Human in vivo-differentiated monocyte-derived dendritic cells

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Abstract

When entering tissues, monocytes can differentiate into cells that share morphological and functional features with either dendritic cells (DC) or macrophages. Monocyte-derived DC have been observed in humans at mucosal tissues and in inflammatory settings, where they are usually referred to as « inflammatory DC ». In this chapter, we review recent studies on the characterization of these cells in humans. We also discuss nomenclature and examine the criteria defining *in vivo*-differentiated human mo-DC.

Keywords : monocyte ; dendritic cell; inflammation ; auto-immune disease ; cancer

1. Introduction

Monocytes have long been used as precursor cells for the *in vitro* generation of human dendritic cells (DC) [1]. However, it has become clear that, *in vivo,* most DC derive from a specific precursor, termed pre-DC, distinct from monocytes [2-8]. The physiological relevance of monocytes as DC precursors was demonstrated in the mouse in a *Leishmania* infection model in which monocytes could differentiate *in vivo* into cells that shared phenotypic and functional features with DC [9]. Numerous subsequent studies in mice have

shown that DC can differentiate *in vivo* from monocytes in various inflammatory settings, including infections and models of auto-immune diseases [10, 11]. Because they arise during inflammation, these *in vivo*-differentiated monocyte-derived DC (mo-DC) were initially termed « inflammatory DC ». However, this term can be misleading as it implies that mo-DC are « inflammatory » by essence. Yet, mo-DC can be found in mice in the absence of inflammation at mucosal sites, such as the intestine and the skin [12-14], and in skeletal muscles [15].

In the classification of DC lineages based on ontogeny [16], mo-DC represent a separate DC subset. Importantly, monocytes can also differentiate into macrophages, which are distinct from "resident" macrophages derived from embryonic precursors [17] (figure 1). Whether mo-DC are fundamentally distinct from monocyte-derived macrophages (mo-Mac), and how to distinguish these two populations has been controversial. In this chapter, we will cover *in vivo*-differentiated human mo-DC, both generated in the steady-state (referred to as « steady-state mo-DC ») and arising during inflammation (referred to as « inflammatory mo-DC »). We will also discuss recent data on the molecular requirements for mo-DC versus mo-Mac differentiation.

2. Definition of human *in vivo*-differentiated mo-DC

2.1 Identification of human inflammatory mo-DC

« Inflammatory dendritic epidermal cells » (IDEC) were first described in the skin of atopic dermatitis patients [18]. These cells were absent from healthy skin and characterized by a surface phenotype distinct from that of Langerhans cells. Subsequently, IDEC were also identified in the skin of psoriasis patients [19]. Another study reported in the skin of psoriasis patients the presence of a population of « inflammatory dermal DC », phenotypically distinct from the dermal DC found in healthy skin [20]. Whether these inflammatory DC represented

an activated form of classical DC or were related to monocytes was not investigated at the time.

Several lines of evidence suggest that in humans, as in mice, monocytes recruited to inflammatory sites can differentiate into DC. Radio-labelled autologous monocytes are recruited to the gut in inflammatory bowel disease patients [21] and monocyte numbers are increased in the intestine of Crohn's disease and ulcerative colitis patients compared to healthy donors [22]. Influx of monocytes were also observed in cantharidin-induced skin blisters [23], in the peritoneum of patients with dialysis-related peritonitis [24] and in the nasal mucosa of subjects with induced allergic rhinitis [25]. In the latter study, monocytes were recruited in the nasal mucosa within hours after allergen challenge while a new population of DC appeared after 3 days, consistent with the potential differentiation of monocytes into mo-DC. Moreover, we have identified in synovial fluid from rheumatoid arthritis patients and in peritoneal ascites from cancer patients a population of « inflammatory DC » with a phenotype different from that of classical blood DC [26]. We showed by transcriptomic analysis that these cells represent a distinct DC subset and share gene signatures with *in vitro*-generated monocyte-derived cells, supporting the idea that these inflammatory DC are more closely related to monocytes than to classical DC [26, 27]. Finally, mo-DC have been evidenced in human lung and colorectal tumors, based on their phenotypic similarity with tumor-associated mo-DC in mice tumor models [28].

