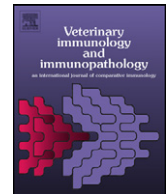




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Review paper

Species-specific properties and translational aspects of canine dendritic cells

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ABSTRACT

Dogs are affected by spontaneously occurring neoplastic and inflammatory diseases which often share many similarities with pathological conditions in humans and are thus appreciated as important translational animal models. Dendritic cells (DCs) represent the most potent antigen presenting cell population. Besides their physiological function in the initiation of primary T cell responses and B cell immunity, a deregulation of DC function is involved in immune-mediated tissue damage, immunosuppression and transplantation complication in human and veterinary medicine. DCs represent a promising new target for cancer immunotherapy in dogs. However, the therapeutic use of canine DCs is restricted because of a lack of standardized isolation techniques and limited information about dog-specific properties of this cell type. This article reviews current protocols for the isolation and *in vitro* generation of canine monocyte- and bone marrow-derived DCs. DCs of dogs are characterized by unique morphological features, such as the presence of cytoplasmic projections and peridolic microstructures. Canine DCs can be discriminated from other hematopoietic cells also based on phenotypic properties and their high T cell stimulatory capability in mixed leukocyte reactions. Furthermore, the classification of canine DC-derived neoplasms and the role of DCs in the pathogenesis of selected infectious, allergic and autoimmune diseases, which share similarities with human disorders, are discussed. Future research is needed to expand the existing knowledge about DC function in canine diseases as a prerequisite for the development of future therapies interfering with the immune response.

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1. Introduction

Dendritic cells (DCs) represent a heterogeneous group of bone marrow-derived cells and serve as sentinels of the immune system, capable of capturing and processing antigens. They represent the most potent antigen presenting cell population, which initiate primary T cell responses and play an important role for B cell immunity (Steinman, 2007; Liu and Nussenzweig, 2010).

In addition to rodent models, there is an increasing interest in spontaneous and experimental canine diseases as translational models for human disorders (Kling, 2007; Paoloni and Khanna, 2008). A deregulation of DC function is involved in immune-mediated tissue damage and immunosuppression in human and veterinary medicine (Vanloubbeeck et al., 2003; Moore, 2004; Abreu et al., 2005; Beineke et al., 2009; Ricklin et al., 2010). Furthermore, DCs have been identified as key initiators of graft rejection and graft-versus host disease, making dogs an important model for hematopoietic cell transplantation (Hägglund et al., 2000; Mielcarek et al., 2007). DCs also represent a promising new target for cancer immunotherapy (Berger and Edelson, 2003). However, despite the fact that dogs are widely used in preclinical trials of transplantation studies and the increasing interest in immunization with DCs, only few clinical trials have been performed in dogs, mainly because of a lack of standardized isolation techniques and limited information about phenotypical and morphological characterization of these cell types (Kolb et al., 1997; Isotani et al., 2006; Tamura et al., 2007, 2008; Liu et al., 2008). Therefore, the intention of this article is to review the current knowledge about *in vitro* generation and characterization of canine DCs. In addition, the role of DCs in the pathogenesis of selected canine neoplastic, infectious, allergic and autoimmune diseases, which share similarities with human disorders and thus have significance for translational medicine, is discussed.

2. Generation of canine dendritic cells *in vitro*

DCs were isolated from the blood and lymph node of dogs for the first time by Goodell et al. (1985). Current protocols for the isolation and generation of canine DCs in culture are listed in Table 1. Canine DCs can be generated *in vitro* from CD14⁺ peripheral blood mononuclear cells (PBMC) and the bone marrow by separation of CD34⁺ blood progenitor cells. Monocytes and bone marrow progenitor cells can be isolated by density gradient centrifugation and purified by their ability to adhere to plastic culture flasks or by antibody mediated cell sorting (de Carvalho et al.,

2004; Ibisch et al., 2005; Bonnefont-Rebeix et al., 2006; Isotani et al., 2006; Wijewardana et al., 2006; Xiong et al., 2010; Fitting et al., 2011). Subsequent differentiation of both cell types into DCs is achieved by application of different cytokines to the culture medium. The most widely used approach to generate monocyte-derived DCs (moDCs) is the stimulation of monocytes with recombinant IL-4 and GM-CSF (Ibisch et al., 2005; Bonnefont-Rebeix et al., 2006; Wang et al., 2007a; Ricklin Gutzwiller et al., 2010). Alternatively, canine DCs have been induced effectively from PBMC by T-cell conditioned media without the application of purified cytokines (Wijewardana et al., 2006). Additional application of human Fms-like tyrosine kinase 3-ligand (Flt3L) to the culture significantly increases the yield of DCs by up to four times compared to cytokine treatment alone (Wang et al., 2007a,b), which is necessary to obtain sufficient numbers of DCs for therapeutic applications (e.g. ~10⁷ DCs per vaccination/injection in human patients; Tuyaeys et al., 2007). Moreover, moDCs can be directly isolated from peripheral blood of dogs stimulated with rhFlt3L *in vivo* (Mielcarek et al., 2007). Canine moDCs have been generated successfully *in vitro* by serum-free methods using X-Vivo15 medium containing recombinant cytokines, recombinant human (rh) Flt3L, calcium ionophore, and Picibanil, respectively (Bund et al., 2010). The use of serum-free culture media reduces the risk of negative side-effects in adoptive DC-based immunotherapies by avoiding anaphylactic reactions to xenogeneic peptides (e.g. bovine serum albumin; Bund et al., 2010; Mackensen et al., 2000).

