ELSEVIER

Veterinary Immunology and Immunopathology
77 (2000) 1–13

Veterinary immunology and immunopathology

www.elsevier.com/locate/vetimm

Mini Review

Dendritic cells, implications on function from studies of the afferent lymph veiled cell

C.J. Howard*, J.C. Hope

Institute for Animal Health, Compton, Near Newbury RG20 7NN, UK

Received 21 June 2000; received in revised form 29 July 2000; accepted 7 August 2000

Abstract

Studies of afferent lymph veiled cells (ALVC) show that the full biological function of dendritic cells in peripheral tissue is not explained by a simple model in which immature dendritic cells at the body surface take up antigen, migrate via the afferent lymph ducts, mature and then effectively present antigens to T-cells in the draining lymph node. Furthermore, it is evident from various investigations that the dendritic cells in afferent lymph draining from the body surfaces are not a homogeneous population of cells. They comprise a mixture of cell phenotypes defined by staining with monoclonal antibodies, and the different sub-populations have distinct biological functions and roles in vivo. The molecular basis for differences between the function of afferent lymph dendritic cell subsets is only now being explored and defined but some progress has been made in understanding the role of co-stimulatory molecules. It should be possible to exploit knowledge of the functions of these cells and aid future vaccination strategies in domesticated animals thereby improving animal health and reducing economic loss, and, as a consequence, improving human health. By deliberately targeting functionally distinct subsets of either precursor or mature dendritic cells in vivo, it should become feasible to achieve an appropriately biased immune response.

Keywords: Afferent lymph; Antigen presenting cells; Bovine leukocytes; Dendritic cells

1. The central role of dendritic cells in immune responses

Dendritic cells (DC) may be regarded as a system of cells that are specialized for the presentation of antigen to T-cells. They are the most potent of the antigen presenting cells and are essential for the initiation of immune responses in naïve animals

Abbreviations: ALVC, afferent lymph veiled cell(s); CLA, cutaneous lymphocyte associated antigen; DC, dendritic cell(s); ITIM, immunotyrosine inhibitory motif; SIRP, signal regulatory protein; VSG, variable surface glycoprotein of *Trypanosoma brucei*

* Corresponding author. Tel.: +44-1635-577239; fax: +44-1635-577263.
E-mail address: chris.howard@bbsrc.ac.uk (C.J. Howard).

0165-2427/00/$ – see front matter
PII: S0165-2427(00)00234-8

(Steinman, 1991; Banchereau and Steinman, 1998). They originate in the bone marrow but recent investigations suggest that they may be derived from either myeloid or lymphoid precursors. DC are a trace population in most tissues but notably form networks underlying major body surfaces such as skin, trachea and intestine, where their function is the uptake of antigens and, after migration to the draining lymph nodes, the presentation of processed antigen. DC were originally identified by their characteristic morphology and tissue-specific locations (Langerhans cells in the epidermis, dermal dendritic cells in skin, afferent lymph veiled cells (ALVC) in afferent lymph and interdigitating cells in lymph nodes and other organized lymphoid tissues). Their isolation from these tissues requires prolonged and tedious purification procedures, although they can also be produced in cytokine driven culture from precursors found in the bone marrow or blood (Caux, 1998; Shortman and Caux, 1997) (Table 1).

A number of properties have been established that are critical to the function of DC as the ultimate antigen presenting cell (APC). These include the ability to effectively take up antigen by a number of routes, which may include endocytosis by clathrin-coated pits or caveolae, macropinocytosis or phagocytosis depending on the maturation stage of the cell. High levels of expression of MHC class II and the prolonged expression of MHC class II and peptide on the cell surface, as well as high levels of expression of a number of co-stimulatory molecules that include CD80, CD86, CD40 and LFA-1, have been considered to contribute to the efficiency of DC as APC (Banchereau and Steinman, 1998). The ability of DC to transfer molecules and antigens derived from apoptotic cells from exogenous to endogenous pathways has been noted. This has been cited as a mechanism for cross priming, and for the absence of the necessity for an active infection of DC by microorganisms for the induction of MHC class I restricted $CD8^+$ T-cell responses (Watts, 1999; Albert et al., 1998; Sauter et al., 2000). Indeed, infection of DC by viruses may affect their function (Estcourt et al., 1998; Karp, 1999; Williams et al., 1998).

Apart from effectively stimulating primary immune responses, DC may also influence the bias of the immune response. Evidence from humans has suggested that monocyte derived DC (DC1) induced Th1 differentiation while DC derived from plasmacytoid cells (DC2) induced Th2 differentiation (Bottomly, 1999; Rissoan et al., 1999). In mice, lymphoid and myeloid DC have been reported to induce Th1 and Th2 responses respectively in vivo (Maldonado-Lopez et al., 1999). However, it has also been reported that immature myeloid DC can be pushed to differentiate into APC that induce Th1 or Th2 types of responses if they are exposed to IFNγ or PGE2, respectively (Vieira et al., 2000). Liu (2000) proposed that immature pre-DC2 could be induced to differentiate into DC2 or DC producing IFNγ by exposing them to different cytokine cocktails. This indicates a plasticity and flexibility in the differentiation pathway of DC that may be affected by the local environment in which the immature cells find themselves rather than following a predestined route of differentiation. Nevertheless, this does not invalidate the observation that DC from different tissues, or within the same tissue, can have distinct properties that will affect the way in which they handle antigen and promote T-cell responses.