Collectively, these results support the notion that monocytes recruited to inflammatory sites can differentiate *in situ* into mo-DC in humans.

2.2 Defining features of *in vivo*-differentiated mo-DC

DC have been traditionnally defined based on 1) their distinctive morphology, with the presence of dendrites ; 2) their superior ability to activate naive T cells ; 3) their capacity to

migrate to lymph nodes. In addition, distinct DC subtypes can be defined based on their ontogeny and their requirement for distinct transcription factors [16]. However, because these properties can be difficult to investigate in humans, most studies rely on the differential expression of phenotypic markers to distinguish DC from other myeloid cells, and DC subsets from one another.

Human inflammatory mo-DC are HLA-DR$^+$CD11c$^+$ cells that express markers found on classical DC such as CD1c, CD1a, CD1b, FcεRI, as well as molecules that are also expressed by macrophages including CD206, CD14 and CD11b [19, 25, 26, 28, 29]. By contrast, they lack the macrophage markers CD16 and CD163 [26] [28]. Inflammatory mo-DC can also be distinguished from macrophages by their morphology (figure 2) and their capacity to stimulate T cell proliferation [20, 26].

2.3 Molecular ontogeny of mo-DC

The relationship of mo-DC to monocytes is suggested by their expression of CCR2, a chemokine receptor essential for monocyte recruitment into peripheral tissues [30]. However, this is not an absolute marker of monocyte-derived cells, as a CCR2$^+$ DC population from mouse intestine has been shown to derive from pre-DC [31].

The ontogeny of human DC is usually inferred from the analysis of gene patterns and specific transcription factors, but can also be analyzed using *in vitro* differentiation models [6, 7, 16]. Inflammatory mo-DC from ascites express a unique combination of transcription factors that are either shared with classical DC (IRF4, BATF3, ZBTB46) or with macrophages (EGR1, EGR2), but they do not express MAFB which is restricted to macrophages [26]. Using a novel model of human monocyte differentiation, we have recently shown that MAFB controls monocyte differentiation into mo-Mac, while IRF4, BLIMP-1 and Aryl Hydrocarbon Receptor (AHR) are essential for monocyte differentiation into mo-DC

[27]. Moreover, patients suffering from systemic juvenile idiopathic arthritis underexpress AHR in their monocytes, which are biaised towards mo-Mac differentiation [32].

Precursors of human classical DC have been shown to be pre-committed to either lineage at an early stage [8]. By contrast, single-cell RNA-seq analysis of human $CD14^+$ monocytes has revealed that monocytes do not contain two populations that would be pre-committed to differentiate into mo-DC versus mo-Mac [27]. Rather, blood $CD14^+$ monocytes are programmed to spontaneously differentiate into blood $CD16^+$ monocytes [33], unless they extravasate into tissues. Monocytes express a partial mo-Mac gene signature, suggesting they may possess in tissues a default differentiation pathway into macrophages unless they encounter specific cues, such as inflammatory cytokines and AHR ligands [27]. Because their differentiation is governed by distinct transcription factors, mo-DC and mo-Mac do not represent variations of a highly plastic cell population as previously proposed [16], but rather are *bona fide* cell lineages derived from monocytes (figure 3).

2.4 Slan-DC

$CD16^+$ cells that express a 6-sulfo LacNAc modification of the P selectin glycoprotein ligand 1 (PSGL-1) have been termed « slan-DC » and proposed to represent an inflammatory subtype of DC based on their capacity to produce large amounts of TNF-α upon stimulation [34]. These cells were initially described in the blood, but can also be found in tissues such as the skin of psoriasis patients [35], tumor-draining lymph nodes [36], intestinal lamina propria [37], mesenteric lymph nodes and intestinal mucosa of Crohn's disease patients [38], renal carcinoma [39], tonsil [36] and brain lesions of patients with multiple sclerosis [40]. Comparative transcriptomic analysis identified slan-DC as a subset of $CD16^+$ monocytes [41, 42] and showed that they are distinct from the DC lineage [43]. These results are further supported by functional analysis showing that slan-DC are poor antigen-presenting cells

compared to classical DC [41, 43]. In addition, slan-DC do not express CCR7 [39] and exhibit a macrophage morphology in tissues [37]. These observations suggest that slan-DC may be a specialized monocyte subset rather than mo-DC.