For the development of bone marrow-derived DCs (bmDCs), isolated CD34⁺ progenitor cells can be stimulated with rhGM-CSF, rhTNF- α and rhFlt3L (Fitting et al., 2011; Hägglund et al., 2000). Using plastic adherence methods, in contrast to feline Flt3L, human Flt3L has been reported not to support the growth and differentiation of canine bmDCs (Ricklin Gutzwiller et al., 2010). Variable Flt3L-induced effects might be related to the cell composition obtained by different isolation techniques and variable responsiveness of different DC subsets, respectively. Moreover, variation of the stimulatory capacity of recombinant human, feline and canine GM-CSF is probably attributed to the degree of sequence identity between the different mammal species (Isotani et al., 2006).

In vitro maturation of immature DCs of dogs can be achieved by LPS as an extrinsic factor or TNF- α as an intrinsic factor (Wang et al., 2007a,b). Additionally, different techniques have been developed to mature canine DCs by combining TGF- β , IL-6, IL-1 β and IFN- γ (Wang et al., 2007b). Notably, IL-12 alone added to immature DCs in culture has the ability to mature canine DCs. Since a stable

Table 1

Methods for isolation and generation of canine dendritic cells.

	Isolation method	Stimulants	References
Monocyte-derived dendritic cells	Plastic adherence	rhGM-CSF + rIL-4	Ibisch et al. (2005), Bonnefont-Rebeix et al. (2006), Ricklin Gutzwiller et al. (2010), Xiong et al. (2010)
	Plastic adherence	rhGM-CSF + rIL4 + rhFlt3L	Wang et al. (2010)
	Antibody mediated	T-cell conditioned media	Wijewardana et al. (2006)
	Plastic adherence	rhFlt3L (<i>in vivo</i> stimulation)	Mielcarek et al. (2007)
	Plastic adherence	Calcium ionophore + rIL-4; rhGM-CSF + rIL-4 + picibanil; rhGM-CSF + rhTNF α + rhSCF + rhFlt3L + rIL-4	Bund et al. (2010)
Bone marrow-derived dendritic cells	Antibody mediated	rhGM-CSF + rIL-4 + rIL-1 β + rhTNF- α	Weber et al. (2003)
	Antibody mediated	rhGM-CSF + rhFlt3L + rhTNF- α	Hägglund et al. (2000)
	Plastic adherence	rhGM-CSF + rIL-4	Isotani et al. (2006)
	Plastic adherence	rhFlt3L	Ricklin Gutzwiller et al. (2010)
	Plastic adherence	rhGM-CSF	Wasserman et al. (2012), Ricklin Gutzwiller et al. (2010)
	Antibody mediated	rhGM-CSF + rhFlt3L + rhTNF- α	Fitting et al. (2011)

Flt3L: Fms-like tyrosine kinase 3-ligand; rh: recombinant human; GM-CSF: granulocyte-macrophage colony-stimulating factor; IL: interleukin; rc: recombinant canine; rf: recombinant feline; TNF: tumor necrosis factor; SCF: stem cell factor.

mature phenotype of DCs is essential for antitumor immunity, the authors suggested strategies to manipulate the tumor environment, such transfection of IL-12-expression-vectors into canine tumor cells to improve the efficacy of DC-based cancer therapy in dogs (Sugiura et al., 2010). T-cell conditioned media also induces DC maturation in dogs, probably due to the presence of CD40L, TNF- α and IFN- γ . In humans, a higher expression of co-stimulatory molecules and IL-12 of DCs, indicative of a mature state, can be induced by T-cell conditioned media than by the use of purified molecules (CD40L, IFN- γ and TNF- α), but only few studies have compared the efficiency of different maturation stimuli upon canine DCs (Kato et al., 2001; Wijewardana et al., 2006).

The maturation state of DCs is critical for their immunological functions. It is therefore recommended to use mature DCs for vaccination strategies, while immature DCs have tolerogenic potential because they induce regulatory T cells and inhibit host immune responses (van Duivenvoorde et al., 2007). Thus, dependant on the application, the purity and maturity of DC preparations should be

assessed based on morphological, phenotypical and functional criteria for each protocol (Figdor et al., 2004).

3. Morphology of myeloid lineage dendritic cells

Similar to other animal species, cultured canine DCs represent non-adherent, balloon-like “veiled cells” with cytoplasmic projections (dendrites), variable size and shape, and large lobulated nuclei (Figs. 1 and 2; Ibisch et al., 2005; Wang et al., 2007a; Sugiura et al., 2010). In addition, binucleation of canine DCs has been described *in vitro* (Sugiura et al., 2010). Cytoplasmic projections are the most characteristic morphological features of DCs, which distinguishes them from macrophages. Moreover, in contrast to macrophages, canine DCs lack large lysosomal organelles or phagocytic vacuoles, respectively, but exhibit an abundant formation of the Golgi apparatus and endoplasmic reticulum (Ibisch et al., 2005). A unique ultrastructural finding that has been described only in dogs is the presence of so called periodic microstructures, which represent variable sized, electron dense granules with a wasps’ nest-like