Table 1
Comparison of surface antigen expression by monocyte derived dendritic cells (MoDC) and afferent lymph veiled cells (ALVC)[a]

Surface molecule	Expression by	
	ALVC	MoDC
CD1W1	+ or ++	+
CD1b	+ or ++	+ or ++
CD1W3	+	+
CD2	−	−
CD3	−	−
CD4	−	−
CD5	− or +	−
CD8	−	−
CD11a	− or +	+
CD11b	−	+
CD11c	± or +	−
CD14	−	+
CD21	− or +	−
CD25	− or ±	−
CD45R′B′	−	−
CD45RO	+	+
CD62L	−	−
WC1	−	−
WC6	++	− or +
WC10	− or ±	−
CD32	− or +	+
Fcγ2R	−	−
MyD-1	− or +	++
Mannose receptor	− or +	±
CC81ag	− or +	−
CD80	++	++
CD86	++	+
CD40	++	+ or ++
MHC II	++	+ or ++

[a] Data taken from Howard et al. (1997), Werling et al. (1999) and Hope et al. (unpublished). Intensity of staining with mAb indicated as no staining (−), weak staining (±), staining clear (+) and strong staining (++). Variable staining of cells within the two populations is also indicated.

2. A model for dendritic cell function

Studies of murine Langerhans cells have provided a model of DC maturation and differentiation. Precursors of dendritic cells, derived from bone marrow, move through the circulation and become resident within the epidermis. The homing of these $CD34^+$ progenitors to the skin may be linked to their expression of the cutaneous lymphocyte-associated antigen (CLA) and CLA^+ $CD34^+$ cells have been shown, in in vitro studies, to be precommitted to the Langerhans cell lineage (Strunk et al., 1997). Langerhans cells serve as ‘sentinels’ in the skin monitoring the exposure of the body surface to antigen. At this site, Langerhans cells represent immature DC with low to moderate levels of MHC

class II and co-stimulatory molecules, and high capacity to phagocytose micro-organisms and other particulate antigens (Reis e Sousa et al., 1993; Pure et al., 1990). As such, Langerhans cells are efficient in antigen uptake, but are poor stimulators of T-lymphocyte responses. Langerhans cells are stimulated to migrate from the body surface by tissue injury, inflammation or infection (Steinman, 1991; Ibrahim et al., 1995). This process of migration, via the afferent lymphatic vessels, to the draining lymph node is associated with functional and phenotypic changes such that, upon arrival in the lymph node the DC have acquired the capacity to effectively stimulate T-cell proliferation. The migration and maturation of Langerhans cells is known to be controlled by cytokines, including IL-1, TNF-α and GMCSF (Heufler et al., 1988; Cumberbatch and Kimber, 1992). Culture of Langerhans cells in vitro with these cytokines mimics the functional and phenotypic maturation of Langerhans cells observed following in vivo stimulation (Heufler et al., 1988; Schuler and Steinman, 1985; Witmer-Pack et al., 1987). The enhanced T-cell stimulatory capacity of cultured Langerhans cells and DC from lymph nodes, compared to freshly isolated Langerhans cells, is related to the up-regulation of MHC II, CD80, CD86, CD40, ICAM-1 and other surface molecules that are involved in the interaction with, and stimulation of, T-lymphocytes by DC (Steinman, 1991; Larsen et al., 1992; Witmer-Pack et al., 1988; Dai et al., 1993). Secretion of co-stimulatory cytokines, such as IL-6, is also increased in lymph node DC, compared to Langerhans cells (Cumberbatch et al., 1996). In parallel with the enhancement of T-cell stimulatory capacity observed upon Langerhans cell migration and maturation, there is a reduction in the capacity for antigen uptake by mature DC (Steinman, 1991). These in vitro data suggest that the processes of antigen uptake and presentation are separate events mediated by cells with distinct functional and phenotypic characteristics. However, other studies have indicated that DC directly isolated ex vivo from afferent lymph draining the skin or intestine are able both to take up antigen and to effectively stimulate proliferation of naïve and memory T-lymphocytes (Howard et al., 1997; Liu et al., 1998). Thus, antigen uptake and presentation are both properties of the one population of DC in vivo, as discussed below.

3. Implications from in vivo studies

The model in which afferent lymph draining the skin is collected by cannulation after removal of the prescapular lymph node (Emery et al., 1987) allows the isolation of large numbers of ALVC from afferent lymph. An advantage of investigating the function of these cells is that they have not been subjected to long periods of culture in vitro and have not been subjected to enzymatic treatment or other separation techniques necessary in other models. The absence of DC in efferent lymph is explained by the proposal that in the absence of stimulation by an interaction with T-cells in the lymph node the interdigitating cells die as a result of apoptosis (Koppi et al., 1997; Haig et al., 1999). McKeever et al. (1992) presented direct functional evidence, from the bovine model, that ALVC could initiate primary T-cell responses in vivo. In this study 500 μg of soluble protein antigen was inoculated intradermally into the area being drained by the afferent lymphatics and pseudoafferent lymph draining the area collected. As early as 30 min after inoculation the isolated ALVC induced maximal proliferative responses in vitro with

immune T-cells from a monozygotic twin calf. When 10^7 ALVC were incubated in vitro with OVA, washed and inoculated back into the calf a proliferative response was detected within 1 week that was maximal 3 weeks later.

These findings gave further support to the view that the interdigitating cells in the lymph node which present antigen to naïve T-cells are derived from ALVC and that the ALVC are physiologically involved in the transport of antigen to lymph nodes. In vitro studies with ALVC from rabbits lent support to this concept (Knight et al., 1982). It was further supported by the observation that surgical ablation of the afferent lymphatics draining into the popliteal lymph node of rats resulted in the loss of dendritic cells from the lymph node within 1 week (Hendriks et al., 1980).

