Chapter 17
Dendritic Cells

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Abstract Dendritic cells (DCs) are the sentinels of the immune system and play a critical role in stimulating immune responses against pathogens and maintaining immune homeostasis to harmless antigens. They can be found in all lymphoid and most non-lymphoid tissues, including mucosal surfaces, like the lung and the gut, where intricate networks of DCs are situated to sense potential harmful exposures. As such, DCs are among the first cells to come into contact with food bioactives in the gastrointestinal tract and thus are instrumental in shaping the immune system's response to such exposures. Here we provide an overview of DC characteristics, with the emphasis on DCs in the mucosal immune system, and discuss in vitro/ex vivo DC culture settings that can be applied for in vitro testing of (food) compounds.

Keywords Dendritic cells • DC subsets • Culture • Mucosa

17.1 Origin

Paul Langerhans was the first to describe DCs in the nineteenth century, when he visualised dendritically shaped cells in the skin (Langerhans 1868). Ralph M. Steinman and Zanvil A. Cohn identified "dendritic cells" as a specific group of white blood cells (Steinman and Cohn 1973), and it was not until 1998 that the central role of DCs as professional antigen presenting cells for the induction of adaptive immunity was defined (Banchereau and Steinman 1998). Over the last few decades the DC-field has developed extensively with enhanced knowledge on DC progenitors, distinctive subsets, and their specific functions depending on the tissues where they reside.

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17.2 Features and Mechanisms

DCs continuously sample antigens from their environment by phagocytosis, receptor-mediated endocytosis, and pinocytosis. After uptake of antigens, DCs migrate to the draining lymph node. During migration they may mature and upregulate co-stimulatory molecules like CD40 and CD86, depending on the maturation signals they have encountered. DCs are equipped with unique pattern recognition receptors (PRRs) for which the ligands may activate or inhibit DC maturation and facilitate antigen recognition, uptake and processing. The extensive sets of PRRs (e.g. Toll-like receptors (TLRs), C-type lectins (CLRs), Nod-like receptors (NLRs) and Retinoic acid induced gene-based (RIG)-I like receptors (RLRs)) are expressed on the cell surface or in intracellular compartments, like endosomes or in the cytoplasm to enable recognition of intra- and extracellular exposures.

Dendritic cells are instrumental for the activation of naïve T cells or reactivation of T cells from the memory pool (McLellan et al. 1996). T cell activation requires antigen presentation in MHC class I (for $CD8^+$ T cell) or II (for $CD4^+$ T cells) molecules (signal 1), in combination with expression of co-stimulatory molecules (signal 2). Cytokines provide a third signal and induce T cell differentiation and effector functions. Depending on the cytokine environment provided by DCs, $CD4^+$ T cells differentiate into Thelper1 (Th1) via IL-12, Th2 via IL-4, or Th17 cells via IL-6 (Kapsenberg 2003; Bettelli et al. 2008). In addition, Th cells can also differentiate into regulatory phenotypes (Tregs, Th3 and Tr1) that dampen immune responses (Sakaguchi et al. 2006). On the other hand, DCs instruct $CD8^+$ T cells, which recognize MHC class I (cross)presented antigens, to develop into cytotoxic lymphocytes (CTLs) (Bhardwaj et al. 1994).

The responsiveness of DCs in terms of expression of co-stimulatory/regulatory molecules and cytokine profiles may thus provide insight into the immune modulating properties of food bioactives. Here, we provide an overview of the different DC subsets in blood, and more specifically in the gastrointestinal tract (Fig. 17.1). Then we will provide information on the in vitro and ex vivo possibilities to study effects of food bioactives on DCs.

17.2.1 DC Subsets

To study immune modulatory effect of bioactives on DCs, different models systems may be considered, e.g. DC cell lines, in vitro-generated DCs, or directly isolated primary DCs, each with their own advantages and disadvantages. It is important to realize that different subsets of DCs exist in peripheral blood and (non)lymphoid tissues and that the differentiation and function of each of these subsets is highly dependent on the interacting cells and mediators in the local microenvironment. So, while this chapter provides experimental settings to study the direct effects of food bioactives on DCs, one should always consider the possible indirect effect via stimulation of the bioactives on for instance epithelial cells, and that different DC subsets may respond differently to the same exposure.