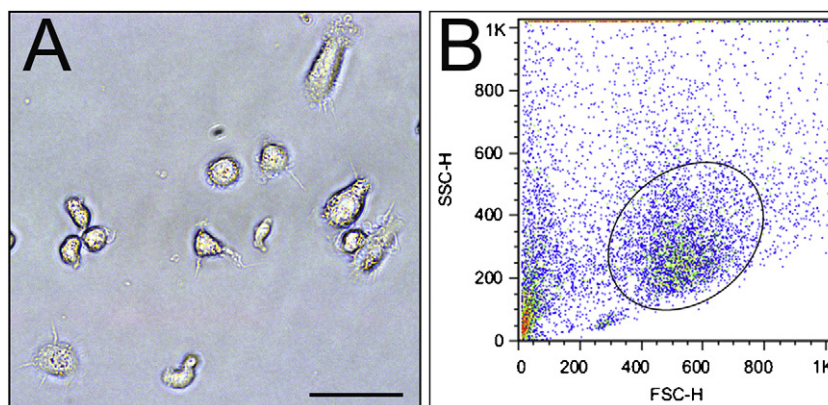


Fig. 1. Canine monocyte-derived dendritic cells stimulated with cytokines *in vitro* for seven days. (A) Non-adherent cells showing cytoplasmic projections characteristic of dendritic cell differentiation. Phase contrast microscopy, scale bar: 50 μ m. (B) Flow cytometric analysis of cultured cells: dot plot showing gated dendritic cell population (encircled cell population). FSC-H: forward scatter height; SSC-H: side scatter height.

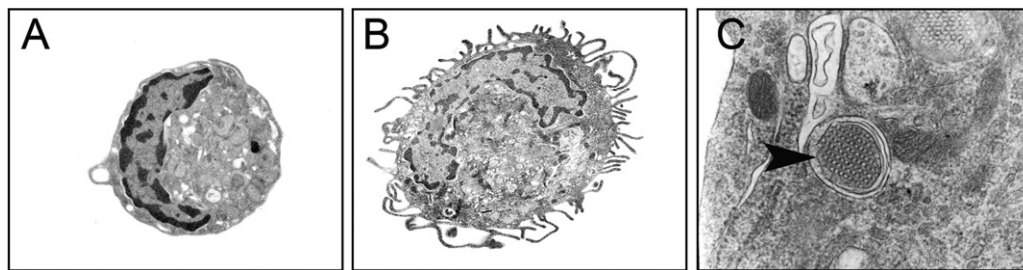


Fig. 2. Ultrastructural analyses of a canine monocyte and monocyte-derived dendritic cell by transmission electron microscopy. (A) Monocyte at day one in culture (magnification 2000 $\times$). (B) Cultured dendritic cell stimulated with cytokines at seven days in culture showing characteristic cytoplasmic projections (magnification 2000 $\times$). (C) Periodical microstructure (arrow head) in the cytoplasm represents a unique feature of canine monocyte-derived dendritic cells (magnification 50,000 $\times$).

appearance in the cytoplasm of moDCs and bmDCs (Fig. 2; Ibisch et al., 2005; Bonnefont-Rebeix et al., 2006; Isotani et al., 2006). In contrast to DCs, periodic microstructures are rarely observed in macrophages and never in granulocytes of dogs (Ibisch et al., 2005). They are expected to result from crystallization of accumulated proteins. However, the primary origin and function of periodic microstructures in DCs is still unclear (Ibisch et al., 2005; Marchal et al., 1995a). Similar structures occur in the cytoplasm of Langerhans cell-derived neoplastic cells, such as in canine cutaneous histiocytoma (Marchal et al., 1995a), while classical Birbeck granules, an ultrastructural feature of human Langerhans cells, are not observed in dogs (Moore et al., 1996).

Microscopic and ultrastructural analyses enable the discrimination between DCs and other cell types and thus give information about the purity of cell cultures. In addition, morphological features such as the density and length of cytoplasmic projections can be used as parameters for DC maturity *in vitro* (Figdor et al., 2004; Winzler et al., 1997).

4. Phenotypic properties of canine dendritic cells

Despite wide-ranging similarities of cell surface marker expression with human and murine DCs, canine DCs exhibit certain species-specific properties. A summary of phenotypic properties of different canine DC subsets is given in Table 2. In contrast to human beings and mice, canine moDCs do not lose the ability to express CD14 upon cultivation (Carrasco et al., 2001; Bienzle et al., 2003; Ibisch et al., 2005; Wijewardana et al., 2006; Ricklin Gutzwiller et al., 2010). However, CD14-negative cells with a DC-like morphology and T cell allostimulatory properties have been isolated from the blood of rhFlt3-stimulated dogs (Mielcarek et al., 2007). After seven days in culture, canine moDCs express high levels of MHC II and CD11c (Ibisch et al., 2005; Bonnefont-Rebeix et al., 2006). In addition, these cells show an increased expression of CD1a, CD40, and CD83 as well as of the co-stimulatory molecules CD80 and CD86 (Wang et al., 2007a; Ricklin Gutzwiller et al.,

Table 2

Expression of different marker molecules by canine myeloid dendritic cells compared to monocytes and macrophages.

