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Review article

Antigen presenting cells in mucosal sites of veterinary species

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Abstract – The ability of antigen presenting cells, in particular dendritic cells, to integrate a variety of environmental signals, together with their ability to respond appropriately by initiating either tolerance or defensive immune responses make them cells of particular relevance and importance in the mucosal environment. They have been demonstrated in a variety of mucosal tissues in veterinary species and have been characterized to varying degrees, showing that fundamental immunological principles apply throughout all species, but also highlighting some species differences. A major advantage of carrying out immunological research in veterinary species is their size: it is possible to cannulate lymphatic ducts and obtain information about cell migration between different tissues. It is also possible to obtain pure populations of relatively rare cell types such as the plasmacytoid dendritic cells or mucosal dendritic cells ex vivo for the study of immune responses to diseases in their natural host and for other thorough functional studies. Two major myeloid antigen presenting cell (APC) (dendritic cells, DC) cell populations have been described in gut draining lymph and other mucosal sites in ruminants and pigs, characterised by the presence or absence of surface molecules, their enzyme profiles, their ability to phagocytose and their different potential as APC. There is evidence that one of these subsets has migrated from the diffuse mucosal tissue, where it is found as a phagocytic as well as stimulatory APC population, which in turn may be derived from blood macrophages. In addition, the presence and role in viral infection of the IFN-α producing plasmacytoid DC in mucosal tissue is discussed, based on studies in pigs.

dendritic cells / plasmacytoid dendritic cells / macrophages

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1. INTRODUCTION

The ability to present antigen is a functional property of a cell and involves the uptake of whole molecular or particulate antigen, the use of enzymatic processes to break it down to small peptide sequences and the re-expression and presentation of these peptide sequences to specific CD4 T cells. These T cells respond by proliferation, changes in cell surface molecules and cytokine secretion, which can in turn affect the presenting cell.

The term "antigen presenting cell" (APC) is therefore defined by its function and not necessarily by any particular characteristic cellular molecule. However, one molecule is required for antigen presentation: MHCII [1, 70]. MHCII is the molecule that carries the re-expressed peptides to the surface of the presenting cells and is recognised, together with its load, by the responding T cell. However, although the presence of MHCII is required, its expression is not always indicative of antigen presentation.

Many cells are capable of antigen presentation and are usually classified as either professional or non-professional APC [70]. As their name implies, antigen presentation is probably the major function of a professional cell, whereas non-professional cells such as epithelial, endothelial or other tissue cells may in vitro, under certain circumstances, present antigen. The significance of such non-professional presentation in vivo is still controversial, and of relevance to this review is the fact that there appears to be considerable between species variation in the types of cells implicated in such non-professional antigen presentation.

The professional presenting cells in turn are a complex family of leucocytes, consisting of monocytes/macrophages, B cells and dendritic cells (DC) and their subsets. Even the subdivision into macrophages and dendritic cells is not unambiguous, as the ontogeny of some DC suggests that they have a lineage relationship with monocytes/macrophages: dendritic cells can be generated in vitro from blood monocytes [14, 108], and immature tissue dendritic cells appear to share many functions with tissue macrophages [79]. In addition, they appear to be derived from at least two different precursors, macrophages and myeloid DC are as their name implies of myeloid origin and plasmacytoid DC are of lymphoid origin [97]. Macrophages are generally thought to be derived from blood monocytes after migration into the tissue environment [71], they are characterised by relatively high phagocytic activity and correspondingly high levels of lysosomal enzymes. DC are characterised morphologically by their long dendritic processes and functionally by their high stimulatory activity, yet are highly plastic, both phenotypically and functionally and therefore not easily defined.

It appears that this functional group of cells called DC is of outstanding importance immunologically [75, 97, 98]. Only DC are capable of presenting antigen to naïve T cells and of triggering their responses, all other APC can only stimulate memory T cells. DC therefore occupy a very unique functional niche in the immune system and have attracted a huge amount of interest and research. Such research has shown that DC appear to integrate a large variety of environmental signals, sensing the presence of bacterial and viral antigens by various specific receptors such as the toll-like receptors (TLR) and others, and that they appear to be capable of a "value" judgement of such prokaryotes as "dangerous" or "harmless" and initiating appropriate responses, although the exact mechanisms are outside the scope of this review and also still largely obscure. Clearly, the decision making ability of an

APC is particularly important in mucosal tissue, as the outcome may be one of two almost diametrically opposite types of response: an active defensive response to mucosal pathogens or the opposite "tolerant" response to harmless environmental agents such as food-derived antigens and commensal bacteria. As most animals are constantly exposed to the latter two harmless agents and on rarer occasions to the pathogenic ones, we must assume that the "default" or "normal" mechanism in such mucosal sites is a state of immunological tolerance. In agreement, it has been found that DC found in and migrating from mucosal tissue are involved in "tolerogenic" mechanisms.

The ability to present to naïve T cells can be mimicked in vitro by the so-called mixed lymphocyte reaction (MLR): DC cause allogeneic T cells of irrelevant specificity to respond by vigorous proliferation, other APC cause little if any such proliferation. This assay gives us a functional handle on the importance of a particular cell as a primary presenting cell, of importance if much other information about phenotype etc. is missing, due to the lack of reagents.

There have been many attempts to categorise APC and match phenotypic characteristics with functional properties [62]. The terminology used to describe such cells is largely dependent on the analytical tools used in their study. In turn, the tools available for such studies are constrained or shaped by the species in which these studies are conducted. An additional problem for such studies is their plasticity [72, 108]; we are probably looking at cells undergoing constant and possibly reversible functional and phenotypic changes as well as cells, which can be divided into discrete subsets of different ontogeny. Another property of APC and particularly DC hampering our studies are their migratory habits [35]: DC are thought to acquire antigen in peripheral tissues and then migrate to the draining lymph nodes for antigen presentation. As the vast majority of immunological research is carried out in rodents and humans, of necessity our knowledge is biased towards these species. Clearly, rodents will remain the focus of scientific research, originally because of cost and ease of handling, now also because of the availability of transgenic animals and other research tools such as immunological reagents. Humans on the other hands are of interest for purely "selfish" reasons, but as far as scientific research is concerned will always suffer from the availability of tissue from healthy donors as controls or for more fundamental studies. However, there is a lot of evidence that many mechanisms we talk about as a general phenomenon may in fact be predominantly a phenomenon in rodents. For example, it has been shown recently that pigs are a better model for humans than rodents to study the so-called plasmacytoid DC (pDC) [36], these studies will be described in detail in this review.