		Blood	Mucosa	TLR expression
pDC		CD11c^{int} CD123+ BDCA2+ BDCA4+		TLR 7,9
BDCA3 cDC		CD11c+ CLEC9A+ (Langerin+)	CD103+ Sirpα-	TLR1,2,3,6,8,10
BDCA1 cDCs		CD11c+ CD1c+	CD103+ Sirpα+ CD11b+ RALDH	TLR1,2,3,4,5,6,8,10
Langerin$^+$ DCs		Langerin+		TLR1,2,3,5,6,10
MoDC		CD11c+ FcεR1+	CD103- Sirpα+ CX3CR1	TLR1,2,4,5,8,9

Fig. 17.1 Surface marker phenotype and TLR pattern expression in DC subsets in blood and mucosa

17.2.1.1 Blood DCs

DCs can be divided in conventional DCs (cDCs) and plasmacytoid DCs (pDCs). In human blood, both cDCs and pDCs can be phenotypically characterized by the lack of lineage marker expression (CD3, CD14, CD19, CD56) and a high expression of HLA-DR. The circulating cDCs are CD11c$^+$ and are divided by the expression of CD1c (BDCA1) or CD141 (BDCA3). Human pDCs lack expression of CD11c and are discriminated by the expression of BDCA2, BDCA4 and/or CD123. CD16$^+$ MHC class II$^+$ cells cluster as a distinct population in the blood (Lindstedt et al. 2005) and have previously been associated with DC origins by some. However, CD16$^+$ cells were recently assigned to the monocyte lineage (Robbins et al. 2008; Ziegler-Heitbrock et al. 2010), confirmed by transcript profiling comparing CD16$^+$ cells with BDCA1$^+$ DCs (Frankenberger et al. 2012).

17.2.1.2 Mucosal DCs

The mucosal immune system is highly specialized: it has to be able to avoid invasion of (commensal) bacteria, and tolerate their presence in the intestine. It should also induce tolerance to non-harmful food antigens and protect against potentially harmful pathogens, toxins and xenobiotics. All of this takes place over a single layer of highly specialized epithelial cells (Chapter 2).

The formulation of antigens determines the main site for antigen uptake in the intestinal tract. Considerable evidence suggests that the gut associated lymphoid tissues (GALT), such as Peyers Patches (PP) and isolated lymphoid follicles, are critical for handling of particulate antigens. The microfold cells (M cells), present in the GALT epithelium, are involved in actively transferring the particulate antigens from the gut lumen into the lymphoid areas. The role for GALT in the induction of oral tolerance to soluble antigens is not entirely clear. Normal oral tolerance could be induced in mice lacking PP. It was therefore suggested that the main function of the GALT is to control immune responses to commensal bacteria. Antigen uptake by DCs underlying the villus epithelium of the lamina propria (LP) in the small intestine has been shown to be crucial for induction of oral tolerance to soluble antigens. How the DCs are provided with the antigens over the epithelial barrier will strongly depend on the compound and is beyond the scope of this chapter. Since both the intestinal tract and its draining lymph nodes contain specific DC subsets we will briefly discuss them before providing detailed information of possible DC systems that could be used to mimic these DCs.

Intestinal DCs: Thorough comparative transcriptional and functional profiling in DCs isolated from a human and murine small intestine LP and peripheral blood showed three DC subsets within the $CD45^{+}lin^{-}MHCII^{+}$ LP population based on the expression of CD103 and Sirpα (Watchmaker et al. 2014). The $CD103^{+}Sirp\alpha^{-}$ DCs (SP) were related to human blood $BDCA3^{+}$ DCs, whereas $CD103^{+}Sirp\alpha^{+}$ (DP) DCs expressed CD11b, CD207 (Langerin), CD209 and high levels of RALDH (coinciding with human blood $CD1c^{+}$ DCs) and supported the induction of ($FoxP3^{+}$) regulatory T cells. The $CD103^{-}Sirpa^{+}$ expressed CX3CR1, but lacked CD64 expression, and clustered with human monocytes indicating that they may have developed from monocytes recruited in response to gut inflammation. Most of these cells are located deeper into the LP when compared to the network of phagocytic cells that is located right beneath the epithelial cells. These phagocytic cells express CD45, HLA-DR, CD14, CD64 and high levels of CX3CR1, and since these cells also do not migrate to the lymph nodes, they have been depicted as intestinal macrophages (Rivollier et al. 2012; Bain et al. 2012; Tamoutounour et al. 2012; Mann et al. 2013).

