identified using the Bio-Toolkit 300 software. The sequence corresponding to the extracellular portion of CD83 (aa 14–152) was ligated into the eukaryotic expression vector pcDNA3.1 (Invitrogen Life Technologies) and the prokaryotic expression vector pGEX-6P-1 (GE Healthcare) containing the GST tag. COS-1 cells was transfected with the pcDNA3.1 construct using Fugene 6 (Roche) following the manufacturer’s instructions. Successfully transfected cells were selected using DMEM (Sigma-Aldrich) containing 10% FCS, 1% glutamine, and 1% penicillin/streptomycin and 800 μg/ml G418 (Sigma-Aldrich) in a humidified atmosphere of 5% CO₂ and air. Cells surviving the initial selection were maintained in medium containing 400 μg/ml G418.

Generation of sheep anti-chCD83-specific polyclonal serum and mouse monoclonal anti-chCD83 Ab

A sheep was immunized i.m. with 1 μg of the pcDNA3.1 construct in three sites. This procedure was repeated three times before the sheep were finally boosted with 300 μg of the extracellular domain of CD83 (CD83e) GST protein at 500 μg/ml in 500 μl of CFA (Sigma-Aldrich) s.c. Monoclonal Ab to chCD83 were raised by standard procedures and using the myeloma myeloma cell line NS0 as fusion partner with murine spleen cells. BALB/c mice were primed i.p. (three times) at intervals of 3 wk with 100 μg of the pcDNA3.1 construct and then finally boosted twice i.v. with 100 μg of CD83e-GST at 3-wk intervals starting 2 wk after the last DNA boost. The supernatants were screened for anti-chCD83 by ELISA and Western blot on the C-terminal peptide. The strongest reacting clones were grown to saturation and cell lines and supernatants were cryopreserved at −70°C.

The sequence data deposit of chicken CD83 (accession number H9262)

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IgG or rabbit anti-sheep IgM (Vector Laboratories) was washed and followed with a HRP-streptavidin tertiary layer (Vector Laboratories). The ELISA was developed using tetramethylbenzidine (Perbio) and stopped using 0.18 mM NaH$_2$SO$_4$. The OD was read with a plate reader set to 450 nm.

Immunostaining and fluorescent immunostaining of chicken tissues

The identification of chCD83 protein using immunohistology was completed using 5-μm paraffin sections that were dewaxed, rehydrated, digested with proteinase K, blocked with 5% BSA plus PBS, and incubated for 2 h with a 1/2000 dilution of chCD83-specific sheep anti-serum in 1% BSA plus PBS. Biotinylated rabbit anti-sheep IgG (5 μg/ml; Vector Laboratories) was used as a secondary and HRP-streptavidin ABC Elite Standard kit (Vector Laboratories) provided the final tertiary layer. Staining was resolved using the diaminobenzidine kit (Vector Laboratories) was used to resolve the stain. Immunofluorescence staining was performed upon 10–16-μM frozen sections. Sections were blocked with 0.2% saponin and 10% normal serum (from the same species in which the secondary Abs were raised) for 1 h. Primary Abs were applied in blocking buffer (1% BSA plus PBS) as follows: sheep anti-chCD83 polyclonal serum (1/400), mouse anti-chicken MHC-II (IgM) (0.5 μg/ml; Southern Biotechnology Associates), mouse anti-chicken CD3 (IgG) (5 μg/ml; Southern Biotechnology Associates), mouse anti-chicken Bu1 (AV20) (IgG) (5 μg/ml; Southern Biotechnology Associates), and mouse anti-chicken IgG (5 μg/ml; Southern Biotechnology Associates). They are followed by the appropriate secondary Abs: donkey anti-sheep IgG Alexa Fluor 488 (5 μg/ml; Molecular Probes), goat anti-mouse IgM Alexa Fluor 647 (5 μg/ml; Molecular Probes), and goat anti-mouse IgG Alexa Fluor 568 (5 μg/ml; Molecular Probes).