Therefore, rather than looking at species differences as an obstacle in the path of our attempts to understand a functional immune system, we can turn this around and interpret it as an opportunity to broaden our understanding of the basic principles underlying immune mechanisms, as well as a chance to utilise different species for the study of different aspects of an immune function, always bearing in mind that species differences do exist.

In fact, this opportunity has already been grasped in the research carried out with veterinary species. Apart from research based on our interest in such species as food sources or as our companions, they offer us the accessibility of rodents, the advantages of size of humans and their phylogenetic diversity for studies designed to complement the knowledge gained from the more traditional objects of our research.

The majority of our knowledge of mucosal APC in rodents is based on inductive sites such as Peyer's patches and mesenteric lymph nodes, which are more suitable as sites of cell isolation. In contrast, studies of APC from the diffuse lymphoid mucosal

tissue in rodents are usually based on artificially expanded populations: because of their small body size, mucosal sites are relatively small, with few APC. Therefore, workers have resorted to expanding their numbers by the application of chemical reagents such as flt-3 ligand, leaving open the question as to how "normal" such an expanded cell population and the resulting immune system is. Therefore, APC from effector sites such as colon and small intestine are not well investigated in rodents, there are only a handful of studies, which have obtained cell preparations of sufficient purity and numbers for such functional studies. In contrast, these sites are easily accessible and yield large numbers of pure cell populations in humans (with the proviso of "normal" tissue sources) and domestic animals such as cattle, sheep and pigs.

DC migration from intestinal tissue has been studied in rats, where the thoracic duct is of sufficient size to be cannulated [60]. However, the thoracic duct also drains nonmucosal tissues, so not all cells are of mucosal origin. Sheep and pigs are eminently suitable for such studies of cell migration; in particular, a model involving the cannulation of re-anastomosed pseudo-afferent lymph ducts after the removal of lymph nodes has yielded information based on cells emigrating exclusively from mucosal tissues [86, 87]. Figure 1 shows this technique and the migratory routes of leucocytes in mucosal tissues.

This review will look at our knowledge of mucosal tissue sites in turn, starting from Peyer's patches, with a brief summary of existing knowledge based on rodents and humans, followed by a discussion of all available studies in veterinary species. As mentioned, there will be a final section dealing with the pDC.

2. MYELOID APC

2.1. Peyer's patches

Peyer's patches (PP) are thought to be the primary inductive sites, where soluble and particulate antigen is taken up by specialised epithelial cells, the so-called microfold or M cells [27]. M cells themselves do not express MHCII and are presumably not involved in antigen presentation, but "hand over" the antigen to professional APC, i.e. DC, resident in the sub epithelial dome (SED), the follicles and the parafollicular T cell areas of the PP.

In mouse PP, it was shown that DC of one phenotype are localized in T cell areas and DC of a different phenotype in the dome and corona region of the follicle, suggesting two distinct roles in antigen uptake, processing, and presentation [57, 88, 89]. It was demonstrated that the sentinel antigen presenting cells at the dome region are immature in terms of their antigen-uptake capacity, display low stimulatory activity for resting T cells, but can be matured by 24 h culture in the presence of granulocyte-macrophage colony-stimulating factor and tumour necrosis factor or anti-CD40, after which the cells undergo dramatic phenotypic and functional changes characteristic of DC maturation. They lose the ability to take up proteins such as ovalbumin, and in parallel with this decline, the biosynthesis of MHCII and invariant chain is dramatically down-regulated or eliminated. Recently, with the availability of more DC specific reagents, at least four different CD11c+ DC subsets have been identified in mice PP [8, 53]. There were only a few macrophages in mouse PP: the surface molecules identified by Mac-1 and F4/80, markers commonly used to identify mouse macrophages, were present only on small subpopulations [57, 88].

Rat PP APC were also characterised and compared to similar cells from LP and draining lymph [61]. Liu and MacPherson also found marked phenotypic heterogeneity in rat PP DC, such as Thy-1, CD2 and the iC3b receptor. Rat PP DC were also shown to be relatively poor at primary antigen presentation and could only stimulate a moderate MLR, but similar to mouse could be activated after short in vitro culture.

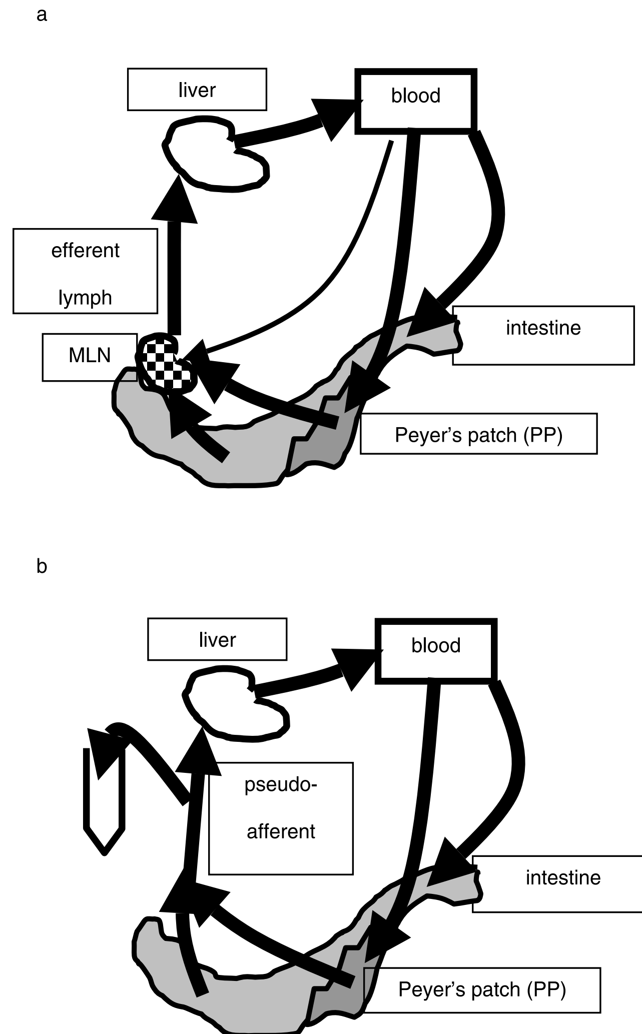


Figure 1. Diagram of gut-associated mucosal tissue and migratory routes of leucocytes. (a) Intact MLN, (b) excised MLN with re-anastomosed lymph ducts, creating pseudo-afferent lymph for cannulation.