Mesenteric lymph node DCs: Soluble food bioactives may also be directly available for internalization by DCs in the draining lymph nodes via the conduit system (Gretz et al. 1997; Anderson and Shaw 2005; Sixt et al. 2005; Roozendaal et al. 2009). The mesenteric lymph node DCs are a mixture of cells found in peripheral blood as well as the LP. As such, peripheral blood DCs and their CD34-derived counterparts could represent these lymph node DCs.

17.2.1.3 Monocyte-Derived DCs

Monocyte-derived (mo)DCs are rare under steady-state conditions in both blood and peripheral tissues. They can be found in increased numbers in vivo under inflammatory conditions. The ability to culture moDCs from monocytes boosted their

popularity as a model to study human DC biology (Sallusto and Lanzavecchia 1994). The gene profile of in vitro-generated MoDCs is enriched for genes also found in in vivo occurring moDC (Segura et al. 2013) and also emphasizes the monocyte origin (not DC origin) with acquired DC features.

17.3 General Protocols

Primary DCs can be isolated from blood, and lymphoid or peripheral tissues, but the presence of DCs in blood is scarce and they are difficult to obtain in sufficient numbers from tissues. In addition, one may encounter large donor variations. The use of cell lines would overcome these challenges, but may hold insufficient similarity with primary DCs with respect to their response to environmental stimuli, ability to capture antigens, mature, migrate, produce cytokines, present antigens and activate T cells. Here we summarize a series of commonly used functional DC assays below and discuss the performance of cell lines and primary cells (summarized in Table 17.2).

17.3.1 DC Cell Lines

Most DC cell lines were differentiated from leukemia-derived cells, originating from myelogenous or monocytic lineage: the cytokine independent cell lines THP-1, U937, KG-1, HL-60 and the cytokine dependent cell line Mutz-3 are the most commonly used (Table 17.1) (Berges et al. 2005; van Helden et al. 2008; Santegoets et al. 2008). The use of these lines has the advantage of the availability of large numbers of synchronized cells with a long life span, but the association with selective DC subsets in peripheral blood and mucosal surfaces is largely unclear. All of these cells are leukemic, representing DC precursor stages and may often need the addition of specific growth and differentiation factors to induce at least some characteristics of differentiated DCs. MUTZ-3 is suggested to have the highest DC differentiation capacity (Santegoets et al. 2008) and may therefore be most representative for in vivo DCs.

Table 17.1 DC cell lines

Cell line	Derived from	DC differentiation	T cell activation capacity	References
THP-1	Monoblastic leukemia cell line	Low	Yes	Tsuchiya et al. (1980) Chap. 14
U937	Monocytic leukemia cells	Moderate	Yes	Chapter 15
KG-1	CD34 myelomonocytic cell line	Incomplete	Yes	Koeffler et al. (1981)
HL-60	Acute pro-myelocytic leukemia cell line	Moderate	Yes	Koski et al. (1999)
MUTZ-3	Myeloid leukemia cell line	Relatively high	?	Hu et al. (1996)

17.3.2 Isolating Primary DCs from Blood

DCs can be directly isolated from PB using their unique surface markers (Fig. 17.1) and utilizing either magnetic bead separation or flow cytometric cell sorting (Vremec and Shortman 1997; Dzionek et al. 2000; Shortman and Liu 2002). Since the percentage of DCs in PB is generally low (cDC: 0.6 % and pDC 0.2 % of all PBMC), DC-enrichment (by depletion of cells expressing lineage markers CD3, CD14, CD16, CD19, CD20, and CD56) is often used. For magnetic bead sorting, separation/isolation kits are commercially available from companies such as Miltenyi Biotec and StemCell Technologies. Preferably, freshly isolated PBMCs obtained by a density gradient of Ficoll-Hypaque, are suspended in cell isolation buffer as per manufacturer's instructions. To optimize survival of primary DCs after isolation it may be helpful to add survival factors to the culture medium: 800 U/ml GM-CSF for cDCs, like CD1c or CD141 DCs, or 10 ng/ml IL-3 for pDCs.