Cell isolation and infection

The spleen and bursa were removed from normal chickens immediately postmortem and immersed in HBSS (Sigma-Aldrich). The 3–4 ml of enzyme mix (DNase I, 0.5 mg/ml; Roche) collagenase D (2 mg/ml; Roche) was injected into the spleen and incubated at 37°C until digestion was complete. RBC were removed by spinning the suspension over an ice-cold 1.09 g/ml Percoll gradient (Amersham Bioscience). A spontaneous nalidixic acid-resistant mutant of *Salmonella* serovar Typhimurium phage type 14 strain F98 was used in the experimental infections. A total of 1 × 10$^5$ cells were transiently infected with *Salmonella* serovar Typhimurium at a multiplicity of infection of 0, 1.5, 7.5, or 30 bacteria per cell. The cells were incubated at 40°C for 2 h, then the infected medium removed and treated with prewarmed medium containing gentamicin (200 μg/ml; Rousell Labs) for 30 min. The gentamicin medium was replaced with RPMI 1640 medium with 10% FCS and 2 mM l-glutamine and the cells were incubated overnight at 40°C and harvested for FACS.

Results

Avian CD83 sequence homology and mRNA expression

To investigate chCD83, five EST clone sequences were identified using public domain databases (603771265F1, 603771889F1, pgpln.pk014.j17, pgpln.pk002.d7, and pat.pk0054.c3.f) that
were homologous to the mature DC marker CD83 in both humans and mice. A consensus sequence was constructed, referred to in our study as chCD83, which had a 39% and 40% identity at the polypeptide level to human and murine CD83, respectively. Four domains were identified in the chCD83 sequence using Interpro scan: a signal peptide, a single Ig-like extracellular domain, a transmembrane domain, and a cytoplasmic region. Thus, the predicted structure of the chCD83 sequence was similar to the structure of other species, which was confirmed by alignment with the full-length human and murine CD83 (Fig. 1), similar to the structure of other species, which was confirmed by alignment with the full-length human and murine CD83, respectively. Four domains were identified in the chCD83 sequence using Interpro scan: a signal peptide, a single Ig-like extracellular domain, a transmembrane domain, and a cytoplasmic region. Thus, the predicted structure of the chCD83 sequence was similar to the structure of other species, which was confirmed by alignment with the full-length human and murine CD83 (Fig. 1a). The N-linked glycosylation sites of chCD83 were predicted using NetNGlyc1.0 and found to be in similar locations in two of the three sites compared with human and murine CD83 as reported previously (14).

The mRNA expression of chCD83 in normal chicken tissues was assessed to establish the potential of chCD83 as a putative DC marker. This assessment was done using a two-step nonquantitative RT-PCR (Fig. 1b) and TaqMan quantitative RT-PCR (Fig. 1c). The highest levels of chCD83 mRNA expression were found in lymphoid tissues such as the spleen and thymus. The chCD83 was also highly expressed in the bursa, which in birds is responsible for primary B cell lymphogenesis. The chCD83 transcripts were undetectable in the skin and brain (Fig. 1b and c). This distribution confirmed an association of chCD83 with both the primary and secondary lymphoid organs of the immune system and select peripheral lymphoid organs, suggests an immune association.

**Polyclonal Abs to chCD83**

To study chCD83 expression further, the specific polyclonal antiserum was generated, as described in Materials and Methods. The specificity of the chCD83 antiserum was confirmed using COS-1 cells transfected with the full-length chCD83 and empty pcDNA3.1 vector as the negative control. An ELISA upon supernatants retrieved from transfected COS-1 cells (Fig. 2a) indicates that the chCD83 polyclonal Ab only reacts with those cells transfected with the chCD83 expression construct, and not with untransfected or transfected empty vector controls. Western blot identified a 39-kDa band reacting with the antiserum and N-terminal sequencing of the proteins from the gel confirmed the bands as chCD83 (data not shown).

Soluble CD83 is detectable in human serum (15) thus chicken serum was assessed for the same property. The low levels of soluble CD83 in human sera (∼150 pg/ml) suggested chicken serum should be concentrated to bring putative soluble chCD83 to the threshold of detection. A 30-kDa cut-off filter was used to fractionate the chicken serum on the basis of the predicted molecular mass for chCD83. Soluble CD83 was detectable in chicken serum and as predicted the majority of the signal was achieved in the <30-kDa fraction (Fig. 2b).