However, Liu and MacPherson suggest that functional differences are less due to maturation, but more to the activation status of the APC.

Human PP and lamina propria (LP) APC have been characterised by immunohistochemistry, using paired labelling of the cytosolic leukocyte L1 protein (or calprotectin) [11], together with CD68 expression to identify DC and macrophages respectively. Bjerke et al. found that CD68 expression and L1 expression were mutually exclusive in healthy tissues. In the dome region of Peyer's patches, CD68+L1-macrophages were abundant, but not in LP. Also subepithetial and interfollicular CD68-interdigitating dendritic cells in Peyer's patches (recognised by antibody to S-100

protein) were usually unreactive with L1 antibody. However, none of the reagents were later found to uniquely identify DC, L1 is expressed by DC and eosinophils and the S-100 protein is also ubiquitous [11].

PP APC cells have also been characterised in pigs. Again, it was apparent that several subsets of APC were present, identified by different intensity of immunohistochemical labelling with the porcine myeloid marker SwC3, now known to be the porcine homologue to CD172a (SIRPα) [69]. In a second study, cells were also isolated from pig PP based on their low buoyant density [68]. A large proportion of these cells expressed high levels of MHC II, indicative of DC, and only a subset expressed CD172a, but there were also plasma cells, eosinophils and others. There was low level expression of the lysosomal enzyme non-specific esterase (NSE) and low ATPase, but acid phosphatase and β-galactosidase were absent. This cell population could induce a MLR, however, as the exact proportion of DC in this cell preparation was not known, an assessment of their stimulatory potential on a per cell basis was not possible.

Interestingly, other pig studies enabled the assessment of the impact of environmental antigens on the immune development of the PP as a whole: newborn animals had only rudimentary PP, which increased in size postnatally by a factor of 100 between days 1 and 10. This development was largely absent in germfree animals [85].

Mucosal DC have also been implicated as "Trojan" horses for various pathogens, the causative agent of bovine spongiform encephalitis (BSE), the abnormal prion protein (PrPSc), was found frequently in APC in Peyer's patches in the distal ileum of infected cattle [103].

2.2. Mesenteric lymph nodes (MLN)

After encountering antigen in PP, it is generally thought that DC migrate out to the draining mesenteric lymph nodes. Cells in such draining lymph can however originate both from the organised lymphoid tissue, i.e. the PP, as well as from the diffuse lymphoid tissue. Such APC in transit will be described in the next section. There is relatively little phenotypic and functional information about DC in the MLN themselves. However, the involvement of the MLN is generally thought to be crucial in the induction of oral tolerance to soluble proteins such as ovalbumen [95]. It has also been shown in germ free mice that live commensal bacteria ingested in PP reach the MLN in DC and not in macrophages [65], using CCR7 as a homing molecule and are retained in this tissue. Therefore, MLN and presumably MLN DC are implicated in tolerogenic mechanisms both to food derived soluble peptides as well as commensal bacteria.

Liu and MacPherson [61] isolated DC from rat MLN and found that fresh lymph node DC stimulated a MLR or oxidative mitogenesis efficiently, without requiring in vitro activation/maturation. This implies that once DC from PP reach the draining lymph node, they have undergone functional changes, rendering them capable of potent T cell stimulation.

Relatively few studies have looked at APC isolated from MLN of veterinary species.

2.3. Diffuse lymphoid tissue

By far the greatest number of mucosal APC are found in the diffuse lymphoid tissue of the large and small intestine.

Until relatively recently, this tissue has received very little attention, the presence of T cells of a memory phenotype led to the conclusion that these tissues had predominantly recall effector functions, the role of large numbers of APC was therefore unexpected and was often ignored.

Additional reasons for this lack of interest was probably the fact that healthy rodents in comparison to other species appear to have relatively few APC in this

tissue, whereas species such as the pig frequently show villi in the small intestine, which are almost filled with a dense network of MHC II + APC [42]. In addition, APC from this diffuse lymphoid tissue have been variously classified as "macrophages" or "DC", depending on the species studied and the criteria used for the characterization of a "macrophage" or a "DC". The first studies identifying MHCII+ DC in LP were done in rats [74], and their antigen sampling potential in mice was suggested by their ability to express tight cell junction proteins and insert themselves into epithelium [80]. However, only when it became possible to isolate pure APC from this site and to study their functional properties, could their stimulatory potential and therefore their dendritic nature be confirmed.

A truly heroic effort was made to isolate such APC from mice, but only after their numbers had been expanded by flt3 ligand [76]. Again, different subsets were identified, including some unusual ones, such as DC expressing both CD11c and MHCII at low intensity. These DC could be loaded by feeding antigen in vivo, and produced immuno-regulatory cytokines such as IL-10 and type I IFN, suggesting a role in immunoregulation and tolerance induction. However, these intestinal DC appear not to be inherently tolerogenic, but "quiescent" in a healthy animal and capable of presenting antigen and inducing tolerance, but being sufficiently responsive to inflammatory stimuli to allow T cell priming and protective immunity when necessary.

In humans [92–94], the presence of large numbers of unusual "macrophages", isolated by counter flow elutriation from healthy intestinal tissue have been reported. These authors also studied the functional properties of such human intestinal APC and show that they express high levels of MHCII, are highly phagocytic, express TLR2 and TLR4, but do not express innate response receptors such as CD14 (LPS receptor) or receptors for Fcα, Fcε or Fcγ, nor did they express growth factor receptors for IL-2 (CD25) and IL-3 (CD123). They also did not produce proinflammatory cytokines, including IL-1, IL-6, IL-10, IL-12, RANTES, TGF-β, and TNF-α, in response to an array of inflammatory stimuli but retained avid phagocytic and bactericidal activity. However, these authors show in vitro that intestinal stromal cell-derived products can induce blood monocytes to adopt the functional and phenotypic properties of such gut macrophages. Surprisingly, the functional test in a MLR was not carried out for the human ex vivo cells.