Markers	Langerhans cells	Bone marrow-derived DCs	Monocyte derived DCs	Monocytes	Macrophages
CD1a	+(1,2)	+high (14,16)	+high (4,11,16)	+low (4,11)	+(7)
CD1b	+(15)	n.d.	n.d.	n.d.	n.d.
CD1c	+(1,8)	+(7,13)	+high (7)	–(7,10)	–(10)
CD4	–(1,8)	+(7,8)	–(2,7)	–(7)	–(7)
CD8	–(8)	+(7, 8)	–(2, 7)	–(7)	–(7)
CD11b	–(1,2,8)	+high (2,7)	+low (7)	+(7)	+(1, 7, 8)
CD11c	+(1,2,8)	+high (7)	+low (7)	+low (3,4,7)	+high (7)
CD14	–(8)	–(13,14)	+(3,9,11)	+(5,9,11)	+(3,7,8)
CD34	n.d.	–(12,13)	n.d.	n.d.	n.d.
CD40	n.d.	+high (7)	+high (4,6,7)	+low (4)	–(7)
CD45RA	–(1)	+high (7)	+low (7)	+low (7)	+high (7)
CD80	+(2)	+high (7)	+high (4,7,11)	+low (4,7,11)	+low (7)
CD83	n.d.	n.d.	+(6)	–(6)	n.d.
CD86	–(2)	+high (7,16)	+high (5,7,11)	+low (5,7,11)	+low (5,7)
CD206	n.d.	+high (7)	–(7)	–(6,7)	+high (7)
CD209	n.d.	n.d.	+(6)	–(6)	n.d.
MHC II	+(1,2,8)	+high (7,14)	+high (4, 7,11)	+low (4,7,11)	+low (7,10)
E-cadherin	+high (15)	–(15)	–(15)	n.d.	n.d.
ICAM-1	–(1,8)	+(1,8)	+(1,8)	n.d.	n.d.
TLR-3	n.d.	n.d.	+high (9)	–(9)	n.d.
Thy1	–(1,8)	+(8)	n.d.	n.d.	n.d.

DCs: dendritic cells; n.d.: not determined; +: molecule present; -: molecule absent; low: low expression level; high: high expression level.; References are given in parentheses: ¹Moore et al. (1996), ²Ricklin et al. (2010), ³Ibisch et al. (2005), ⁴Wang et al. (2007a), ⁵Bonnefont-Rebeix et al. (2006), ⁶Bund et al. (2010), ⁷Ricklin Gutzwiller et al. (2010), ⁸Affolter and Moore (2002), ⁹Bonnefont-Rebeix et al. (2006), ¹⁰Goto-Koshino et al. (2011), ¹¹Sugiura et al. (2010), ¹²Fitting et al. (2011), ¹³Häggglund et al. (2000), ¹⁴Weber et al. (2003), ¹⁵Baines et al. (2008), ¹⁶Moore et al. (2006).

2010). CD1 family molecules, which facilitate the presentation of non-protein antigens, are highly expressed on canine DCs (Affolter and Moore, 2002). All of these surface markers are present on canine macrophages/monocytes, too, but at a lower density compared to canine moDC as demonstrated by flow cytometry (Affolter et al., 2002; Ibsch et al., 2005; Wang et al., 2007a; Goto-Koshino et al., 2011). Accordingly, none of the typical markers which are used to differentiate DCs from monocytes/macrophages in human beings and mice, such as CD1c, CD11c and CD14, enable the precise identification of canine DCs. Noteworthy, Toll like receptor (TLR)-3 is abundantly expressed in moDCs of dogs, but not in monocytes. Thus, detection of TLR-3 by immunological or molecular techniques helps to discriminate between both cell types (Bonnefont-Rebeix et al., 2007). In conclusion, since markers exclusively specific for canine DCs are not available yet, a panel of different antibodies directed against surface molecules, such as CD1a, CD11c, CD40, CD80, CD83, CD86, CD206, CD209, and TLR-3 must be used to classify canine DCs by flow cytometric analyses (Moore et al., 2006; Bund et al., 2010; Ricklin Gutzwiller et al., 2010; Schwens et al., 2011).

Regarding the phenotype of mature canine moDCs differing results have been published. For example, following stimulation with LPS or IL-12 the levels of CD1a, CD40, CD80 and CD83 significantly increase in mature DCs compared to immature DCs, while MHC II and CD86 expression remain unchanged (Bonnefont-Rebeix et al., 2006; Wang et al., 2007a; Sugiura et al., 2010). Conversely, it has been demonstrated that LPS- and TNF- α -stimulated moDCs show a prominently increased expression of MHC II, CD11c, CD80, and CD86, while CD1a and CD40 on the cell surface are only slightly elevated (Table 3; Wang et al., 2007b; Ricklin Gutzwiller et al., 2010). These differences in surface marker expression might be a consequence of different isolation protocols and culture conditions used in these experiments.

Surprisingly, canine bmDCs and moDCs express low levels of CD4 and CD8, which is also observed in splenic DCs of mice (Yoshida et al., 2003; Montoya et al., 2005; Ricklin Gutzwiller et al., 2010). Similar to moDCs, bmDCs of dogs express CD1c, CD11c, CD11b, CD80 and MHC II, while upon LPS stimulation only CD80 has been reported to increase on bmDCs (Table 3; Ricklin Gutzwiller et al., 2010). It has been shown that canine bmDCs lack CD14 expression (Hägglund et al., 2000; Weber et al., 2003). However, other publications described the presence of CD14 expressing DCs derived from the bone marrow of dogs (Ricklin Gutzwiller et al., 2010). CD11b expression is higher in bmDCs than in moDCs. In contrast, the mannose receptor CD206 is expressed only in bmDCs of dogs and therefore represents

a useful marker for discriminating different subsets of DCs *in vivo* (Ricklin Gutzwiller et al., 2010).

Canine Langerhans cells express CD1c, CD11c, CD80, and MHC II *in situ*, which parallels the surface molecule expression pattern of human Langerhans cells (Zaba et al., 2009; Ricklin et al., 2010). Furthermore, canine Langerhans cells characteristically express the cell adhesion molecule E-cadherin (Baines et al., 2008; Pires et al., 2008), whereas in contrast to other species, canine Langerhans cells do not express S100, ATPase and ICAM-1 (Glick et al., 1976; Moore and Mariassy, 1986; Moore et al., 1996).