2.5 A practical key to identify human mo-DC from biological samples

Most studies rely on a small number of surface markers to distinguish cell populations. Because macrophages, monocytes, mo-DC and classical DC subsets display overlapping phenotypes, identifying these different populations in biological samples can be challenging. Based on the results discussed above, we propose that several categories of criteria should be combined for identifying human *in vivo*-differentiated mo-DC :

1) *Phenotype*. Mo-DC are HLA-DR^{+}CD11c^{+}CD14^{int}CD206^{+}CD1c^{+} cells. Additional useful markers to distinguish them from other myeloid cells are shown in Table 1.

2) *DC Function*. Mo-DC possess typical DC functions, including the ability to efficiently stimulate naive T cells and the capacity to express CCR7 (potentially enabling their migration to lymph nodes).

3) *DC morphology*. Mo-DC display a typical DC morphology : they are of small size, possess dendrites and lack large cytoplasmic vacuoles (as opposed to macrophages) (figure 2).

4) *Ontogeny.* Mo-DC derive from monocytes, which can be assessed by analyzing gene signatures or the expression of CCR2. Mo-DC can be distinguished from mo-Mac by the expression of specific transcription factors such as IRF4 and AHR, and the absence of MAFB expression.

3. Distribution of human mo-DC

3.1 Human steady-state mo-DC in mucosal tissues

Several studies have analyzed the composition of myeloid cells at mucosal sites, including cells displaying the phenotype of mo-DC. Lungs of healthy donors comprise a population of $CD14^+CD206^+CD1c^+CD1a^+$ cells that express IRF4 [44]. $CD14^+CD206^+CD1c^+CD163^-$ cells are also present in the broncho-alveolar lavage (BAL) of healthy volunteers and express IRF4 and CCR2 [45]. Another study reported the presence of $CD14^+CD1c^+CD1a^+$ DC in the BAL of healthy volunteers and showed that these cells can upregulate CCR7 [46]. In addition, transcriptomic analysis indicated that this population overexpresses CCR2, CD206 and CD11b [46]. These results suggest that mo-DC are present in human lungs at the steady-state. $CD14^{low}CD206^+CD1c^+CD1a^+FcεRI^+CD11b^+$ cells can also be found in the peritoneum as shown through the analysis of dialysate of patients, with or without dialysis-induced peritonitis [24]. These cells express CCR2, IRF4 and upregulate CCR7 upon activation [24], suggesting they are mo-DC .

Three main DC populations were identified in the small intestine, including a $CD1c^+$ $CD11b^+$ population that was increased in patients with hyperemic mucosa (i.e. with increased redness and blood flow, consistent with inflammation) [47]. The transcriptomic signature of this population was closer to blood monocytes than to classical blood DC, suggesting they are mo-DC.

Finally, whether mo-DC are present in human skin in the steady-state remains unclear. Dermal $CD14^+$ DC were initially proposed to be mo-DC based on their reconstitution by donor-derived cells after hematopoietic stem cell transplantation and on their transcriptional proximity with blood monocytes [48, 49]. However, this population may be heterogeneous and a large proportion of them have been shown to be actually monocyte-derived macrophages [50].

3.2 Human mo-DC in cancer

Several studies in mouse models have shown that circulating monocytes are recruited to tumors and can differentiate at the tumor site [51-53]. It is therefore likely that human tumors contain inflammatory mo-DC within the myeloid infiltrate, but this question has been little studied. Tumor-associated mo-DC have been identified based on their phenotype in lung and colorectal tumors [28]. $CD14^+CD1c^+$ cells, which may be mo-DC, have also been observed by histology in melanoma skin lesions and colon metastasis [54]. Finally, a single-cell analysis of lung adenocarcinoma has evidenced that some $CD1c^+$ DC express monocyte/macrophage markers (CD14, CCR2, CD206, CD64, CD11b), which could indicate the presence of a population of mo-DC [55] .