However, as has been pointed out, the simple model based on studies of Langerhans cells does not take into account and does not explain all of the observations that have been made from in vivo or ex vivo studies (Liu and MacPherson, 1995). This model proposes that immature DC at the body surface, that are poor at stimulating T-cells, actively take up antigen and transport it to the draining lymph node, and become highly active stimulatory cells in the process that are poor at antigen uptake. Although Langerhans cells cultured for 1–3 days loose their ability to process native protein antigen and acquire the ability to stimulate resting T-cells, this is not so for ALVC. DC in lymph draining the intestine of rats that had undergone mesenteric lymphadenectomy were obtained by cannulation of the thoracic duct. It was shown that lymph-borne DC that had been cultured for 20–72 h were able to take up and present antigen to immune T-cells in vitro as effectively as fresh DC. The OVA pulsed rat ALVC were also able to present to naïve T-cells in vivo (Liu and MacPherson, 1995), as was the case with bovine ALVC noted above (McKeever et al., 1992). Previous studies reported that ALVC draining the intestine could present antigen that had been inoculated orally to naïve T-cells in vivo (Liu and MacPherson, 1993). Thus, DC in afferent lymph, that have taken up antigen and would arrive in the draining lymph node within a matter of seconds, are able to both take up native protein and present processed antigen to T-cells in vivo and in vitro. Furthermore, they retain the ability to take up and present antigen for 72 h in culture. Taken together, these observations suggest that the model proposed to account for the development of Langerhans cells does not represent the full range of DC behavior observed in vivo and ex vivo. Rapid down-regulation of antigen uptake and processing is not necessarily a property that is common to all peripheral DC. In addition, McFarlin and Balfour (1973) while investigating the afferent lymphatic drainage in pigs, and Hall and Smith (1971) in their studies of afferent and efferent lymph from sheep, reported that after painting skin with a sensitizing dose of dinitrofluorobenzene almost all (>90%) of the chemical was found in the afferent lymph bound to protein rather than to cells. Some was evident as early as 15 min post-exposure. Thus, although DC in the periphery take up antigen when soluble antigen is inoculated into or onto the skin, most of the antigen is found free in the lymph. Furthermore, bacteria or particulate antigens may reach the lymph node without necessarily having been taken up by dendritic cells. Retention of the ability to take up antigen and stimulate T-cells is clearly advantageous to the host. Thus, there appear to be several mechanisms whereby antigens either enter or attach to DC in the appropriate location in lymph nodes, and are then presented to naïve lymphocytes that enter from the blood via the high endothelial venules.

4. Phenotypic and functional diversity within ALVC

DC in afferent lymph draining from the skin or intestine are not a homogeneous population of cells, neither can they be regarded as a single cell population that is simply maturing as they migrate. The ALVC appear to comprise a population that is phenotypically diverse, and which is also functionally diverse.

Studies on ALVC of cattle have defined two major sub-populations (McKeever et al., 1991; Howard et al., 1997). The majority, probably all, of the ALVC within afferent lymph can be identified and distinguished from monocytes and macrophages or B-cells using flow cytometry by their high level of expression of the 210 kDa antigen currently known as WC6 (Howard et al., 1996; Howard and Naessens, 1993). Within this $WC6^{+}$ ALVC population, two major sub-populations are evident. The larger, comprising about 80% of the ALVC, can be identified by expression of the bovine MyD1 antigen and lack of expression of CD5 as well as the WC10 antigen and the antigen recognized by mAb CC81. The $MyD1^{-}$ ALVC express CD11a at a high level while the $MyD1^{+}$ ALVC either do not express CD11a or express it at a low level (Fig. 1). Expression appears to change with culture or inflammation (Stephens et al., unpublished). The $MyD1^{+}/CC81^{-}$ ALVC sub-population is heterogeneous and contains a mixture of cells. Some are derived from