The paucity of specific DC markers in dogs demonstrates the need for further research, such as global analyses of the DC proteome and gene expression (Becker et al., 2012; Lehtonen et al., 2007). The identification of unique DC molecules represents a prerequisite for the generation of novel antibodies, which will enable adequate phenotyping of different canine DC subsets and the development of cell sorting techniques.

5. Functional properties of canine dendritic cells

In order to initiate T cell responses, DCs have to undergo a maturation process, which includes functional changes (Platt et al., 2010). For instance, while immature DCs primarily have the ability to capture and process antigens, increased co-stimulatory and antigen presenting capacities of mature DCs lead to the activation of naïve T cells (Banchereau et al., 2000; Lambotin et al., 2010). Therefore, appropriate characterization of canine DCs should include a phenotypical and morphological description as well as functional analyses, such as the measurement of phagocytic activity and quantification of the T cell stimulatory capacity in mixed leukocyte reactions (MLR).

5.1. Phagocytosis

Several signals, such as bacterial and viral products as well as cellular components following tissue injury can initiate the maturation of DCs (Ibrahim et al., 1995). Antigen uptake is performed by endocytosis or pinocytosis ("cell drinking"), respectively (Albert et al., 1998). The phagocytic activity of canine DCs can be measured by their ability to incorporate fluorescent-labeled microbeads or by the uptake of self-quenching fluorescence-labeled ovalbumin (Isotani et al., 2006; Wijewardana et al., 2006; Wang et al., 2007a). Similar to the response to antigen capture *in vivo*, all above mentioned methods for canine DC maturation in culture cause functional changes, including a decline of the phagocytic activity (Isotani et al., 2006; Wijewardana et al.,

Table 3

Expression of different cell surface markers in canine matured dendritic cells *in vitro*.

	CD1a	CD11c	CD40	CD80	CD83	CD86	MHC II
Bone marrow-derived mature dendritic cells	n.d.	↑(1)	↑(1)	↑(1,5,6)	n.d.	↑(1,5)	↑(1,5,6)
Monocyte-derived mature dendritic cells	↑(2,3,7)	↑(4)	↑(1,7)	↑(1,3,7)	↑(4)	↑(1)	↑(1,2,4)

↑: increased expression compared to immature dendritic cells; n.d.: not determined; References are given in parentheses: ¹Ricklin Gutzwiller et al. (2010), ²Wijewardana et al. (2006); ³Sugiura et al. (2010); ⁴Wang et al. (2007b); ⁵Isotani et al. (2006); ⁶Wasserman et al. (2012); ⁷Wang et al. (2007a).

2006; Wang et al., 2007a; Platt et al., 2010; Xiong et al., 2010; Wasserman et al., 2012).

5.2. T cell stimulatory capacity

MLR is based on the ability of DCs to induce activation and proliferation of T cells, mainly CD4⁺ T cells, which serves as a functional assay to determine the antigen presenting function of DCs (Banchereau and Steinman, 1998). As in humans and rodents, MLR of dogs is performed by co-incubation of antigen presenting cells with allogenic leucocytes, followed by measuring of the proliferation rate (e.g. by thymidine incorporation assay; Inaba et al., 1992; Garderet et al., 2001; Wijewardana et al., 2006; Wang et al., 2007a; Xiong et al., 2010; Ricklin Gutzwiller et al., 2010). As demonstrated in rodents, DCs have a high ability – more than 100 fold than macrophages and B cells – to stimulate allogenic lymphocytes and therefore represent the major cell type which initiates MLR (Banchereau and Steinman, 1998). In dogs, mature DCs have a higher ability to stimulate allogenic leukocytes than immature DCs (Weber et al., 2003; Ibsch et al., 2005; Bonnefont-Rebeix et al., 2006; Isotani et al., 2006; Wijewardana et al., 2006; Wang et al., 2007a; Bund et al., 2010; Wasserman et al., 2012). Moreover, canine bmDCs compared to moDCs have a higher ability to induce MLR (Ricklin Gutzwiller et al., 2010), which contrasts with findings in humans, where no difference in the T cell stimulatory capacity between bmDCs and moDCs have been observed (Syme et al., 2005).

Since information about time-dependent changes of DC maturation *in vitro* in dogs is scarce, attention must be given to potentially different behaviors among DCs subsets, and to the fact that the maturation state and especially functionality of DCs are reverse processes (e.g. after removal of stimulatory cytokines), which has implications for therapeutic applications and experimental studies using canine DCs (Romani et al., 1996; Nelson et al., 1999; Wijewardana et al., 2006).