3.3 Human mo-DC in blood

Human mo-DC have been identified in a variety of tissues (Table 2), but whether circulating mo-DC are present in the blood remains unclear. Blood $CD14^+CD1c^+CD11b^+$ cells have been described, but these cells do not express CD206 nor CD1a [54]. This population is elevated in the blood of melanoma patients compared to healthy donors. Comparative transcriptomic analysis showed that this population is closely related to monocytes, but whether they are a subpopulation of monocytes or *bona fide* mo-DC requires further investigation.

In addition, single-cell RNA-seq analysis showed that blood $CD1c^+$ DC comprise two distinct subgroups, one of which was termed « inflammatory » [5]. These « inflammatory » $CD1c^+$ DC express mRNA for CD14, but not the protein at the cell surface. They can be separated from the other $CD1c^+$ DC subgroup by their differential expression of CD163 and CD36. Despite being termed « inflammatory », these cells are transcriptionnally closer to classical DC rather than to monocytes, and are most likely derived from pre-DC. The properties of these two $CD1c^+$ DC populations remain to be characterized.

4. Functional properties of *in vivo*-differentiated mo-DC

Due to the difficulty of isolating human mo-DC directly from biological samples, there is limited data on their functional properties. Human mo-DC efficiently activate CD4 and CD8 T cells in *ex vivo* assays [20, 24, 26]. Peritoneal mo-DC secrete IL-6, TNF-α, IL1-β and IL-12p70 upon *ex vivo* restimulation [24]. Mo-DC from BAL also secrete TNF-α upon restimulation, but their ability to secrete other cytokines was not reported [45]. By contrast, inflammatory mo-DC from tumor ascites secrete IL-6, TNF-α, IL1-β without the need for *ex vivo* restimulation, suggesting they are already activated *in situ* by the inflammatory environment [26]. Ascites mo-DC, but not macrophages, also secrete IL-12p70 and IL-23 upon *ex vivo* restimulation [26].

Consistent with their ability to produce IL-23, inflammatory mo-DC from tumor ascites and from synovial fluid from rheumatoid arthritis patients are potent inducers of Th17 polarization *ex vivo* [26]. Inflammatory mo-DC from the skin of psoriasis patients also induce Th17 polarization *ex vivo* [20]. Because Th17 cells play a major role in tissue damage in these diseases, these results suggest that inflammatory mo-DC could be invoved in the pathogenesis by fueling the inflammation. Nevertheless, the properties of inflammatory mo-DC will likely be influenced by their micro-environment, and their T cell-polarizing ability may be different in a Th1- or Th2-driven pathology.

Conclusion and perspectives

The literature discussed in this chapter indicates that mo-DC are present *in vivo* in humans and represent a *bona fide* DC subset. Recent technical advances, such as single-cell transcriptomic analysis, may reveal mo-DC populations in additionnal tissues or pathological situations. A major challenge is to better understand their functional properties and potential

role in the physiopathology of inflammatory disorders or cancer. This would pave the way for manipulating mo-DC differentiation or function for therapeutic intervention.

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Figure legends

Figure 1. Ontogeny of macrophages and dendritic cells. Macrophages and dendritic cells (DC) populations can be divided into two categories based on their ontogeny: derived from monocytes or from dedicated precursors.

Figure 2. Morphology of human DC versus macrophages. DC and macrophages from the synovial fluid of rheumatoid arthritis patients were isolated and their morphology analyzed after cytospin and Giemsa/May-Grünwald staining. Typical DC morphology includes a small size, the presence of dendrites and the lack of internal vacuoles. Bar = 10 μm.