It seems possible or indeed likely that the unusual human "macrophages" and the unusual rodent "DC" are the same cells, with properties shared by immature DC and macrophages. As blood monocytes are used to derive DC cells in vitro, it seems reasonable to assume that blood monocytes can also give rise to DC in vivo.

Studies of similar cells in the pig, isolated by flow cytometric cell sorting and therefore representing pure cell populations, tend to support the hypothesis that LP APC have functional and phenotypic properties of both "macrophages" as well as "DC". Our group has isolated large numbers of such APC from the jejunum of normal pigs [42], characterised by high levels of cell surface and intracellular MHCII and co-expression of CD172 (SIRPα) [2] and high levels of CD16. Other surface molecules expressed on the majority of these cells were CD11R1 (the porcine homologue of CD11b [24]) and CD11c. They also carried the porcine myeloid marker SwC8 [38, 40]. These characteristics were confirmed by immunohistochemistry. Previous studies of less pure cell populations [55, 56] showed cells with large round or irregular nuclei and endocytic vacuoles, with cells with round nuclei showing more vacuoles and cells with irregular nuclei more RER, probably representing cells of different maturation/ activation status. In parallel, cells with variable proportions of cytoplasmic and cell surface MHCII could

be seen [39]. Lysosomal enzymes indicative of phagocytic activity were also present, such as non-specific esterase, acid phosphatase and β-galactosidase. In agreement, these APC were highly phagocytic, ingesting both bacterial ghosts and cellular debris. Interestingly, we could also demonstrate entire ingested CD3+ cells inside such MHC II+ cells isolated from pig jejunum (Fig. 2) [39], suggesting a potential mechanism for the removal of T cells prone to apoptosis [6]. This may well be a mechanism linked to the maintenance of tolerance. However, these cells were also potent stimulators of an MLR and therefore functional DC. Although generally considered as a monocyte/macrophage marker, the presence of the Fcγ receptor CD16 as well as high levels of cytoplasmic MHCII may be a reflection of their "hybrid" status: the presence of Fcγ receptors has been demonstrated on immature DC in humans [79] and on DC from draining lymph in sheep [20], where it was shown that engagement of these receptors cause maturation and a substantial modulation of the dendritic cell surface phenotype after immune complex uptake. A close association of these DC with CD4+ but not with CD8+ T cells has recently been demonstrated [51], using an automated image analysis technique [52]. Therefore, it appears that these DC are not just sampling antigen in the gut, in agreement with the studies in mice, where the uptake of food-derived antigen by these cells has been described [76], but also interact with local CD4+ T cells. Figure 3 shows a multicolour image, where the close juxtaposition between T cells and APC can be seen in a pig jejunal villus. Figure 4 is a diagrammatic representation of the immune structure of the pig jejunum, illustrating the distribution of all major leucocyte subsets found in this site.

As it has been shown that local T cells are all of advanced memory phenotype and prone to apoptosis when stimulated in vitro [5, 6, 41], a mechanism of tolerance by the elimination of food specific T cells can be envisaged.

In addition, these studies of sorted "pure" cell populations highlighted a potential confounding phenomenon: it proved almost impossible to detach these DC from accompanying T cells, possibly because the process of phagocytosis had started. Therefore, the reports of T cell surface molecules on DC based purely on flow cytometry have to be treated with caution.

MHCII expression has also been studied in the jejunal LP of other veterinary species, with essentially similar cellular distribution to that in rats and pigs: most MHCII is located in a dense network underlying the epithelium. In cats, MHCII expression was studied in healthy and IBD cats, it was found elevated in the inflamed tissue [105]. Similarly in dogs, MHCII+ networks of DC were observed [28] in healthy dogs, which was increased in dogs with ulcerative colitis, but not in dogs with dietary hypersensitivity [29, 30, 107]. A similar picture of APC underlying the gut epithelium was observed in chicken [73], however, characterisation was by a relatively uncharacterised antibody said to recognise monocytes, macrophages and interdigitating cells.

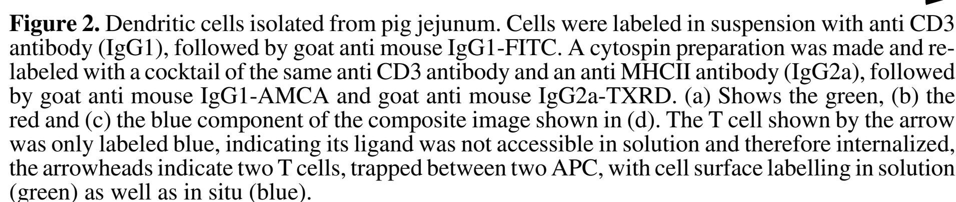
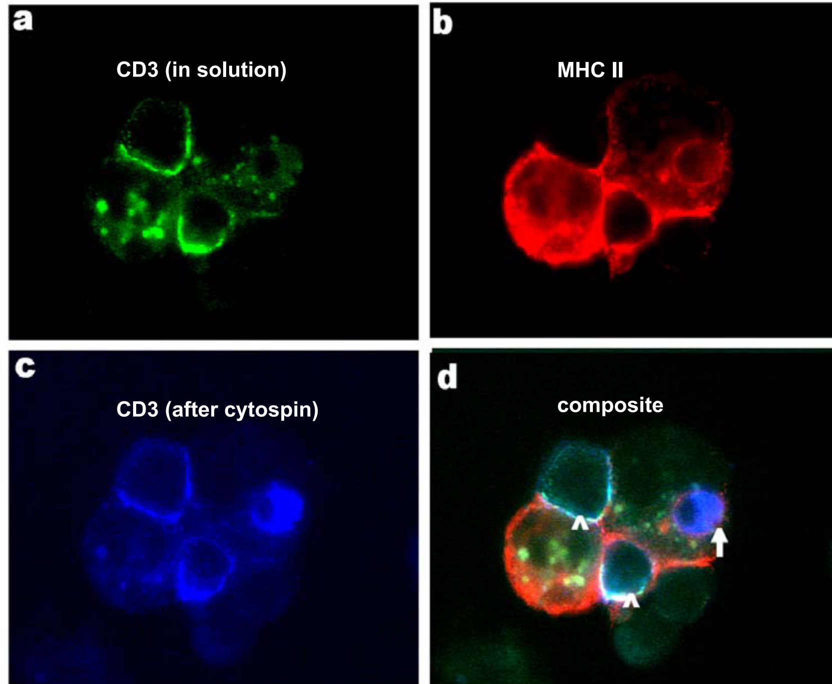


