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Research paper

Presence of dendritic cells in chicken spleen cell preparations and their functional interaction with the parasite *Toxoplasma gondii*

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ABSTRACT

Toxoplasmosis is a worldwide epizootic disease of mammals. Chickens, albeit being less susceptible, can be contaminated in free-range flocks and may have an important role in parasite transmission. Plastic adherence selection of chicken spleen cells enriched 8F2+ (putative chicken CD11c) MHC II+ cells of the myeloid type; however, we did not succeed to separate dendritic cells from macrophages using their feature to become loosely adherent after culture as in mammals. Still we clearly identified dendritic-like cells being morphologically distinguishable from macrophages in the KUL01 (macrophage marker) negative fraction, exhibiting responsiveness to LPS and parasite extracts by developing characteristic cellular protrusions as well as a minor phagocytic incorporation of dead parasites. Live *T. gondii* tachyzoites were able to invade the two different types of myeloid adherent cells, to replicate, and to induce an overall decrease in the expression of MHC II and co-stimulatory molecules, CD80 and CD40. Our data indicate that dendritic cells in addition to macrophages may have a role in hiding viable replicating *T. gondii* tachyzoites from the immune system and in shuttling them to different organs in the chicken as previously described for different Apicomplexa infecting mammals.

1. Introduction

Toxoplasmosis is a world-wide spread zoonotic disease of warm-blooded animals including man that is characterized by abortions and neurological malformations in neonates and thus imposes a serious threat on public and animal health (Montoya and Liesenfeld, 2004). Avian species, notably chickens, can be infected, but clinical symptoms are usually absent or mild in infected birds (Dubey, 2009). Nevertheless they may serve as a non-negligible source for feed contamination for free-ranging carnivores due to extensive outdoor breeding practices in some countries as well as a source for human alimentary infections (Dubey, 2009; Dubey et al., 2010; More et al., 2011; Yan et al., 2010; Zhao et al., 2011). Among carnivores, felids are the only source of infective oocysts (Cenci-Goga et al., 2011). Following ingestion, *Toxoplasma gondii* (*T. gondii*) disseminates to various organ sites in mammals. Macrophages and dendritic cells have been shown to be involved in the dissemination process. Recent studies confirm the major role of dendritic cells since infection with the parasite increases the motility of dendritic cells. Macrophages and dendritic cells are antigen-presenting cells, with dendritic cells linking innate and adaptive

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immunity due to their migration capacities. Dendritic cells are also able to secrete high quantity of cytokines to orientate the immune response. These functions can thus be hijacked by *T. gond*ii (Lambert et al., 2006, 2009; Morisaki et al., 1995). One important question that has not been addressed so far is whether the parasite can invade myeloid cells with similar consequences for birds as for mammals.

Macrophages and dendritic cells derive from a common myeloid precursor (Liu and Nussenzweig, 2010). As a glint of great complexity in mammals, different sub-populations of dendritic cells defined upon their localization, cell surface marker expression and function, have been identified (Ardavin et al., 2001; Naik, 2008; Novak and Bieber, 2008). Moreover, upon inflammation and the formation of the respective cytokine environment, cells from the monocyte/macrophage lineage can differentiate in dendritic-like cells (Lopez-Bravo and Ardavin, 2008; Naik et al., 2006). This observation highlights the relationship between the macrophage and dendritic lineages.

In the chicken, studies on the identification of dendritic cells and their biological functions are still scarce, mostly due to the lack of specific immune reagents. However, recent *in vitro* studies appear to confirm the existence of dendritic-like cells, given the functional properties of cultured bone marrow cells treated with chicken GM-CSF and chicken IL-4: upon maturation, these cells loose some immature markers (cell surface DEC-205), decrease their endocytosis capacity (Wu et al., 2009b), increase DC-LAMP gene expression (Wu et al., 2009a), and shift from CCR6 gene expression to CCR7 gene expression (Wu et al., 2011), exactly as previously described for mammalian dendritic cells.