6. Cytokine profile of canine dendritic cells

DCs produce a broad range of cytokines in order to initiate or modulate the immune response. Immature DCs recognize pathogens through pathogen-associated molecular patterns (PAMPs) using TLRs (Medzhitov et al., 1997; Scott et al., 2005). The interaction between PAMPs and TLRs induces the secretion of cytokines, such as IL-10, IL-12 and IFN- α , as observed in humans (Banchereau et al., 2000; Scott et al., 2005). Based on the stimulus, DCs show different cytokine profiles. LPS-stimulated canine DCs produce increased amounts of IL-1 β , IL-10, IL-12, IL-13 and TNF- α , while TNF- α -stimulated DCs secrete elevated amounts of IL-2, IL-4, IL-12, IL-13, TNF- α , TGF- β and IFN- γ (Wang et al., 2007b). LPS-stimulated DCs produce more IL-12 than TNF- α -stimulated cells. Neither LPS nor TNF- α has an impact on expression level of IL-15 and IL-18 in immature DCs and mature DCs, respectively (Wang et al., 2007b). The cytokine expression of LPS-matured canine DCs largely resembles the cytokine profile of LPS-matured DCs in humans and mice (Morelli et al., 2001; Elkord et al., 2005; Wang et al., 2007b). However, in contrast to other species, a prolonged

IL-10 expression together with increased levels of IL-13 has been observed in LPS-stimulated canine DCs (Wang et al., 2007b). Usually, an increased IL-13 expression in LPS-stimulated cells represents a compensatory mechanism upon decreased expression of IL-10, as demonstrated in human beings (D'Andrea et al., 1995). The expression of IL-10 and IL-12 in LPS-stimulated human DCs have been shown to be time-dependent. However, so far, dynamics of these cytokines have not been investigated in canine DCs. In contrast to mice, canine TNF- α -stimulated DCs have a higher ability to initiate Th2 immune response than LPS-stimulated DCs due to an elevated production of IL-4 and IL-13 (Langenkamp et al., 2001; Efron et al., 2005; Wang et al., 2007b).

In summary, cytokine expression analyses enables to determine the polarization (DC1 vs. DC2 phenotype) and maturation state of DCs and provides information about the preferential induction of Th1 or Th2 biased immune responses by canine DCs *in vitro* and *in vivo*.

7. Dendritic cells in canine diseases and translational models

Dogs are affected by spontaneously occurring neoplastic and inflammatory diseases which often share many similarities with pathological conditions in humans and are thus – besides their veterinary relevance – appreciated as important translational animal models (Kling, 2007; Spitzbarth et al., 2012). Further, there is an increased use of dogs for the development and application of cancer treatment in humans (Paoloni and Khanna, 2008; Wasserman et al., 2012).

7.1. Neoplastic diseases

Canine DC-derived neoplasms include benign cutaneous histiocytoma as well as malignant tumors, such as histiocytic sarcomas and DC leukemia (Schwens et al., 2011). Histiocytic sarcomas in dogs occur as localized and disseminated forms. Based on phenotyping analyses they express CD1, CD11c and MHC II (Moore, 1984; Laeng et al., 1986; Moore and Rosin, 1986; Kerlin and Hendrick, 1996; Affolter and Moore, 2000, 2002). Canine cutaneous histiocytoma is a benign epidermal neoplasm of Langerhans cell origin, which, similar to histiocytic sarcomas, expresses CD1, CD11c and MHC II. Additional detection of the adhesion molecule E-cadherin, which, among leukocytes is unique to Langerhans cells, enables the discrimination of this neoplasm from other dermal histiocytic tumors (Glick et al., 1976; Marchal et al., 1995a,b; Moore et al., 1996; Baines et al., 2008). Similar to Hashimoto-Pritzker disease, a human Langerhans cell disorder, canine cutaneous histiocytomas spontaneously regress due to Th1-mediated immune responses (Affolter and Moore, 2002; Kaim et al., 2006). Thus, canine cutaneous histiocytoma represents an interesting naturally occurring animal model for investigating tumor immunity and tumor regression (Kaim et al., 2006). Canine histiocytic tumors have to be discriminated from reactive histiocytosis (cutaneous and systemic), which represents a non-neoplastic immunoregulatory disorder, responsive

Table 4
Phenotypical analyses of canine neoplastic and reactive dendritic cell disorders.

Disease	Origin	Expression of marker molecules
Cutaneous histiocytoma	Langerhans cells	CD1a (F) ¹ , CD1b (FC) ² , CD1c (F) ¹ , CD11a (F) ¹ , CD11c (F) ¹ , CD18 (P, FC) ^{1,2} , CD45 (F, FC) ^{1,2} , MHC I (FC) ² , MHC II (F, FC) ^{1,2} , E-cadherin (P) ²
Histiocytic sarcoma (localized and disseminated)	Myeloid dendritic cells	CD1b (F) ³ , CD1c (F) ³ , CD11c (F) ³ , CD18 (P) ³ , CD45 (F) ³ , ICAM-1 (F) ³ , MHC II (F) ³ , Thy-1 (±) (F) ³
Dendritic cell leukemia	Immature interstitial dendritic cells	CD1c (F, FC) ⁴ , MHC II (F, FC) ⁴ , CD11c (F, FC) ⁴ , CD45 (F) ⁴ , CD80 (F) ⁴ , CD86 (F) ⁴ , Thy-1 (±) (F) ⁴
Reactive histiocytosis (cutaneous and systemic)	Activated interstitial dendritic cells	CD1a (F) ⁵ , CD1b (F) ⁵ , CD1c (F) ⁵ , CD4 (F) ⁵ , CD11c (F) ⁵ , MHC II (F) ⁵ , Thy-1 (F) ⁵

Marker expression was determined by immunohistochemistry using frozen (F) or paraffin embedded (P) tissue sections or by flow cytometry (FC), respectively. ±: variable expression. References are given as superscripts: ¹Moore et al. (1996); ²Baines et al. (2008); ³Affolter and Moore (2002); ⁴Allison et al. (2008); ⁵Affolter and Moore (2000).

to immunosuppressive therapies. Immunophenotyping of cells derived from reactive histiocytosis revealed the expression of CD1, CD11c, MHC II, and CD90 (Thy-1) as well as CD4, indicative of an activated state of dermal dendritic cells. Phenotypical characteristics of canine neoplastic and reactive DC disorders are summarized in Table 4. Detailed analyses of cell surface markers of histiocytic cells by flow cytometry and immunohistochemistry, respectively, enable the classification of DC proliferative disorders (neoplastic vs. reactive) and discrimination of histiocytic tumors from other hematopoietic neoplasms in dogs, which has importance for the treatment and prognosis of tumor patients (Moore et al., 1996; Moore, 2004; Fulmer and Mauldin, 2007; Allison et al., 2008).