Culturing peripheral blood-derived CD1c (BDCA1) DCs for 2 days with GM-CSF, vitamin D and Retinoic Acid (RA) induced high expression of RALDH as well as the ability to induce gut-homing receptors resembling the gut-resident $CD103^{+}Sirp\alpha^{+}$ (DP) DCs to some extent (Sato et al. 2013).

Although using primary DCs directly isolated from the blood is feasible for most academic research labs and will probably resemble the in vivo DC biology most closely, the limited amount of primary DCs in PB is an obvious obstacle when many conditions and functional effects need to be assessed.

17.3.3 $CD34^{+}$-Derived DCs

Another alternative is to generate DCs from myeloid precursors. CD34 is a marker expressed on haematopoietic stem cells and early myeloid progenitors that contain high proliferative potential. The $CD34^{+}$ cells can therefore first be expanded followed by a differentiation towards DCs. $CD34^{+}$ cells can be isolated from bone marrow (BM), peripheral blood (PB) or from umbilical cord blood (CB) using magnetic labelling (Kato and Radbruch 1993).

Firstly, $CD34^{+}$ cells can be expanded using different combinations of cytokines and growth factors. Flt-3L, TPO, SCF, IL-3, and IL-6 are early-acting cytokines that support the proliferation of DC precursors from $CD34^{+}$ HPC. Two different cocktails, Flt3-L, TPO, SCF and Flt3-L, SCF, IL-3, IL-6 (FS36), were compared for their capacity to induce proliferation of $CD34^{+}$ HPC (Bontkes 2002). FS36 showed the greatest ability to expand the $CD34^{+}$ HPCs. Expanded $CD34^{+}$ cells can be differentiated towards different DC subtypes i.e. $BDCA3^{+}$ DCs up to only 3 % (Poulin et al. 2010), or pDCs up to 5 % (Demoulin et al. 2012). Addition of TGF-beta during differentiation enhances the formation of $langerin^{+}$ LC (Soulas et al. 2006; Caux et al. 1992; Szabolcs et al. 1995).

Established protocols to culture LCs, dermal DCs, $BDCA3^{+}$ DCs and pDCs can be found in literature and are summarized below (Fig. 17.2).

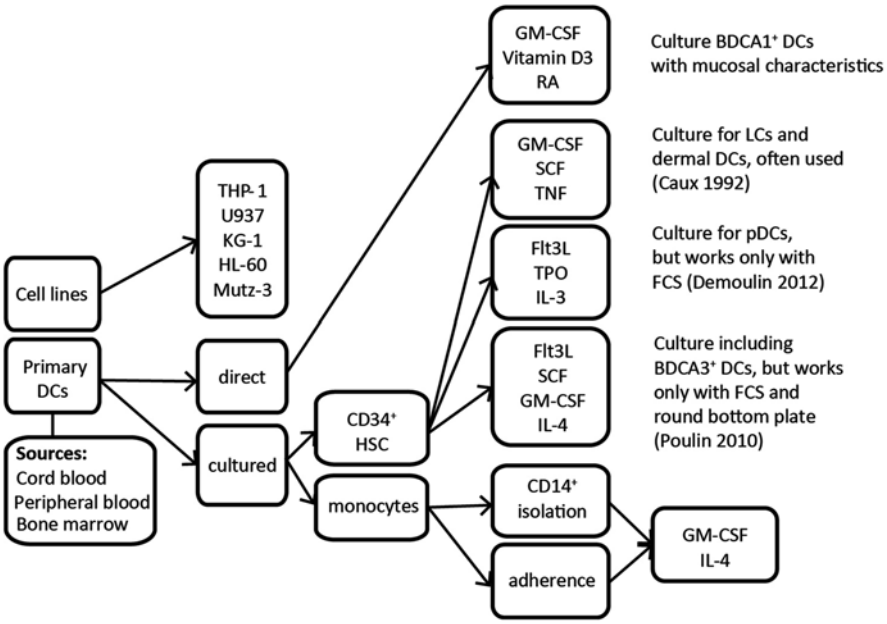

Fig. 17.2 Different DC sources

17.3.4 Monocyte-Derived DCs

Monocytes can be selected via adherence or $CD14^+$ isolation with magnetic beads. For adherence, PBMCs are resuspended in culture medium and placed in a humidified incubator maintained at 37 °C and 5 % CO_2, to allow the monocyte precursors to adhere. After 1 h, non-adherent cells are simply removed by firmly tapping the flask and isolating non-adherent cells. For bead selection, PBMCs are resuspended in cell isolation buffer as per manufacturer's instructions, followed by isolation using a commercially available kit. The isolated monocytes can be frozen for later use.