Figure 2. Dendritic cells isolated from pig jejunum. Cells were labeled in suspension with anti CD3 antibody (IgG1), followed by goat anti mouse IgG1-FITC. A cytospin preparation was made and relabeled with a cocktail of the same anti CD3 antibody and an anti MHCII antibody (IgG2a), followed by goat anti mouse IgG1-AMCA and goat anti mouse IgG2a-TXRD. (a) Shows the green, (b) the red and (c) the blue component of the composite image shown in (d). The T cell shown by the arrow was only labeled blue, indicating its ligand was not accessible in solution and therefore internalized, the arrowheads indicate two T cells, trapped between two APC, with cell surface labelling in solution (green) as well as in situ (blue).

Figure 3. Photomicrograph of pig jejunum, showing close juxtaposition of CD16+ DC and CD4+ T cells in the LP, labeled in green (some green background staining, only dark green staining is specific); CD8+ T cells in blue in epithelium.



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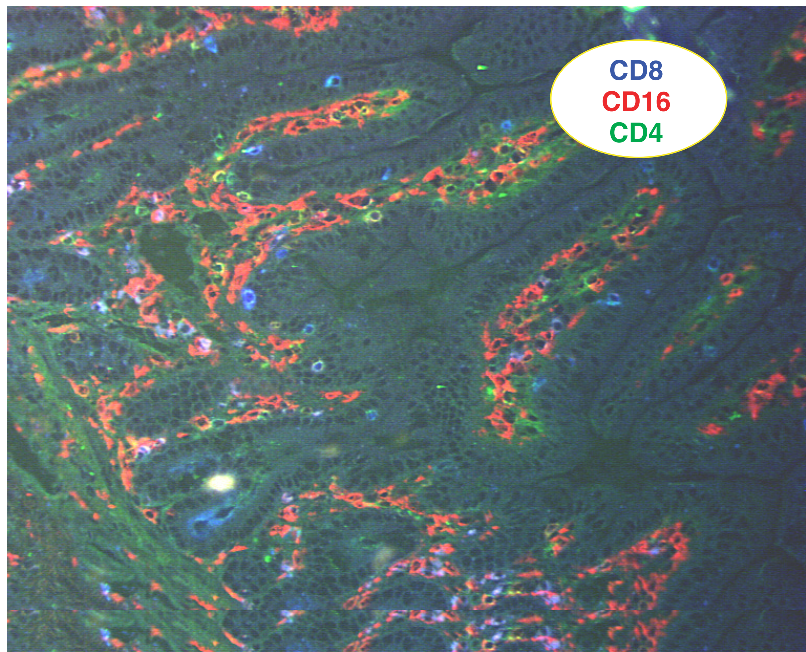

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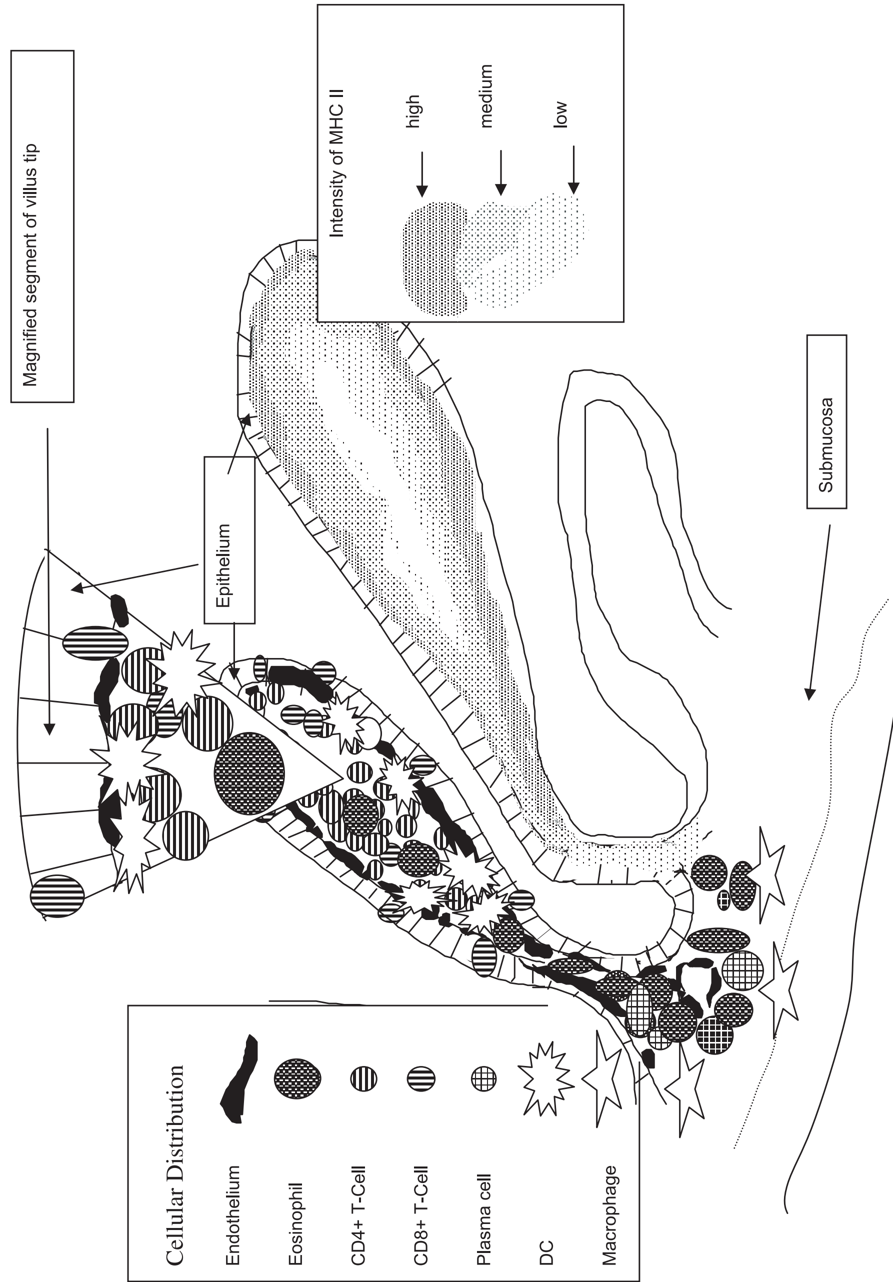

Figure 4. Diagram of major leucocyte subsets in pig jejunum.