As in mice (Steinman et al., 1979), the existence of dendritic-like cells in the chicken has also been presumed previously from histological studies, but the results are still puzzling with respect to the cell surface antigen marker expression. The chicken is devoid of lymph nodes, but the chicken spleen is a secondary lymphoid organ, even though it is organized quite differently than the spleen of mammals. Antibodies raised against cells from the chicken monocyte/macrophage lineage (named 68.1 and KUL01) have been shown to stain different types of cells according to their spatial localization, either macrophage-like cells in the red pulp and around periellipsoid lymphoid sheaths (PELS; B cell area) or interdigitating dendritic cells in periarteriolar sheaths (PALS; T cell area) (Jeurissen, 1991; Jeurissen et al., 1992; Mast et al., 1998). Another type of dendritic-like cells identified as ellipsoid cells using a cell type-specific antibody 68.2 is detected in the PELS (Jeurissen et al., 1989). The ellipsoid cells appear to have no equivalent in mammals. A different type of cells from non-hematopoietic origin, the follicular dendritic cells, is found in spleen germinal centres in close contact to PELS and is able to trap immune complexes. However, the phylogenic relationship between all these cells in the chicken is still speculative, with some authors claiming that ellipsoid dendritic cells could give raise to interdigitating cells and follicular dendritic cells migrating then in spleen germinal centres (Igyarto et al., 2007; Janse and Jeurissen, 1991; Jeurissen et al., 1992). After intravenous injection, antigens are localized first in PALS and are fixed on ellipsoid cells and then immune complexes are found in germinal centres taken up by follicular dendritic cells (Igyarto et al., 2007; Jeurissen et al., 1992). More recently, Del Cacho et al., identified in another highly organized lymphoid structure only found in birds, the cecal tonsils, two types of cells with a dendritic morphology, the CD45 negative follicular dendritic cell from a non-hematopoietic origin trapping immune complexes, and CD45 positive interdigitated dendritic cells from a hematopoietic origin (Del Cacho et al., 2009). Epidermal dendritic cells have also been identified from chicken skin explant and have proved their capacity to be recruited as antigen presenting cells. A proportion of these cells were partially stained with 68.1 and KUL01 antibodies (Igyarto et al., 2006).

In summary, at present, it appears quite difficult to discriminate between the two types of antigen presenting cells, macrophage and dendritic-like cells, in the chicken. However, dendritic cells obtained from lymphoid organ such as spleen would be closer to the reality than cells derived from bone marrow precursors *in vitro* in order to study their functional properties and thus their biological role *in vivo*. In the present study, we hence started with spleen adherent cells with the aim to identify putative dendritic cells through their morphological characteristics and to further analyze their phenotype in comparison to macrophages in addition to the role these cells may play as natural targets for intracellular parasites such as *T. gondii*.

2. Materials and methods

2.1. Chickens

GB1 (B13/B13) white leghorn chickens were maintained in a pathogen free environment at the INRA experimental animal facilities (PFIE, Nouzilly, France). All experiments were performed with females from 7 to 8.5 weeks of age. Six chickens were used for each experiment to obtain the adherent cells from spleens. The chickens were euthanized according to protocols approved by the Animal Ethics Committee (Région Centre, France).

2.2. Parasite and antigen

Tachyzoites of the RH strain of *T. gondii* were grown on human foreskin fibroblasts. Live tachyzoites were prepared on the day of experiment and used to infect spleen cell cultures. Soluble antigen extracts, referred as *T. gondii* extracts, were prepared exactly as previously described. Briefly, tachyzoites were washed in 5 ml of PBS and then centrifuged at 1250 × *g* for 15 min at 4 °C. The pellet was re-suspended in distilled water at a concentration of 10^9 tachyzoites/ml and then sonicated 2 times at 60–80 W for 10 min on ice. After centrifugation at 1500 × *g* for 30 min at 4 °C, the supernatant containing the soluble *T. gondii* antigens was used as the source of antigen. The concentration was determined by a protein assay reagent kit (Bio-Rad, Marnes-la-Coquette, France) with BSA as the standard. *T. gondii* extracts were stored as aliquots at −80 °C and sonicated 2 times before use. For phagocytosis assays, tachyzoites were killed by incubation at 56 °C for 30 min