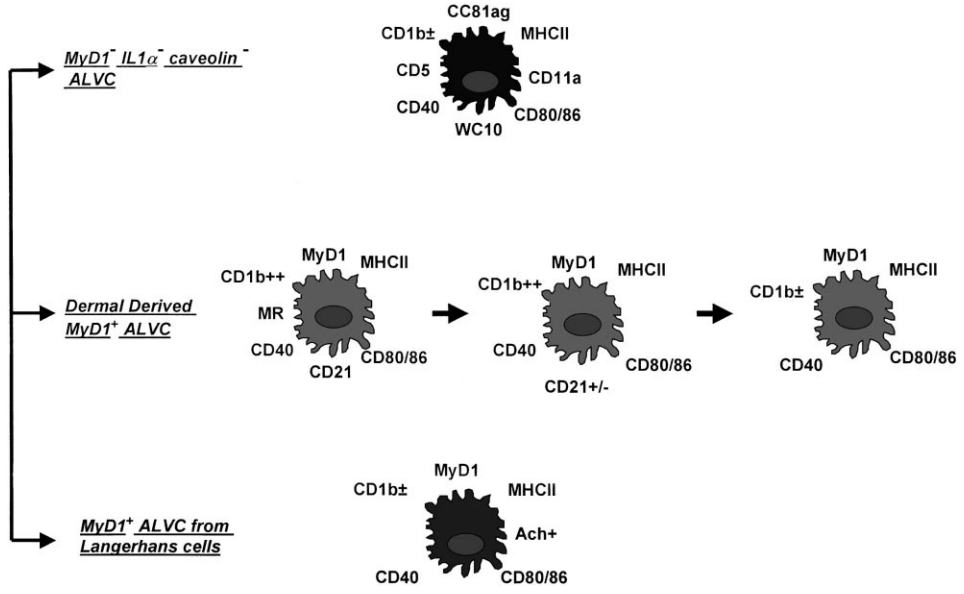

Fig. 1. Subsets of dendritic cells in afferent lymph (ALVC). Our current proposal for the three major subsets of ALVC and some of the discriminating antigens that are expressed are shown. The $MyD1^{+}/CC81^{-}$ and acetylcholine esterase negative (Ach−) ALVC are depicted suggesting different maturational stages of the same population. The MyD1 antigen, mannose receptor (MR), workshop cluster 10 (WC10) antigen, and the antigen recognized by mAb CC81 are as described (Howard et al., 1997; Brooke et al., 1998). Staining with mAb to caveolin-1 (Werling et al., 1999; Howard et al., 1999) indicated the presence of caveolae. High levels of expression (++), low levels of expression (±) and the presence of expressing and non-expressing cells (+/−) are indicated for some antigens.

Langerhans cells (as defined by the presence of acetylcholine esterase) but most come from the dermal dendritic cells, the latter appearing to exhibit varying degrees of maturation (Stephens et al., unpublished). The $MyD1^-/CC81^+$ ALVC appear to be homogeneous (see Fig. 1).

The importance of these phenotypic differences is that the sub-populations of ALVC that they delineate have been shown to interact with and stimulate T-cells differently. Both $MyD1^+/CC81^-$ and $MyD1^-/CC81^+$ ALVC can take up native protein (OVA) and present processed antigen effectively to resting memory $CD4^+$ T-cells. However, the $MyD1^+$ population was found to be much more effective than the $MyD1^-$ population at presenting respiratory syncytial virus antigen or variable surface glycoprotein (VSG) from *Trypanosoma brucei* to memory $CD4^+$ T-cells (McKeever et al., 1991; Howard et al., 1997). Efficient presentation of the respiratory syncytial virus antigen has been related to uptake via caveolae rather than clathrin coated pits or macropinocytosis (Werling et al., 1999). The VSG may enter largely by the same route as it is a GPI linked molecule and molecules with this structure have been reported to be selectively concentrated in caveolae (Anderson, 1998). The $MyD1^+$ and $MyD1^-$ ALVC also differ in ability to stimulate proliferative responses in allogeneic $CD8^+$ T-cells. This difference has been related to synthesis of IL-1α by the more effective $MyD1^+$ population, and lack of IL-1α synthesis by the ineffective $MyD1^-$ ALVC (Hope et al., 2000). Thus, presentation of antigens predominantly by one population or the other in vivo may markedly affect the type of response induced.

Apart from the likely importance of IL-1α in the stimulation of $CD8^+$ T-cells, the molecular basis for these differences in ALVC sub-population function is not clear. Both $MyD1^+$ and $MyD1^-$ ALVC express similar high levels of MHC class II and of CD80 and CD86, originally shown by staining with a CTLA4 fusion protein and recently confirmed with anti-CD80 and CD86 mAb (Howard et al., 1997; Stephens et al., unpublished). Differences in MyD1 expression may be important. This molecule is a member of the recently described family of signal regulatory proteins (SIRP) and the presence of an immunotyrosine inhibitory motif (ITIM) in the long cytoplasmic tail implies an important role for the molecule in the modulation of activation signals (Brooke et al., 1998). Of further significance is the observation that the MyD1 molecule mediates the binding of $CD4^+$ and $CD8^+$ T-cells to the APC and this property is likely to affect the function of the APC. A ligand for the homologue of the bovine MyD1 antigen on human myeloid cells is the broadly expressed CD47 antigen (Seiffert et al., 1999) as has been recently shown in cattle (Brooke and Howard, unpublished). CD47 is known to have a variety of roles in signaling and activation of cells.

Studies of ALVC draining the intestine of rats have shown that the DC from this source are also phenotypically heterogeneous. Rat ALVC were shown to fall into two major sub-populations. One was stained with mAb to CD4 and with a mAb OX41, the other sub-population was not (Liu et al., 1998). Of some significance was the finding that the OX41 antigen was also a member of the SIRP family of ITIM containing molecules and is the homologue of the bovine MyD1 antigen (Adams et al., 1998). Thus, there is a molecular basis for believing that at least some of the DC within the cattle and rat ALVC may be equivalent populations. Of potentially great significance for understanding the biology of DC is the report that the $CD4^+/OX41^+$ ALVC are strong antigen presenting cells while

the $CD4^-/OX41^-$ ALVC are weak and contain cytoplasmic apoptotic DNA, epithelial cell-restricted cytokeratins and non-specific esterase inclusions. Similar DC were evident in the intestinal lamina propria, Peyer's patches and mesenteric (but not other peripheral) lymph nodes. It has been suggested that a distinct DC subset ($CD4^-/OX41^-$ cells) constitutively endocytoses and transports apoptotic cells to T-cell areas and that they have a role in the maintenance of peripheral tolerance (Huang et al., 2000). There is no evidence at present that the $OX41^-/CD4^-$ ALVC derived from the rat intestine are equivalent to the $MyD1^-/CC81^+$ cells present in bovine afferent lymph and they may well represent a different subset altogether. However, the observation emphasizes the differences in properties of ALVC subsets and how the differing DC sub-populations are involved in the control of the immune response.