Once the monocytes are selected, they can be differentiated towards DCs with culture-medium (e.g. X-vivo) supplemented with 800–1,000 U/ml GM-CSF and 300–500 U/ml IL-4 (37 °C and 5 % CO_2) for 7 days. After the 6/7-day culture period, the immature DCs can be used for testing the immune modulatory effects of food bioactives.

To generate mucosal $CD103^+Sirp\alpha^+$ (DP) resembling moDCs, RA, the even more potent RARalpha agonist AM580 or TGFbeta, can be added to the culture (Hartog et al. 2013; Martin et al. 2014). RA-induced moDCs express CD103 and can induce increased percentages of $FoxP3^+$ in allogeneic $CD4^+$ cells. RA-induced moDC also express high levels of IL-22BP which is comparable to the $CD11b^+CD103^+$ DCs found in mouse LP and MLN (Martin et al. 2014).

17.4 Asses Viability

To validate the generation of DCs in these cultures, the purity of DCs can be assessed by flow cytometric analysis of the surface markers CD11c and HLA-DR. This staining can be expanded with markers, depending on the specific protocol used (for instance CD14 expression on MoDC or CD1c on CD34-derived DC.) Viability can be checked by adding a viability dye (e.g. 7AAD) to the FACS panel, or count the amount of cells with Tryphan blue with a microscope (Table 17.2).

17.5 Experimental Readout

Although bioactive food components have been shown to interact with many different types of cells, it remains a legitimate question whether DCs exposed to food bioactives may in turn affect features of the innate or adaptive immune system, in particular T cells. Some food bioactives may be associated with the development of allergic or autoimmune-like clinical symptoms in susceptible individuals. The clinical manifestation, ranging from mild to severe and life-threatening, vary considerably for different compounds and for the same compound in different subjects and may be idiosyncratic in nature. They comprise pseudo-allergic reactions (interference with immunological effector mechanisms and cells, such as mast cells), compound-allergic reactions (activation of compound-specific T and B cells),

Table 17.2 Summary of (dis)advantages of using differentially generated DCs

Type of DCs	Advantages	Disadvantages
Cell lines (*THP-1*, *U937*, *KG-1*, *HL-60*)	Large number of cells. Relatively long life span.	Association to primary DCs unclear (different stages of differentiation, discrepancies in DC-functions)
Primary DCs		
CD1c	Largely comparable to in vivo counterpart. CD1c can be treated to differentiate into gut-like DCs.	Low cell numbers. Isolation procedures might affect phenotype and function.
BDCA3		
pDCs		
CD34-derived DCs		
Dermal DCs/ LCs	$CD34^+$ cells can be expanded to increase cell number. Resemble primary DCs very well.	Acquisition requires specialized centres. % of pDC and BDCA3 DC are still low in these cultures, thus requiring large numbers of cells for selection.
pDCs		
BDCA3		
Monocyte-derived DCs		
	Large number of cells. Relatively easy to obtain and culture. Very well studied: extensive set of data available.	Represent inflammatory monocytes with DC-features, which may hamper translation for primary DCs.

and compound-induced autoimmune reactions. Although the induction of allergic or autoimmune responses requires DC activation, food bioactives may also inhibit DC activation. This may represent an advantageous effect of food bioactives in reducing (chronic) inflammatory response like asthma, inflammatory bowel disease, etcetera, by reducing the DC activity and possibly inducing regulatory T cells.

Different assays can be performed to test the DC's responsiveness to bioactives for the prediction of the subsequent immune modulatory effect of that specific compound. These assays include the monitoring of phenotypic markers expressed on the surface of DCs indicating their differentiation, activation and their potentially stimulating effect on T cells. Nevertheless, even with a clear outcome of these assays, it remains to be seen whether indeed antigen-specific T cells with the potential to induce adverse effects in vivo will be induced. In this regards it is important to consider whether the food bioactive contains potential antigens, are able to form neo-antigens or merely function as adjuvants for responses against other components in the food or play a role in sensitization to auto-antigens.