**The unique distribution of chCD83 in the avian spleen**

To define the distribution of chCD83 cells, immunohistochemistry was performed using the CD83 polyclonal Ab upon a variety of normal tissues. The avian spleen has several unique features, which distinguish it from its mammalian counterparts. These features are summarized in Fig. 3. The avian spleen has B cell- and T cell-rich regions just like its mammalian counterpart. The T cell-rich zone also termed the periarteriolar lymphoid sheath (PALS)
surrounds the central arterioles and just like the mammalian spleen, avian B cell germinal centers form within this region. However the chicken PALS lacks a marginal zone and instead Ag is deposited via the ellipsoidal capillaries (16), which in this sense are equivalent to the marginal sinus. Surrounding these capillaries is a network of nonlymphoid cells termed the ellipsoid and around the ellipsoid a second B cell compartment termed the periellipsoid lymphoid sheath (PELS). Collectively the ellipsoid, PELS and surrounding macrophages are thought to be functionally equivalent to the mammalian marginal zone (17, 18).

The chCD83\(^+\) cells are almost exclusively associated with avian splenic B cell regions. The chCD83\(^+\) cells with a dendritic morphology were identified surrounding the outer edge of the ellipsoid (Fig. 3a) closely associated with the PELS (Fig. 3b). The chCD83\(^+\) DC could also be identified in the germinal centers in the PALS area (Fig. 3c), but were only rarely identified in the PALS outside of these structures (Fig. 3d). The chCD83\(^+\) cells could also be identified in the red pulp (data not shown). This distribution of chCD83\(^+\) cells is in contrast to mammalian CD83\(^+\) cells, which are associated with T cells in the PALS area of the spleen.

The identification of chCD83\(^+\) cells in the avian primary lymphoid organs

As chCD83\(^+\) cells were found to have such a close association with B cell areas in the spleen we investigated the distribution of chCD83 in the bursa, the organ responsible for B cell lymphopoiesis. The bursa is divided into numerous follicles (Fig. 4), each follicle is further subdivided into the central medulla region and the outer cortex, separated by the corticomedullary boundary in much the same fashion as the mammalian thymus.
Developing B cells of the medulla give rise to cortical B cells. It has been suggested that Ag deposited in the medulla, derived from the blood and gut contents via the follicle-associated epithelium, may be necessary for the positive selection of medullary cells and the facilitation of this process (19–21). Using immunohistochemistry, chCD83/H11001 DCs were identified in the medulla of normal bursal follicles (Fig. 4a). Using anti-B cell and anti-MHC-II mAbs, it was evident that the distribution and frequency of B cells contrasted markedly with the distribution of chCD83/H11001 cells (Fig. 4b and data not shown), suggesting chCD83/H11001 cells were of a non-B cell lineage. Although chCD83/H11001 cells could be readily identified in the bursa, in the thymus chCD83/H11001 cells were identified only rarely and found near Hassal’s corpuscles, which also expressed chCD83 (data not shown).

The identification of chCD83+ cells in other tissues

The chCD83+ cells could also be identified in other B cell areas in other secondary lymphoid organs. In the caecal tonsil, chCD83+ cells with a dendritic morphology could be identified in the germinal centers (Fig. 5a) and in the diffuse lymphoid tissue immediately below the epithelia (data not shown).

The chCD83+ cells could be identified throughout the periphery including the ileum and jejunum, where positive cells were mainly localized in the subepithelial lamina propria (Fig. 5b). In the liver CD83+ cells were detected in the interstitium of the portal triad and in the parenchyma and in the lung CD83+ cells were detected within the wall of the alveolar capillaries and throughout the interstitium (data not shown). Brain and skin tissues were also tested and were found to be chCD83+ in accordance with results from the TaqMan quantitative RT-PCR experiments (data not shown).

The chCD83 cell was also extensively expressed in the Harderian gland, which is responsible for the production of Ab in the lachrymal fluid of the avian eye and has an immune function in the chicken. The Harderian gland has a system of primary and secondary canals that drain into the central canal and contains lymphocytes and plasma cells between the glandular structures (Fig. 5c). The entire epithelial lining of the Harderian gland canal system strongly expressed chCD83 (Fig. 5d).

The modulation of chCD83 expression by Salmonella serovar Typhimurium

Although chCD83 expression was readily detectable in normal tissues, tissues taken from Salmonella serovar Typhimurium free specific pathogen-free chicken tissues were consistently found to be chCD83+, including the spleen but also the bursa, liver, ileum, and caecal tonsil (data not shown). Although differences in diet may have contributed to this phenomenon, it was hypothesized that the up-regulation of chCD83 in non-specific pathogen-free normal chicken tissues was due to exposure to unidentified environmental pathogens. This hypothesis suggested that CD83 in the chicken might be up-regulated during inflammation as identified in other species. To test this hypothesis, isolated splenic nonadherent cells (Fig. 6a) were transiently exposed to live Salmonella serovar Typhimurium strain F98, and the expression of CD83 upon their surface was measured by FACS. Using the chCD83 polyclonal Ab, a significant up-regulation of chCD83 could be detected as indicated.