It might be expected that studies of sheep DC would give identical findings to those in cattle. This appears not be the case. Thus, Walsh et al. (unpublished) have found that sheep ALVC draining the skin are $WC6^+$, as is the case with cattle. The sheep ALVC are $MyD1^+/CC81^-$ or $MyD1^-/CC81^+$. However, some of the $MyD1^+/CC81^-$ ALVC express the CD4 antigen and some the CD8 antigen. The important question is do the $CD8^+$ ALVC relate to the lymphoid DC of mice, discussed below, and do the $CD4^+$ DC equate to the major population of DC in rat lymph. Clearly, it will be necessary to investigate functional properties of the subsets before a clear picture emerges.

5. Subsets of DC with different functional roles

Heterogeneity of DC biology has been a feature reported in many studies. Differences in tissue location (Nestle et al., 1993; Egner and Hart, 1995; Agger et al., 1992; Kelsall and Strober, 1996; Hart, 1997), or maturation induced differences in DC phenotype and function may account, in part, for this. Although, it is clear that DC progenitors may directly differentiate into cells with distinct characteristics typical of specific types of dendritic cells such as Langerhans cells or dermal DC (Caux et al., 1996). Culture of $CD34^+$ haematopoietic progenitor cells with GMCSF and TNF-α results in the generation of two distinct dendritic cell populations: epidermal Langerhans cells, and another that closely resembles both dermal and monocyte-derived dendritic cells (Caux et al., 1996, 1997; Sallusto and Lanzavecchia, 1994). The dermal DC progenitors express the myeloid antigen CD14 and can differentiate to either macrophages or DC depending upon culture conditions (Caux et al., 1996, 1997). The majority of DC in the periphery are of myeloid origin.

Evidence from humans and mice indicates that precursor cells of the lymphoid lineage, which are present in both bone marrow and thymus, can also differentiate into DC (Res et al., 1996; Galy et al., 1995). These DC express lymphoid markers including $CD8\alpha$ in mice (Vremec and Shortman, 1997), are situated in anatomical compartments distinct from myeloid DC, and display different functional activities when compared to myeloid derived DC. For example, mouse lymphoid DC ($CD8\alpha^+$) were reported to display suppressive, rather than stimulatory, effects on both $CD4^+$ and $CD8^+$ T-lymphocytes (Suss and Shortman, 1996; Kronin et al., 1996). Recent evidence suggested that myeloid and lymphoid DC are able to prime T-cells with equal efficiency, but that they differed in

the induction of distinct cytokine profiles in T-lymphocytes. Thus, murine lymphoid DC preferentially stimulated Th1 responses (Maldonado-Lopez et al., 1999; Pulendran et al., 1999), and secreted both IFN-γ (Ohteki et al., 1999) and IL-12 (Reis e Sousa et al., 1999). In contrast, murine myeloid derived DC preferentially induced Th2 biased responses characterized by the secretion of IL-4 and IL-10 by T-lymphocytes (Pulendran et al., 1999). In humans, a putative lymphoid DC derived from the $CD4^+$ $CD11c^-$ $CD3^-$ plasmacytoid T-cell, has recently been isolated from lymphoid tissue and blood (Grouard et al., 1997). These cells, like the mouse lymphoid DC, depend on IL-3, rather than GMCSF, for their survival and maturation (Grouard et al., 1997; Saunders et al., 1996). Rissoan et al. (1999) reported in studies of human DC that monocyte derived DC, termed DC1, induced Th1 type responses and DC2, derived from plasmacytoid cells, stimulated Th2 biased lymphocyte responses. Thus, human myeloid and lymphoid DC appeared to induce Th1 and Th2 cells, respectively, in contrast to the mouse where myeloid DC induced Th2 and lymphoid DC, Th1 responses. DC1, synthesized IL-1α but DC2, derived from plasmacytoid cells, did not (Rissoan et al., 1999). A similar disparity in IL-1α secretion has been demonstrated for subsets of cattle ALVC (Hope et al., 2000). A relationship of the cattle ALVC subsets to the DC1 or DC2 of Rissoan et al. (1999) would therefore have important consequences for T-cell stimulation and differentiation. However, the MyD-1^-/$CC81^+$ ALVC express CD45RO (Howard et al., 1997) and not the high MW CD45R isoform, which is expressed by plasmacytoid DC in humans, and this is consistent with a myeloid rather than lymphoid origin. The MyD-1^-/$CC81^+$ ALVC also express CD5, which has been reported on human myeloid DC (Howard et al., 1997; Reid et al., 2000). Thus, we cannot conclude that the MyD-1^-/$CC81^+$/$CD11a^+$ bovine ALVC are the plasmacytoid equivalent although it remains possible. However, our current hypothesis is that both bovine ALVC populations are of myeloid origin and neither is the equivalent of the plasmacytoid DC that migrate from blood into inflamed lymph nodes (Cella et al., 1999) or the lymphoid DC of mice (Shortman and Caux, 1997; Caux, 1998).

6. Questions to be addressed

A number of important questions remain to be addressed, including the in vivo function of DC and the precise role of DC subsets, and future studies in ruminant models will continue to provide valuable insights. Comparative studies of sheep and cattle are likely to reveal that, even between closely related species, significant differences exist in DC phenotype and function. Also, in order to establish common functions and generic models of DC biology, it will be important to establish how ruminant DC subsets relate to the subsets identified in humans and mice.