17.5.1 Co-stimulation

DCs may undergo a number of phenotypical changes, following maturation by the uptake and processing of an antigen. The process of DC maturation, in general, involves an increase in the surface expression of co-stimulatory molecules, like CD40, CD80, CD83, and CD86, which can be measured by flow cytometry. As a negative control unloaded DCs can be taken along, as well as a DC positively stimulated with a known maturation cocktail, like IL-1beta, IL-6, TNF and PGE-2 or TLR ligands.

17.5.2 Cytokine Production

Besides up-regulation of co-stimulatory molecules, DCs produce a large variety of cytokines that could be either pro/anti-inflammatory or skew the phenotype of T cells. Therefore, a quite simple procedure to test the activity of the DCs towards the food bioactives is the measurement of cytokine levels. A wide variety of cytokines may be expressed by mature DCs including IL-1 alpha, IL-1 beta, IL-4, IL-6, IL-8, IL-10, IL-12, IL-15, IL-16, IL-17, IL-18, IFN-alpha, IFN-beta, IFN-gamma, and TNF. These cytokines can be measured by ELISA, or multiplex assays.

17.5.3 Other DC Readouts

Co-stimulation and cytokine production are effect parameters used in the vast majority of studies on effects of food bioactives on DCs (see Table 17.3 for an overview). Studying the induction of specific T cells is complicated due to the low

Table 17.3 In vitro studies using food-associated antigens in in vitro DC models

DC source	Food bioactive group	Specific food bioactives	Additional DC activator	Expression costimulation	Cytokine production	Other	MLR	References
CD34+ derived DC	Probiotics	*L. paracasei* CNCM I-4034 *B. breve* CNCM I-4035	*S. typhi* CECT 725 or LPS	No	Yes: various	TLR expression	No	Bermudez-Brito et al. (2012, 2013)
pDC (PBMC)	Probiotics	*L. lactis* JCM5805		Yes: HLA-DR and CD86	Yes: IFN-alpha	IFN and IRF expression	No	Sugimura et al. (2013)
Lamina propria and PBMC	Probiotics	Probiotic preparation VSL#3	LPS	Yes: CD40 and CD80	Yes: IL-10 and IL-12	Effect on T cell responses	No	Hart et al. (2004)
Lamina propria and PBMC	Probiotics	*L. plantarum* BMCM12		No	Yes: various		Yes	Bernardo et al. (2012)
MoDC	Probiotics	*Lactobacillus rhamnosus* Lcr35		Yes: CD83, CD86, CD209 and HLA-DR	Yes: various	Microarray gene expression profile	No	Evrard et al. (2011)
MoDC	Polyphenols	Protocatechuic acid	LPS	Yes: CD83, CD86 and HLA-DR	Yes: various	Migration PPARg activation	No	Del Cornò et al. (2014b)
THP-1-derived	Polyphenols	Apple polyphenol extract	OVA (endotoxin?)	Yes: CD86 and HLA-DR	Yes: IL-1b, IL-10, IL-12 and TNF-a	Antigen uptake	No	Katayama et al. (2013)
MoDC	Isoflavones	Genistein and Daidzein	LPS, TNFa, CT	Yes: CD83, CD80, CD86, and HLA-DR	Yes: IL-6, IL-12, TNF-a and IL-10	DC–NK interaction	Yes	Wei et al. (2012)
MoDC	Isoflavones	Genistein, Daidzein and Glycitein	CT	Yes: CD83, CD80 and CD86	Yes: IL-6 and IL-8	Th-cytokine induction (IL-5, IL-9, IL-13)	Yes	Masilamani et al. (2011)