FIGURE 7. Relationship between chCD83+ cells and T and B lymphocytes. Two-color immunofluorescence microscopy of normal spleen (a–c and g) and normal ileum (d–f) is used at an original magnification of ×630 objective. Splenic ellipsoid (open arrowhead) and splenic artery (asterisk) are indicated. All chCD83 staining is represented in green. a, Splenic ellipsoid stained for chicken CD83 vs MHC-II (red). b, PALS region of the spleen stained for chicken CD83 vs, MHC-II (red). c, PALS region of the spleen stained for chicken CD83 vs CD3 (red). d, CD83 vs pan-Ig anti-κ L chain (red). e, CD83 vs CD3 (red). f, CD83 vs MHC-II (red). g, Original objective magnification at ×100 of chCD83+ DCs in a splenic ellipsoid.
by an increase in mean fluorescence intensity taken across the whole population (Fig. 6a). Representative FACS plots from this study indicated that a minority of cells became positive following exposure to Salmonella (Fig. 6c) vs uninfected controls (Fig. 6b). The chCD83+ population was analyzed further using a chCD83-specific mAb reagent. The chCD83+ cells within the splenic non-adherent cell fraction were found to have a large and granular appearance as indicated by high forward scatter and side scatter profiles (Fig. 6, d and e). A profile such as this is typical of complex cells such as classical DC of mammalian immune systems.

Two-color immunofluorescence microscopy

Two-color immunofluorescence microscopy was used to establish the relationship between chCD83+ cells and T and B lymphocytes. The chCD83+ cells of the splenic ellipsoid were MHC-IIlow (Fig. 7a), and IgM- and IgG- (data not shown). The chCD83+ cells were virtually excluded from the PALS where the majority of MHC-IIhigh cells reside (Fig. 7b). All chCD83+ cells of the spleen were CD3- (Fig. 7c). Therefore the chCD83+ cells of the spleen were morphologically dendritic, associated with B cell zones, excluded from T cell zones, but were neither of the T cell nor B cell lineage.

Immunofluorescence studies on the gut identified chCD83+ populations of similar morphology. Gut chCD83+ cells were Ig- and CD3- with a dendritic morphology (Fig. 7d). However the gut chCD83+ cells found as individual cells rather than foci, throughout the tissue in particular the lamina propria but also intraepithelial sites. This population of chCD83+ cells was further distinguished from the other chCD83+ populations by their consistent expression of MHC-II (Fig. 7f).

Discussion

DC in humans and mice are the critical link between innate signals and an appropriate and proportional T cell response. The study of this cell type in humans and mice has been greatly accelerated by the identification of DC-specific markers such as CD83. CD83 is expressed by the epithelial cells of the thymus and in the T cell-rich regions of the secondary lymphoid organs. It was this pattern of expression that led Zhou et al. (7) to correctly identify CD83 as the chicken equivalent of the DC marker CD83.

Evidence concerning the existence of avian DC is limited to the identification of putative Langerhans cells (epidermal DC), based upon the localization of MHC-II+ cells in the chicken epidermis and the detection of Birbeck granules (a Langerhans cell-specific organelle) (4, 5, 22). However the absence of DC-specific markers in the chicken has prevented progress in this field. To address this issue, we generated specific polyclonal Abs and mAbs to the avian DC-specific markers such as CD83. CD83 is expressed by the epithelial cells lining the ducts of a unique ocular lymphoid organ called the Harderian gland or the gland of Harder, which is remarkable for the large numbers plasma cells resident within the tissue and is responsible for the secretion of Abs into the lachrymal fluid. At present this epithelial expression is not understood but it further indicates the association of CD83 with an organ involved in Ab formation and is under further investigation.