2.4. MHC II on stromal tissue

In rodents and humans, mucosal epithelial cells can express MHCII, with increased levels in inflamed tissues. Their ability to present antigen has been demonstrated in vitro. However, the relevance of an equivalent in vivo mechanism is not clear. It cannot be a universal mechanism, as pigs never appear to express MHCII on epithelium [42], even in inflamed tissues, whereas cats have been reported to express MHCII only in diseased epithelial tissues [105]. In dogs, MHCII was variably expressed on enterocytes in the small and large intestine, which increased in animals with ulcerative colitis [29].

However, normal endothelium in pigs expresses high levels of MHCII [42, 64] and isolated endothelial cells can trigger recall but not primary responses in vitro [91].

2.5. Migration of APC out of mucosal tissues

The study of cells migrating out from mucosal tissues alone has been a particularly strong field for veterinary mucosal immunologists. Here, the advantages of size, allowing the cannulation of afferent and efferent lymphatics in cattle and sheep and "pseudo-afferent" lymphatics in pigs have yielded valuable information.

The only rodent from which similar information is available is the rat, however, as mentioned, such thoracic duct leucocytes may well originate from non-mucosal tissues. Two major subsets in such draining lymph have been identified [62, 66, 67] in the rat. DC co-expressing CD4 and OX41 (SIRPα) represent about 50–60% of thoracic duct DC and are more potent APC in MLR, but are excluded from T cell areas of MLN. They express short fine processes and low non-specific esterase, whereas CD4-OX41- DC are less potent, strongly positive for B7, express long pseudopodia, have high non-specific esterase levels, and many cytoplasmic inclusions in the form of material from apoptotic enterocytes. However, they cannot present native Ag but can stimulate strong MLR, can phagocytose allogeneic cells in vitro, are poor stimulators of CD8(+) T cells, and can lyse NK- sensitive target cells [49]. These differences are stable in culture; therefore probably represent two discrete subsets. Both populations express similar amounts of MHCII, ICAM-1, CD11b/c and OX62.

Very similar SIRPα– and SIRPα+ DC subsets have been isolated from cattle and sheep draining lymph. In cattle, the presence of CD26 on the CD4-SIRPα– subset has also been demonstrated, and a greater proportion of this subset was shown in lymph draining the gut mucosa. in comparison to draining lymph from skin [31]. In addition, the bovine homologue to human CD205 was shown to be present on both DC subsets, thereby confirming their DC nature [32].

Again, the SIRPα+ cell was the most potent stimulatory cell, causing both CD4 and CD8 T cells to proliferate. These cells also contained more of both transcripts and protein for IL-1 and of transcripts for IL-6 [99]. In contrast, the SIRPα– cell effectively stimulated CD4+ but not CD8+ T lymphocytes [48], but did not induce anergy or death nor secreted an inhibitory factor. This subset produced considerably more interleukin-12 [99]. Interactions between both DC subsets were also shown: supernatant from CD8(+) T cells cultured with the SIRPα+ DC significantly enhanced proliferation of CD8(+) T cells in response to the first subset DC, an effect that was blocked by interleukin (IL)-1 α, but not IL-1 β, specific mAb. The proliferation of such SIRPα– DC and CD8+ T cell co-cultures was also enhanced by adding IL-1 α, but not by IL-2, IL-6, IL-12, or IL-15. It was therefore concluded that the failure to stimulate CD8+ T cell proliferation resulted from the lack of IL-1 α synthesis by this population. The cytokine secretion of both subsets could be increased by culture with CD40L(+) cells, but quantitative differences between the subpopulations remained.

In sheep, equivalent sub-populations of DC are present in afferent lymph. Studies in this species have been helped by the cross-reactive CD26 antibody raised to bovine CD26 [31]. Here, a comparison between lymph draining from the head mucosa (cervical DC (CerDC)) versus cutaneous microenvironments on the constitutive DC release was made, again by lymph cannulation [25]. Again, the proportion of the lymph draining the mucosal site contained a higher proportion of the CD26(hi) signal regulatory protein (SIRP)(–) DC subset, and again, cytoplasmic apoptotic DNA as well as cytokeratin-positive inclusions were primarily detected in this subset of DC. Cholera toxin (CT) administered onto the oro-nasal mucosa accelerated migration, indicating that the effect of CT on DC mobilization is not subset- specific.

The significance of the presence of CD26 in this DC subset was investigated [31]: CD26 is an exopeptidase with specificity for motifs within the receptor-binding domain of several chemokines including monocyte derived chemokine (MDC). CD26 mediated truncation of MDC affects the Th cell response affected by the chemokine and may produce a Th1 bias. CD26 mediated modification of MDC may bias the immune response induced in naive T cells by DC, leading to the suggestion that this subset is involved in self-antigen presentation and tolerance induction.

The rat, bovine and ovine CD4-SIRPα-populations are reminiscent of the DC isolated directly from jejunal LP of the pig. This DC subset has high NSE levels and phagocytic activity in rats, shows epithelial and apoptotic inclusions in vivo in rats and sheep and is capable of phagocytosis of allogeneic lymphocytes in vitro in the case of rat DC. Ex vivo isolated pig LP DC, albeit with relatively low levels of SIRPα [42], were shown to have avid phagocytic activity and high NSE levels [56], suggesting the origin of this DC subset in the diffuse lymphoid tissue of the gut.

In the pig, a recent study, using the cannulated pseudo-afferent lymph model, has confirmed this and demonstrated that large numbers of DC, expressing CD11R1 (CD11b) and CD172 are found in efferent lymph [10] and are phenotypically similar to DC from the diffuse lymphoid tissue (see also review article by Bimczok and Rothkötter [9]).