MoDC	Curcuminoids	Curcumin	LPS or Poly I:C	Yes: CD86, CD83, CD54 and HLA-DR	Yes: various	Migration Endocytosis	Yes	Shirley et al. (2008)
MoDC	Curcuminoids	Curcumin	LPS	Yes: CD40, CD83, CD80, CD86 and HLA-DR	Yes: various	FOXP3 induction Negative regulatory molecules (PDL1/2)	Yes	Rogers et al. (2010)
MoDC	Vitamins	1alpha,25-(OH)2 D3	LPS or PGN	Yes: CD40, CD83, CD80, CD86 and HLA-DR	Yes: IL-6, IL-8, IL-12p70 and IL-10		No	Brosbøl-Ravnborg et al. (2013)
MoDC	Vitamins	1,25-(OH)2 D3	LPS or CD40L	Yes: CD40, CD83, CD80, CD86 and HLA-DR	Yes: IL-12p70	Endocytosis	Yes	Piemonti et al. (2000)
MoDC	Proteins	Peptic fragments of gliadin, pRQP and pDAC (decapeptides)		Yes: CD83, CD80, CD86, CCR7 and HLA-DR	Yes: IL-12, TNF-a and IL-10		Yes	Giordani et al. (2014)
MoDC	Proteins	Soluble peanut Ag (PNAg) and purified Ara h 1	LPS and CT (positive controls)	Yes: CD83, CD40, CD86, and HLA-DR	No	Th-cytokine induction (IFN-g, IL4 and IL-13)	Yes	Shreffler et al. (2006)
MoDC	Proteins	Bet v 1, Mal d 1, Api g 1 and Dau c 1		Yes: CD40, CD83, CD80, CD86 and HLA-DR	No	Th-cytokine induction (IFN-g, IL4, IL-10 and IL-13)	No	Smole et al. (2010)
MoDC	Proteins	Peptic fragments of gliadin, soya protein, and OVA		Yes: CD83, CD80, CD86 and HLA-DR	Yes: various	Endocytosis and signal transduction	Yes	Palova-Jelinkova et al. (2005)

frequency of T cell precursors with the corresponding TCR and requires extensive culture conditions and broad read-out tools as the TCR specificity in these conditions is unknown. With regard to the T cell stimulatory **potential** of DCs the mixed lymphocyte reaction (MLR), in which DCs are used to stimulate alloreactive (naïve) T cells, can be considered. Proliferation in combination with cytokine production can be used as a read-out for T cell skewing.

17.6 In Vitro Studies on Food Bioactives Using DCs (Table 17.3)

The immunomodulatory effects of food bioactives can be broadly divided in 'activation', 'suppression' or 'no detectable effect'. Table 17.3 shows that with regard to food proteins immune activation is generally the anticipated endpoint. It should however be considered that other food constituents instead of the protein itself, may stimulate DCs to internalize and present food proteins, and therefore may be involved in the risk for hypersensitivity. Immune suppression in DCs assays is mostly studied in combination with a trigger known to activate the DCs (TLR ligands i.e. LPS, CT or CD40 ligation). Inhibition of DC activation has been studied using probiotics, vitamins, polyphenols (Del Cornò et al. 2014a), isoflavones and curcuminoids. Inhibition of DC activation may be relevant for people suffering from chronic inflammation not only by reducing inflammation directly but also by preventing the risk for developing cancer, which is associated with chronic inflammation. Potential harmful effects of non-specific immune suppression include an increased risk of infection and also here the development of cancer. When there is no direct effect of the food bioactives on the DCs with the assays mentioned above, this does not mean that exposure to the compound is without risk. Indirect effects of the bioactives through their effects on other cells or the effects of their metabolites must be taken into consideration.

17.7 Critical Notes

Extensive comparative studies using different DC assays with proven immune active compounds and food bioactive compound are lacking.

With regard to some of the DC parameters used to study immune modulatory effects of food bioactives, the expression of co-inhibitory molecules (e.g. PDL1 and PDL2) has not been intensively studied. This family of molecules has however a large impact on the immune response and may therefore also be considered in future testing.

As mentioned before, the DCs are the sentinels of the immune system that gather not only by taking up or sensing the food bioactives only, but continuously receive input from cells and factors in their microenvironment. Potential responses should

therefore be evaluated in the light of the local environment. For instance, the choice for a particular DC model/subset may be dependent on the availability of certain compounds/entry site, which may relate to the size, solubility and formulation of the compound of interest.

In conclusion, studying the effects of food bioactives on DC (subsets) is an important step in addressing the immune modulating potential of these compounds in vivo, but should be part of a more extended monitoring program involving studies on the compound's availability, factors of the microenvironment, and effects of metabolites/neo-epitopes.

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