The consistent association of chCD83+ cells with B cells encourages the hypothesis that these two cell types interact in vivo. If this hypothesis were true, we might expect chCD83+ cells to resemble cell types of other species known to interact with B cells. Thus the chCD83+ cells found in chicken genital cells appear similar to the follicular DC (FDC) found in other species. FDC are thought to be the site of Ag deposition in the B cell follicle and are necessary for the affinity maturation of B cell responses (27). Certainly in terms of morphology, localization and their MHC-IIlow phenotype, chCD83+ cells and FDC are similar, however FDC only occur in B cell follicles and germinatal centres and so fail to explain extrafollicular chCD83+ populations. There are a variety of explanations for this discrepancy and to discuss them we must firstly consider the unique structure of the chicken B cell compartment and secondly we must look to a growing body of evidence that describes DC as not one single homogenous population but a series of subsets, which are phenotypically and functionally distinct.

The unusual phenotype of the extrafollicular splenic chCD83+ cell may be due in part to the unusual nature of the chicken splenic B cell compartment. Splenic chCD83+ cells are identified surrounding the ellipsoid, which has been identified as the site of Ag deposition in the chicken spleen (16), and combined with the PELS and the surrounding macrophages are collectively thought to be the functional equivalent of the mammalian marginal zone (17). Indeed, traceable Ag administered i.v. results in its deposition into the ellipsoid via the penicillary capillaries (17). However PELS B cells also make up the entire resting population of avian splenic B cells, suggesting that rather than being strictly equivalent, these
cells are a functional hybrid of marginal and follicular B cells. Therefore splenic chCD83+ DC of the ellipsoid are well positioned to both encounter Ag deposited in the spleen and to interact with a functionally unique and naive PELS B cell compartment. It is possible therefore that this unique resting population of B cells also has a resident FDC-like population associated with this structure. It has long been considered that FDC and T cells were the only cell types capable of providing Ag-specific help to B cells. However, recent evidence suggests that in addition to their activation of naive T cells, some DC subsets can also interact with B cells (28–30).

Numerous DC subsets have been identified in mice and humans separable by defined markers. In the mouse the DC marker CD11c used in combination with other markers such as CD8, DEC205, and CD11b have identified at least five different DC subsets in the spleen alone (31). These populations occupy separate morphological niches and are functionally distinct; for instance, the murine CD8+ DC of the PALS are more phagocytic and bias Th cells more readily to TH1 as a result of their enhanced capacity for IL-12 production (32). The idea of DC subsets in the chicken is a relevant one. The identification of Langerhans cells in the chicken epidermis through the identification of Birbeck granules is a clear indication that some DC subsets are conserved across species.

There are several murine DC subsets, which share a similar distribution to some of the chCD83+ populations and more importantly have been shown to interact with B cells. The DC subsets of the murine marginal zone are known to capture Ag. Furthermore, they have been suggested to bind and transport intact Ag to the bridging channels of the murine spleen and interact with B cells during the early initiation phase of the B cell response (33, 34). These studies are complemented by the identification of another DC subset in murine blood, which is specifically capable of binding and transporting intact Ag from the blood to the splenic marginal zone, whereupon these cells induce a T-independent marginal zone B cell response (28). Both populations could be considered equivalent, in terms of localization, to the ellipsoid-associated chCD83+ population, and their capacity to interact with B cells makes them an attractive explanation for B cell/chCD83+ cell colocalization. This hypothesis can be broadened to encompass the chCD83+ cells of the germinal center, which are morphologically similar to the murine DC subset termed the germinal center DC, and are known to have enhanced capacity to drive B cells toward plasma blast formation (35).

The chCD83+ cell population in the ileum may also be considered equivalent of DC subsets in other species, the intraepithelial localization of the chCD83+ echoes the distribution of DC in the guts of other mammalian species (36) and their expression of relatively high levels of MHC-II indicates that they may be capable of interactions with CD4+ T cells. The chCD83+ cells found in the bursa defy classification because there is no equivalent organ in other species. However, it is conceivable that this organ may also be populated with DC.

In conclusion these results suggest that the avian immune chCD83+ cells have a novel phenotype combining characteristics of FDC and DC. Their exclusion from T cell zones and association with marginal cells is indicative of FDC; however, there are many similarities between the localization of chCD83+ populations and DC subsets of other species, in particular the marginal zone and germinal center DC. These DC subsets are capable of interacting with and activating B cells. Thus, chCD83 can also define a subset of DC specialized in the activation of chicken B cells.

In a field hindered by the absence of suitable monoclonal reagents, our demonstration of an immunization strategy and its use in the generation of specific polyclonal and monoclonal anti-chCD83 reagents is significant, particularly as we have validated its potential in flow cytometry on isolated cells from the spleen that have a DC-like profile.

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Disclosures
The authors have no financial conflict of interest.

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