7.2. Dendritic cell-based cancer immunotherapy

Due to the ability of DCs to process tumor-associated antigens and to induce antitumor immune responses, DC-based immunotherapies gain increasing interest in veterinary oncology (Bergman, 2009; Bergman and Wolchok, 2008). A widely used approach in cancer immunotherapy is to immunize cancer patients with autologous, patient-derived DCs being loaded with tumor antigen *ex vivo* (Palucka and Banchereau, 2012). Preclinical studies revealed the induction of antigen-specific CD4⁺ and CD8⁺ T cell responses in dogs vaccinated with DCs pulsed with canine melanoma or squamous cell carcinoma lysates (Tamura et al., 2007, 2008). In contrast, lysates of canine histiocytic sarcoma and neoplastic B cells fail to induce antitumor immunity, probably attributed to reduced immunogenicities of these tumors (Tamura et al., 2007). In laboratory beagles, fusion vaccines obtained by hybridization of cultured DCs and mammary cancer cells elicit tumor-specific immune responses without detectable side-effects (Bird et al., 2008, 2011). Moreover, injection of DC/tumor cell fusion hybrids have been demonstrated to foster adaptive immune responses and natural killer cell activity in dogs suffering from transmissible venereal tumors, accelerating tumor regression (Pai et al., 2011). Preliminary clinical studies also showed the induction of antigen-specific T cell responses in dogs with malignant melanoma which have been vaccinated with *ex vivo* generated DCs transduced with a viral vector encoding human melanoma antigen gp100 (Gyorffy et al., 2005).

Alternatively, tumor-associated antigens or mRNA encoding tumor-specific proteins can be directly delivered to professional antigen presenting cells, particularly DC *in vivo* (Palucka and Banchereau, 2012; Fitting et al., 2011; Thacker et al., 2009). Clinical trials using tyrosinase DNA vaccination in order to treat canine malignant melanoma, demonstrated the safety and efficacy of this technique with induction of tumor-specific humoral and T cell immunity as well as prolonged survival of affected dogs (Bergman et al., 2003; Grosenbaugh et al., 2011; Liao et al., 2006). In several canine neoplastic diseases, tumor-derived factors can alter the maturation of DCs, thereby suppressing antitumor immune responses or inducing immunological tolerance to tumor-associated antigens, respectively (Wasserman et al., 2012; Goulart et al., 2012). The development of novel strategies, such as CD40-targeted adenovirus vectors as an antigen delivery system, are underway to circumvent this problem by promoting proper activation and maturation of canine DCs which is crucial for adequate antigen-specific immune responses (Thacker et al., 2009).

In order to utilize DC in clinical trials it is necessary to have standardized, reproducible and easy to use protocols. A precise description of the preparation, cell viability before application and phenotypical analyses to determine the purity and maturity of DC populations will allow to compare observations from different clinical studies (Figdor et al., 2004).

7.3. Infectious diseases

Several pathogens, including human herpesvirus type 1 and measles virus as well as human and feline immunodeficiency viruses target DCs and have evolved strategies to modulate their cytokine expression and antigen presenting capacity, thereby promoting virus immune evasion and persistence (Tompkins and Tompkins, 2008; Steinman and Banchereau, 2007). Similar to human measles, canine distemper virus infection induced a profound and long-lasting immunosuppression in affected dogs (Beineke et al., 2009). During the chronic disease stage, DCs seem to serve as the primary host cells for the virus, which promotes viral persistence in lymphoid organs (Wünschmann et al., 2000). This process is assumed to inhibit terminal differentiation of DCs, responsible for disturbed repopulation of lymphoid tissues and diminished antigen presenting function in dogs, as described for measles virus infection (Kerdiles

Table 5

Comparison of canine, mouse and human dendritic cells.

	Canine	Mouse	Human	References
CD14 expression of cultured dendritic cells	+	–	–	Ibisch et al. (2005), Morelli et al. (2001), Zhou and Tedder (1996), Jiang et al. (2005)
CD8 expression of cultured dendritic cells	+	+	–	Ricklin Gutzwiller et al. (2010), Shortman and Liu (2002), Ardavin et al. (2001)
Periodic microstructures in cultured dendritic cells	+	– ^a	– ^a	Ibisch et al. (2005)
Birbeck granules in Langerhans cells	–	+	+	Moore et al. (1996), Valladeau et al. (2002), Wolf (1967)
Expression of ICAM-1, ATPase and S100 in Langerhans cells	–	+	+	Moore et al. (1996), Glick et al. (1976), Moore and Mariassy (1986)

+: present; –: absent.