Such cannulation experiments have also shown that pathogens can modulate the type of APC leaving the mucosal tissue [45]: in normal sheep and those infected with the parasitic intestinal nematode *Trichostrongylus colubriformis*, only veiled cells, labelled with a DC specific antibody [78] and of dendritic morphology were seen, whereas APC with clear macrophage morphology were seen in lymph from sheep infected with *Mycobacterium avium* subsp. *paratuberculosis*, the causative agent of Johne's disease. This latter finding may be of interest in the context of the hypothesis, that parasitic burdens bias the host responses towards tolerance, whereas pathogenic microbial infection may lead to hypersensitivities.

2.6. Migration of APC into mucosal tissues

A variety of chemokines, adhesion molecules and their receptors have been shown to be involved in mucosal homing of many cell types including DC. The vast majority of such studies have been carried out in PP and MLN of rodents and are outside the scope of this paper. We know very little about the mechanisms, which drive the recruitment of APC into the diffuse GALT. However, some recent studies in veterinary species are of interest in this context. The neonatal pig is an excellent model in which to study the immigration of leucocytes into the gut, as probably due to the impervious nature of the pig placenta, the gut of neonatal animals are completely devoid of leucocytes [44, 85]. This contrasts with human infants, where small numbers of leucocytes are already present at birth [106]. It can be

shown in such animals that such migration is totally dependant on the presence of gut flora, as germ-free animals show no such DC or any other leucocytes in the diffuse lymphoid tissue of the jejunum [43]. In addition, large numbers of DC arriving rapidly within the first few days in conventional animals [104] still express the LPS receptor CD14 [52] and CD163 (personal observation), both monocyte/macrophage specific molecules, which are absent in older animals [51, 52]. This would support the hypothesis that LP DC are derived from blood monocytes and develop their particular characteristics in situ. A differential effect of the gut flora on the DC phenotype could also be shown: DC arriving in gnotobiotic animals mono-associated with commensal *E. coli* had vastly increased levels of MHCII and maintained this even after a prolonged period of time, whereas animals colonised with a cocktail of commensal anaerobes had DC of a "normal" phenotype [43]. This suggests that homing of APC to this site is driven totally by the presence of commensal microbes, and that the phenotype and therefore presumably the function of LP DC is shaped in response to the composition of such microbial flora.

2.7. Other mucosal tissues

The immunological structure of the larynx of the pig has also received some attention, and MHC II+ DC were reported both in and underneath the epithelium [34]. Other mucosal tissues studied in health and disease were the oral cavity of cats, where MHCII+ cells with DC morphology were reported [37], as well as in the reproductive tract in FIV infection, where CD1+ DC increased and macrophages decreased after infection [13].

ATPase-positive and major histocompatibility complex class II-positive Langerhans cell-like dendritic cells have been studied at the mucosal surface of the eye, tongue and oesophagus of the chicken [77]. Ultrastructurally, these cells qualified as Langerhans cells except that they lack Langerhans cell granules. Thus, as in mammalian skin and mucosa, chicken mucosa contains mucosal dendritic cells with morphological and phenotypic features for the engagement of incoming antigens within epithelium and lamina propria.

3. PLASMACYTOID DENDRITIC CELLS OR NATURAL INTERFERON PRODUCING CELLS

3.1. Discovery of NIPC-pDC

Natural Interferon Producing Cells (NIPC) were first defined in the early eighties by their capacity to secrete large amount of IFN-α (1-2 UI per cell) in response to a number of different viral, bacterial or tumor cell components [26]. Despite conflicting data regarding the cellular origin of IFN-α, attention quickly focused on an original but rare subset of blood leukocytes, tentatively termed NIPC [84]. For many years, the phenotype of NIPC was defined by what they were not (i.e. non T, non B, non NK, non monocytes, non progenitor cells) rather than by what they are (low buoyant density, CD4+ and MHCII+). The first comprehensive study of the surface phenotype of human blood NIPC was achieved in 1996 [102]. The authors concluded that NIPC are part of the DC family, probably representing an immature subset of blood DC. The identity of NIPC was definitely solved in 1998, by scientists working on another rare population of cells with plasmacytoid morphology but expressing some T cell markers called plasmacytoid T cells, then renamed plasmacytoid monocytes and finally plasmacytoid pre-dendritic cells (pDC). PDC are low-density cells negative for CD11c and lineage markers (CD3, CD19, CD56 and CD14), positive for MHCII, CD4 and CD123 (IL-3 receptor) [63]. Siegal et al. [90] finally proved that purified pDC produce 200 to 1 000 times more IFN-α than other blood cells after microbial challenge, therefore corresponding to the NIPC [90].

Since then, NIPC-pDC have been characterized in mouse [4], rat [50] monkey [19] and in one farm animal species: pig. The story of porcine NIPC has been nearly as long and prolific as the human one. The porcine NIPC were identified based on their IFN-α production in response to transmissible gastro-enteritis virus (TGEV) [17] only a few years after human NIPC were discovered. The porcine model has generated original data pertaining to mechanisms of IFN-α induction by viral glycoproteins [7, 16, 18, 82], their ontogeny [96] and their localization in secondary lymphoid organs [3, 81]. The identification of porcine NIPC as pDC could be established only recently [100]. To isolate porcine DC from blood, Summerfield et al. used a myeloid marker, SwC3 now known to be the homologue of human CD172a, a member of the SIRP family. Two CD172a+ DC subsets could thus be identified in PBMC: the CD4– CD14– and the CD4+ CD14– subsets, both being non adherent and able to mature in vitro (up-regulation of MHCII and CD80/86). Only the CD172a+low CD4+ subset contained IFN-α producing cells (10–25%) after exposure to UV-inactivated TGEV and could efficiently bind IL-3. These two features make the porcine DC subset CD172a+ CD4+ CD14– a population equivalent to the human NIPC-pDC population.

Recent attempts were made to characterize IFN-α producing cells in cattle. Infection of post-natal cattle with a non cytopathic strain of bovine viral diarrhea virus (ncpBVDV) resulted in elevated circulating IFN-α/β [15]. Although the bovine IFN-producing cells share some features with porcine NIPC (they are found in lymph node draining the site of infection and they are distinct from BVDV infected cells), their phenotype does not match the one expected for typical plasmacytoid DC [12]. In particular they lack CD4 and express CD11b and low CD14, indicating a myeloid lineage rather than a plasmacytoid one. Therefore leukocyte subsets other than pDC can be involved in IFN-α production in different species. Besides, the nature of the IFN-inducing signal may also target different subsets of responding cells as was shown for Sendaï virus compared to herpes simplex virus [33].