We should also ask how can information about DC be exploited to improve animal health, reduce economic loss and benefit humans in terms of improving world wide food production. The ability to use DC as vehicles to deliver antigens, stimulate immune responses and protect from disease is being considered and used in treatment of humans it seems unlikely that this would be economic for domesticated animals. However, there is clear potential to improve the efficacy of antigen delivery to DC in vivo, perhaps by targeting DC specific uptake mechanisms. A possible modification of this type of

approach would be to target particular DC subsets, which would bias responses towards a type 1 or 2 response or a $CD4^+$ or $CD8^+$ T-cell response. Another way in which the adjuvant effect of DC could be exploited would be to include a signal with the antigen so that DC1 or DC2 are recruited into the microenvironment in which the DC was functioning. Alternatively cytokines that provide signals to stimulate non-committed DC to mature into either DC1 or DC2 could be included with antigens (Vieira et al., 2000). With so many potential avenues for immune intervention opening up, it is certain that studies of DC biology will continue to be the subject of intensive investigation over the next decade.

References

Adams, S., van der Laan, L.J., Vernon Wilson, E., Renardel de Lavalette, C., Dopp, E.A., Dijkstra, C.D., Simmons, D.L., van den Berg, T.K., 1998. Signal-regulatory protein is selectively expressed by myeloid and neuronal cells. J. Immunol. 161, 1853–1859.

Agger, R., Witmer-Pack, M., Romani, N., Stossel, H., Swiggard, W.J., Metlay, J.P., Storozynsky, E., Freimuth, P., Steinman, R.M., 1992. Two populations of splenic dendritic cells detected with M342, a new monoclonal to an intracellular antigen of interdigitating dendritic cells and some B-lymphocytes. J. Leukoc. Biol. 52, 34–42.

Albert, M.L., Sauter, S., Bhardwaj, N., 1998. Dendritic cells acquire antigen from apoptotic cells and induce class-I restricted CTLs. Nature 392, 86–89.

Anderson, R.G.W., 1998. The caveolae membrane system. Annu. Rev. Biochem. 67, 199–225.

Banchereau, J., Steinman, R.M., 1998. Dendritic cells and the control of immunity. Nature 392, 245–252.

Bottomly, K., 1999. T-cells and dendritic cells get intimate. Science 283, 1124–1125.

Brooke, G.P., Parsons, K.R., Howard, C.J., 1998. Cloning of two members of the SIRPα family of protein tyrosine phosphatase binding proteins in cattle that are expressed on monocytes and a subpopulation of dendritic cells and which mediate binding to CD4 T-cells. Eur. J. Immunol. 28, 1–11.

Caux, C., 1998. Pathways of development of human dendritic cells. Eur. J. Dermatol. 8, 375–384.

Caux, C., Vanbervliet, B., Massacrier, C., Dezutter-Dambuyant, C., de Saint-Vis, B., Jacquet, C., Yoneda, K., Imamura, S., Schmitt, D., Banchereau, J., 1996. $CD34^+$ progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GMCSF + TNF-α. J. Exp. Med. 184, 695–706.

Caux, C., Massacrier, C., Vanbervliet, B., Dubois, B., Durand, I., Cella, M., Lanzavecchia, A., Banchereau, J., 1997. $CD34^+$ progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GMCSF + TNF-α. II. Functional analysis. Blood 90, 1458–1470.

Cella, M., Jarrossay, D., Facchetti, F., Alebardi, O., Nakajima, H., Lanzavecchia, A., Colonna, M., 1999. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type 1 interferon. Nature Med. 5, 919–923.

Cumberbatch, M., Kimber, I., 1992. Dermal tumour necrosis factor-α induces dendritic cell migration to draining lymph nodes and possibly provides one stimulus for Langerhans cell migration. Immunology 75, 257–263.

Cumberbatch, M., Dearman, R.J., Kimber, I., 1996. Constitutive and inducible expression of interleukin-6 by Langerhans cells and lymph node dendritic cells. Immunology 87, 513–518.

Dai, R., Grammer, S.F., Steilein, J.W., 1993. Fresh and cultured Langerhans cells display differential capacities to activate hapten specific T-cells. J. Immunol. 150, 59–66.

Egner, W., Hart, D.N.J., 1995. The phenotype of freshly isolated and cultured bone marrow allostimulatory cells: possible heterogeneity in bone marrow dendritic cell populations. Immunology 85, 611–620.

Emery, D.L., MacHugh, N.D., Ellis, J.A., 1987. The properties and functional activity of non-lymphoid cells from bovine afferent (peripheral) lymph. Immunology 62, 177–183.

Estcourt, M.J., Ramshaw, I.A., Ramsay, A.J., 1998. Cytokine responses in virus infections: effects on pathogenesis, recovery and persistence. Curr. Opin. Microbiol. 1, 411–418.

Galy, A., Travis, M., Cen, D., Chen, B., 1995. Human T, B, natural killer, and dendritic cells arise from a common bone marrow progenitor cell subset. Immunity 3, 459–473.

Grouard, G., Rissoan, M.C., Filgueira, L., Durand, I., Banchereau, J., Liu, Y.-J., 1997. The enigmatic plasmacytoid T-cells develop into dendritic cells with interleukin (IL)-3 and CD40 ligand. J. Exp. Med. 185, 1101–1111.

Haig, D.M., Hopkins, J., Miller, H.R.P., 1999. Local immune responses in afferent and efferent lymph. Immunology 96, 155–163.

Hall, J.G., Smith, M.E., 1971. Studies on the afferent and efferent lymph of lymph nodes draining the site of application of fluorodinitrobenzene (FNDB). Immunology 21, 69–79.

Hart, D.N.J., 1997. Dendritic cells: unique leukocyte populations which control the primary immune response. Blood 90, 3245–3287.

Hendriks, H.R., Eestermans, I.L., Hoefsmit, E.C., 1980. Depletion of macrophages and disappearance of post-capillary high endothelial venules in lymph nodes deprived of afferent lymphatic vessels. Cell Tissue Res. 211, 375–389.