^a Not described in literature.

et al., 2006; Schneider-Schaulies and Dittmer, 2006). In addition, canine distemper virus infection of thymic epithelial DCs may result in compromised maturation of T cells, promoting the release of immature, potentially autoreactive lymphocytes (Wünschmann et al., 2000). In canine and human visceral leishmaniasis an impairment of follicular DCs leads to an aberrant regulation of humoral immunity with increased susceptibility to opportunistic bacterial infection (Smelt et al., 1997; Silva et al., 2012). Follicular cell damage of dogs infected with *Leishmania infantum* is supposed to result in reduced CXCL13 production, which leads to deficient B cell migration and follicular atrophy in lymphoid organs (Silva et al., 2012). On the other hand, prolonged antigen presentation by intralosomal DCs in canine and human trypanosomiasis and aspergillosis, is responsible for delayed type hypersensitivity and pathogen-induced immunopathology, respectively (Andrade et al., 2000; Day, 2009).

7.4. Immune mediated diseases

Inflammatory bowel disease (IBD) in human beings and dogs is assumed to result from an inappropriate immune response to dietary antigens (Hugot et al., 2001; German et al., 2003; Abreu et al., 2005; Cerquetella et al., 2010; Junginger et al., 2012). Accumulating evidence suggests that DCs may be responsible for the breakdown of immunological tolerance (German et al., 1998, 1999, 2000; Hugot et al., 2001; Peters et al., 2005). However, the precise role of DCs in the pathogenesis remains poorly understood, since different studies came to different conclusions, probably as a consequence of the heterogeneity of DC population (Bernstein et al., 1998; Landers et al., 2002; Baumgart et al., 2009; Kathrani et al., 2011). Referring to this, depletion of CD11c⁺ cells, including DCs, in IBD-affected dogs might cause a loss of immunological tolerance, leading to chronic inflammation (Kathrani et al., 2011). Comparable to this, a loss of CD11c⁺ DC has been observed in pediatric Crohn's disease patients, which might induce an imbalance between anti- and pro-inflammatory intestinal DC populations and immoderate immune responses (Silva et al., 2004).

An immune dysregulation as a consequence of an imbalance between different DC subpopulations is assumed to also account for hypersensitivity in atopic dermatitis

of dogs, a chronic inflammatory skin disease of genetically predisposed animals (Hillier and Griffin, 2001; Bieber, 2008; Marsella and Girolomoni, 2009; Ricklin et al., 2010). Clinical and pathological findings in naturally occurring and experimentally induced atopic dermatitis (epicutaneous sensitization with house dust mite) in dogs are remarkably similar to the human counterpart. In addition, immunological reaction in atopic dermatitis, characterized by a dominance of an initial Th2 response, prominent IgE expression, Langerhans cell hyperplasia and infiltration of inflammatory epidermal DCs can be observed in affected humans and dogs, indicative of a similar pathogenesis in both species. Moreover, IgE-bearing DCs in canine and human atopic skin lesions increases the antigen presenting capacity of these cells (facilitated antigen presentation), which initiates and maintains inflammatory responses (Moore and Schrenzel, 1991; Olivry et al., 1999; Nuttall et al., 2002a,b; Marsella and Olivry, 2003; Pucheu-Haston et al., 2008; Marsella and Girolomoni, 2009; Ricklin et al., 2010).

The pathogenesis of autoimmune diseases is complex, often with an undetermined primary etiology. Canine autoimmune hemolytic anemia, autoimmune thyroiditis, and systemic lupus erythematosus, represent spontaneous models for human medicine. In these disorders, autoimmunity is associated with an abnormal interaction between DCs and T cells which potentially triggers the onset of the disease and initiates excessive inflammatory processes (Happ, 1995; Day, 1999; Gershwin, 2007; Wilbe and Andersson, 2012). Understanding the heterogeneity of DCs and their functional alteration by local factors will provide essential clues for the pathogenesis of inflammatory disorders.

The generation of regulatory DCs, e.g. by probiotics, represents an applicable treatment of immune disorders, as demonstrated in murine models for IBD, atopic dermatitis and rheumatoid arthritis (Kwon et al., 2010). Similarly, the adoptive transfer of tolerogenic DCs has the potential to restore immune tolerance to self-antigens in autoimmune disorders, as observed in experimental rodent models for rheumatoid arthritis (van Duivenvoorde et al., 2007). Moreover, the beneficial effect of allergen-specific immunotherapy in veterinary and human immune mediated diseases is supposed to be based on the induction of regulatory T cells by DCs (Loewenstein and Mueller, 2009).

8. Concluding remarks

With the increasing interest and emerging knowledge about DC-based immunotherapy, dogs have become a suitable large animal model for translational research. The awareness of similarities with other species and species-specific properties of canine DCs facilitates the comparability of human and canine diseases. Examples of phenotypical and morphological features which discriminate canine cells from rodent and/or human DCs are listed in Table 5. Functional differences include an increased IL-13 expression by canine DCs following LPS stimulation, which has not been reported in other species (see chapter 6; Wang et al., 2007b; D'Andrea et al., 1995) and the higher lymphocyte stimulatory capacity of bmDCs compared to moDCs of dogs, which contrasts with findings in humans (see chapter 5.2; Ricklin Gutzwiller et al., 2010; Syme et al., 2005). Different from mice, canine TNF- α -stimulated DCs can lead to Th2 polarization, which potentially promotes immune tolerance in DC-based therapies (see chapter 6; Efron et al., 2005; Langenkamp et al., 2001; Wang et al., 2007b). Future research has to expand the existing knowledge about DC function in canine diseases as a prerequisite for the development of future therapies. In this context, novel methodological approaches such as canine specific DNA microarrays and proteomics (Greer et al., 2010; Klose et al., 2011) will offer a great opportunity to gain a more sophisticated insight into the function of canine DCs.

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