As well as their unique ability to secrete IFN-α, NIPC-pDC can also differentiate into mature DC albeit with poor antigen-presenting ability compared to DC from myeloid origin [63]. Since the connection between NIPC and pDC has been established, this DC subset has been involved in several immunological disorders and infectious diseases (AIDS, SLE, cancer). Thus, the study of NIPC-pDC has become very "fashionable" [63].

3.2. NIPC-pDC at mucosal sites

As stated above, NIPC-pDC are found in blood and in secondary lymphoid organs. Far less is known about their presence at mucosal sites, the true sites of interaction between NIPC and microbial IFN-α inducers. The first study looking for mucosal NIPC was done by our group in piglets infected with TGEV [83]. TGEV oral-infection causes an acute gastro-enteritis to piglets. The virus replicates primarily in enterocytes of the intestinal tract (expressing aminopeptidase N, the TGEV receptor) and viral infection is accompanied by a massive transient (24 h) production of IFN-α in blood and intestinal fluid [59]. TGEV-induced IFN-producing cells were investigated in situ by immunohistochemical staining of duodenum, jejunum, ileum, mesenteric lymph node, popliteal lymph node and spleen cryosections. This showed that the vast majority of IFN-α producing cells were located in the small intestine (inside lamina propria and surrounding Peyer's patches) and accumulated in the mesenteric lymph nodes [83]. It was therefore concluded that most if not all circulating IFN-α in TGEV-infected piglets originates from gut and mesenteric lymph node. These intestinal IFN-producing cells express MHCII, and are in contact with but distinct from TGEV-antigen positive cells,

Table I. Distribution of major cell surface molecules on DC from various mucosal sites.

	Thoracic duct / afferent (pseudo-afferent) lymph				Small intestinal LP			
	Rat	Pig	Cattle	Sheep	Rat	Pig	Cattle	Sheep
MHC II	+++	+++	+++	+++	+++	+++	?	?
CD4*	+/–	?	+/–	?	?	?	?	?
CD11b**	+	+	+	+	+/–	+/–	?	?
CD11c	+	?	+	+	+/–	+/–	?	?
CD14***	?	?	?	?	?	–	?	?
CD16	?	+/–	?	+	?	+	?	?
CD26*	?	?	+/–	+/–	?	?	?	?
CD172*	+/–	+/–	+/–	+/–	?	+(low)	?	?

* CD4 and CD172 are largely co-expressed, but mutually exclusive with CD26.
** The homologue for CD11b in pigs is CD11R1 [23].
*** There is a small CD14+ subset in LP of very young pigs.

therefore resembling potential intestinal porcine pDC. Nevertheless, the frequency of intestinal NIPC is very low (one or two per villus at best) compared to “ordinary” DC that are extremely numerous, sometimes filling the whole lamina propria of a villus. One can wonder about their function at such site: their small number makes them unlikely to be a major antigen-presenting DC subset. On the other hand, even rare intestinal NIPC in mesenteric lymph node will flood the T-cell area with IFN-α, which is very likely to influence the outcome of the immune response. These data raise the central question of how different DC subsets cooperate to defend against acute viral infection. Biron’s group addressed this issue by isolating splenic murine DC during the course of MCMV infection in order to test their functions ex vivo (antigen presentation to CD4 and CD8 cells, cytokines secretion and NK activation) [21]. In such an approach, pDC appear specialized for initiation of innate immunity, and as a result of their production of IFN-α regulate other DC for induction of adaptive immunity. It is likely to be the same for intestinal pDC during the course of enteritic viral infection.

PDC have also been studied at mucosal sites independently of their ability to make IFN-α. For instance, pDC can be isolated from human tonsils [101] or lung [23]. Experimentally elicited allergic rhinitis in human results in a massive recruitment of pDC to nasal mucosa [54]. Lambrecht’s group addressed the question of their immunoregulatory/tolerogenic function in lung mucosa in an experimental mouse model of asthma [22]. They demonstrated that lung pDC can present harmless antigen to draining lymph node T cell in a tolerogenic manner and are essential to prevent asthmatic reactions. The capacity of pDC to produce IFN-α can build up with pDC tolerogenic function. For instance, murine gut-derived CD8α+ pDC when matured by CpG into IFN-α producing cells, induce regulatory T cell [8].

4. CONCLUSIONS

The study of antigen presenting cells in mucosal sites in domestic species has yielded valuable information about their phenotype, function and migration routes. Mucosal tissues contain large numbers of APC, consisting of two major and possibly several other minor subsets of DC. Table I is an attempt to summarise the various phenotypic surface molecules for different species described in draining lymph and the diffuse lymphoid tissue. The first major subset has functional and phenotypic characteristics of immature myeloid DC: these DC are phagocytic, yet capable of some antigen presentation. Similar DC are found

in the LP of the diffuse lymphoid tissue. These LP DC appear to be highly mobile, they appear rapidly in neonates in response to microbial colonization and can also be found in the efferent lymph, migrating to the MLN. The second subset is less phagocytic and a more potent antigen-presenting cell population, expressing high levels of CD172 and CD4 and possibly originating from Peyer's patches. Studies in other species have implicated immature DC in the induction of tolerance, however, direct functional evidence of this is still lacking in these studies of mucosal tissues in domestic species.

In comparison, relatively small numbers of NIPC-pDC are also found at mucosal sites. Their function in mucosa is just being revealed. According to experimental models in rodents, mucosal NIPC-pDC stimulate regulatory T cells, thereby preventing excessive inflammatory responses damaging for the mucosal integrity. However much is still unknown about their role during infectious processes. Contrary to their murine counterpart but in common with humans, porcine NIPC-pDC are the only DC subset able to sense bacterial/viral components via TLR9, TLR7 or yet unknown receptors [36, 47, 58]. Mucosal porcine pDC could be the target for studies aimed at elucidating their function in vivo, including their migratory behavior between the peripheral mucosal sites and inductive sites where they accumulate. Such knowledge will be particularly relevant for studies using natural ligands of TLR9 (bacterial and viral DNA) but also for pre-clinical trial using CpG-ODN as immunomodulators [46].

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