Heufler, C., Koch, F., Schuler, G., 1988. Granulocyte-macrophage-colony stimulating factor and interleukin-1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulatory dendritic cells. J. Exp. Med. 167, 700–705.

Hope, J.C., Sopp, P.A., Collins, R.A., Howard C.J., 2000. Differences in the induction of $CD8^+$ T-cell responses by sub-populations of dendritic cells from afferent lymph are related to IL-1α secretion. Submitted for publication.

Howard, C.J., Naessens, J., 1993. Summary of workshop findings for cattle. Vet. Immunol. Immunopathol. 39, 25–47.

Howard, C.J., Sopp, P., Brownlie, J., Parsons, K.R., Kwong, L.S., Collins, R.A., 1996. Afferent lymph veiled cells stimulate proliferative responses in allogeneic $CD4^+$ and $CD8^+$ T-cells but not $\gamma\delta$ TCR^+ T-cells. Immunology 88, 558–564.

Howard, C.J., Sopp, P., Brownlie, J., Kwong, L.S., Parsons, K.R., Taylor, G., 1997. Identification of two distinct populations of dendritic cells in afferent lymph that vary in their ability to stimulate T-cells. J. Immunol. 159, 5372–5382.

Howard, C.J., Werling, D., Taylor, G., Kwong, L.S., Sopp, P., 1999. Association of caveolae and presentation of respiratory syncytial virus antigen by subsets of dendritic cells in afferent lymph. Immunology 98 (Suppl. 1), 103.

Huang, F.P., Platt, N., Wykes, M., Major, J.R., Powell, T.J., Jenkins, C.D., MacPherson, G.G., 2000. A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T-cell areas of mesenteric lymph nodes. J. Exp. Med. 191, 435–443.

Ibrahim, M.A.A., Chain, B.M., Katz, D.R., 1995. The injured cell: the role of the dendritic cell system as a sentinel receptor pathway. Immunol. Today 14, 181–186.

Karp, C.L., 1999. Measles: immunosuppression, interleukin-12 and complement receptors. Immunol. Rev. 168, 91–101.

Kelsall, B.L., Strober, W., 1996. Distinct populations of dendritic cells are present in the subepithelial dome and T-cell regions of murine Peyers patch. J. Exp. Med. 183, 237–247.

Knight, S.C., Balfour, B.M., O'Brian, J., Buttifant, L., Sumerska, T., Clarke, J., 1982. Role of veiled cells in lymphocyte activation. Eur. J. Immunol. 12, 1057–1060.

Koppi, T.A., Tough-Bement, T., Lewinsohm, D.M., Lynch, D.H., Alderson, M.R., 1997. CD40 ligand inhibits Fas/CD95-mediated apoptosis of human blood derived dendritic cells. Eur. J. Immunol. 27, 3161–3165.

Kronin, V., Winkel, K., Suss, G., Kelso, A., Heath, W., Kirberg, J., von Boehmer, H., Shortman, K., 1996. A subclass of dendritic cells regulates the response of naive $CD8^+$ T-cells by limiting their IL-2 production. J. Immunol. 157, 3819–3827.

Larsen, C.P., Ritchie, S.C., Pearson, T.C., Linsley, P.S., Lowry, R.P., 1992. Functional expression of the costimulatory molecule B7/BB1 on murine dendritic cell populations. J. Exp. Med. 176, 1215–1220.

Liu, A.-J., 2000. Proceedings of the 6th International Meeting on Dendritic Cells, Australia, 2000, http://wehiz.wehi.edu.au/dc.

Liu, L.M., MacPherson, G.G., 1993. Antigen acquisition by dendritic cells: intestinal dendritic cells acquire antigen administered orally and can prime naive T-cells in vivo. J. Exp. Med. 177, 1299–1307.

Liu, L.M., MacPherson, G.G., 1995. Antigen processing: cultured lymph-borne dendritic cells can process and present native protein antigens. Immunology 84, 241–246.

Liu, L., Zhang, M., Jenkins, C., MacPherson, G.G., 1998. Dendritic cell heterogeneity in vivo: two functionally different dendritic cell populations in rat intestinal lymph can be distinguished by CD4 expression. J. Immunol. 161, 1146–1155.

Maldonado-Lopez, R., De Smedt, T., Michel, P., Godfroid, J., Pajak, B., Heirman, C., Thielemans, K., Leo, O., Urbain, J., Moser, M., 1999. CD8α^+ and CD8α^- subclasses of dendritic cells direct the development of distinct T-helper cells in vivo. J. Exp. Med. 189, 587–592.

McFarlin, D.E., Balfour, B., 1973. Contact sensitivity in the pig. Immunology 25, 995–1009.

McKeever, D.J., MacHugh, N.D., Goddeeris, B.M., Awino, E., Morrison, W.I., 1991. Bovine afferent lymph veiled cells differ from blood monocytes in phenotype and accessory function. J. Immunol. 147, 3703–3709.

McKeever, D.J., Awino, E., Morrison, W.I., 1992. Afferent lymph veiled cells prime CD4$^+$ T-cell responses in vivo. Eur. J. Immunol. 22, 3057–3061.

Nestle, F.O., Zheng, X.G., Thompson, C.B., Turka, L.A., Nickoloff, B.J., 1993. Characterisation of dermal dendritic cells obtained from normal human skin reveals phenotypic and functionally distinctive subsets. J. Immunol. 151, 6535–6545.

Ohteki, T., Fukao, T., Suzue, K., Maki, C., Ito, M., Nakamura, M., Koyasu, S., 1999. Interleukin-12 dependent interferon-γ production by CD8α^+ lymphoid dendritic cells. J. Exp. Med. 189, 1981–1986.

Pulendran, B., Smith, J.L., Caspary, G., Brasel, K., Pettit, D., Maraskovsky, E., Maliszewski, C.R., 1999. Distinct dendritic cell subsets differentially regulate the class of immune response in vivo. Proc. Natl. Acad. Sci. U.S.A. 96, 1036–1041.

Pure, E., Inaba, K., Crowley, M.T., Tardelli, L., Witmer-Pack, M.D., Ruberti, G., Fathman, G., Steinman, R.M., 1990. Antigen processing by epidermal Langerhans cells correlates with the level of biosynthesis of MHC class II molecules and expression of invariant chain. J. Exp. Med. 172, 1459–1469.

Reid, S.D., Penna, G., Adorini, L., 2000. The control of T-cell responses by dendritic cell subsets. Curr. Opin. Immunol. 12, 114–121.

Reis e Sousa, C., Stahl, P.D., Austyn, J.M., 1993. Phagocytosis of antigens by Langerhans cells in vitro. J. Exp. Med. 178, 509–519.

Reis e Sousa, C., Hieny, S., Scharton-Kersten, T., Jankovic, D., Charest, H., Germain, R.N., Sher, A., 1999. In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin-12 by dendritic cells and their redistribution to T-cell areas. J. Exp. Med. 186, 1819–1829.

Res, P., Martinez-Carceres, E., Jaleco, A.C., Staal, F., Nobeboom, E., Weiger, K., Spits, H., 1996. CD34$^+$ CD38^{dim} cells in human thymus can differentiate into T, NK and dendritic cells but are distinct from pluripotent stem cells. Blood 87, 5196–5206.

Rissoan, M.C., Soumelis, V., Kadowaki, N., Grouard, G., Briere, F., Malefyt, R.D.W., Liu, Y.J., 1999. Reciprocal control of T-helper cell and dendritic cell differentiation. Science 283, 1183–1186.

Sallusto, F., Lanzavecchia, A., 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony stimulating factor plus interleukin-4 and downregulation by tumour necrosis factor-α. J. Exp. Med. 179, 1109–1118.

Saunders, D., Lucas, K., Ismaili, J., Wu, L., Marascovsky, E., Dunn, A., Shortman, K., 1996. Dendritic cell development in culture from thymic precursor cells in the absence of granulocyte/macrophage colony stimulating factor. J. Exp. Med. 184, 2185–2196.

Sauter, B., Albert, M.L., Francisco, L., Larsson, M., Somersan, S., Bhardwaj, N., 2000. Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue culture cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. J. Exp. Med. 191, 423–434.

Schuler, G., Steinman, R.M., 1985. Murine epidermal Langerhans cells mature into potent immunostimulatory cells in vitro. J. Exp. Med. 161, 526–546.

Seiffert, M., Cant, C., Chen, Z., Rappold, I., Brugger, W., Kanz, L., Brown, E.J., Ullrich, A., Buhring, H.J., 1999. Human signal regulatory protein is expressed on normal, but not subsets of leukemic myeloid cells and mediated cellular adhesion involving its counter-receptor CD47. Blood 94, 3633–3643.

Shortman, K., Caux, C., 1997. Dendritic cell development: multiple pathways to nature's adjuvants. Stem Cells 15, 409–419.

Steinman, R.M., 1991. The dendritic cell system and its role in immunogenicity. Annu. Rev. Immunol. 9, 271–296.

Strunk, D., Egger, C., Leitner, G., Hanau, D., Stingl, G., 1997. A skin homing molecule defines the Langerhans cell progenitor in human peripheral blood. J. Exp. Med. 185, 1131–1136.

Suss, G., Shortman, K., 1996. A subclass of dendritic cells kills $CD4^+$ T-cells via Fas/Fas-ligand-induced apoptosis. J. Exp. Med. 183, 1789–1796.

Vieira, P.L., de Jong, E.C., Wierenga, E.A., Kapsenberg, M.L., Kalinski, P., 2000. Development of Th-1 inducing capacity in myeloid dendritic cells requires environmental instruction. J. Immunol. 164, 4507–4512.

Vremec, D., Shortman, K., 1997. Dendritic cell subtypes in mouse lymphoid organs: cross correlation of surface markers, changes on incubation and differences between thymus, spleen, and lymph nodes. J. Immunol. 159, 565–573.

Watts, C., 1999. Dendritic cells spill the beans. Nature Cell Biol. 1, 152–154.

Werling, D., Hope, J.C., Chaplin, P., Collins, R.A., Taylor, G., Howard, C.J., 1999. Involvement of caveolae in the uptake of respiratory syncytial virus antigen by dendritic cells. J. Leukoc. Biol. 66, 50–58.

Williams, N.J., Harvey, J.J., Duncan, I., Booth, R.F.G., Knight, S.C., 1998. Interleukin-12 restores dendritic cell function and cell mediated immunity in retrovirus-infected mice. Cell Immunol. 183, 121–130.

Witmer-Pack, M.D., Olivier, W., Valinsky, J., Schuler, G., Steinman, R.M., 1987. Granulocyte/macrophage colony stimulating factor is essential for the viability and function of cultured murine epidermal Langerhans cells. J. Exp. Med. 166, 1484–1498.

Witmer-Pack, M.D., Valinsky, J., Olivier, W., Steinman, R.M., 1988. Quantitation of surface antigens on cultured murine epidermal Langerhans cells: rapid and selective increase in the level of surface MHC products. J. Invest. Dermatol. 90, 387–394.