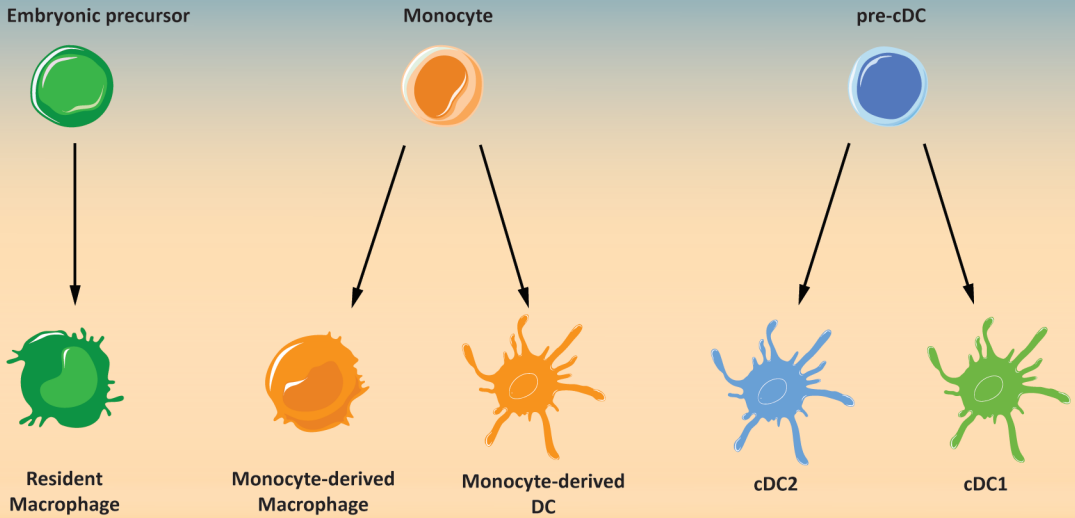
Figure 3. Molecular ontegeny of monocyte-derived cells. In response to external signals (cytokines and AHR ligands), monocytes differentiate either into macrophages or dendritic cells. Transcription factors governing these two pathways are shown.

Table 1. Phenotypic markers for classical DC, mo-DC, monocytes and macrophages.

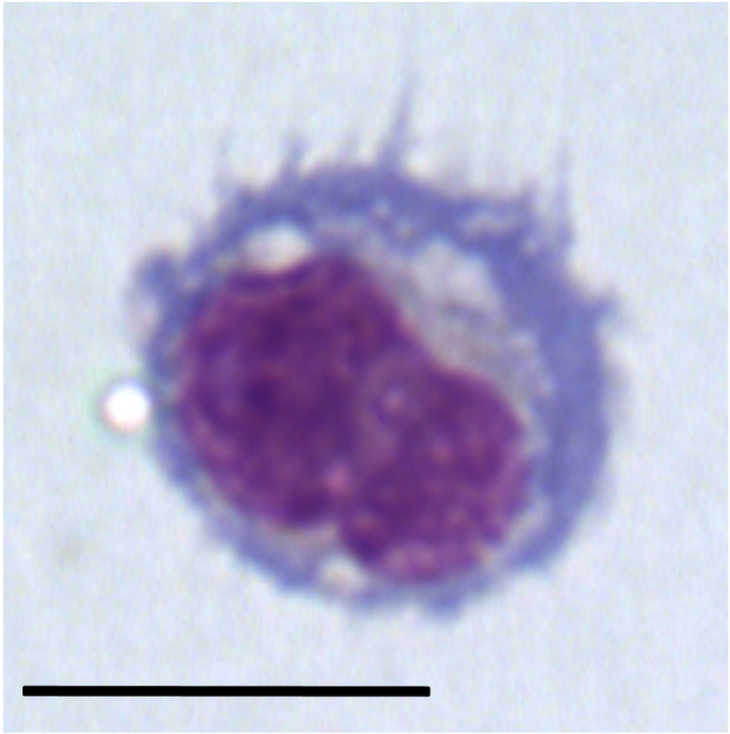
Surface markers	cDC1	cDC2	Macrophage	mo-DC	CD14$^+$ monocytes	Slan-DC
HLA-DR	+	+	+	+	+	+
CD11c	+	+	+	+	+	+
CD1c	-	+	-	+	-	-
CD1a	-	Tissue-dependent	-	+	-	-
CD1b	-	Tissue-dependent	-	+	-	-
CD141	+	low	low	+	-	-
CD14	-	-	+	intermediate	+	-
CD16	-	-	Tissue-dependent	-	-/low	+
CD206	-	Tissue-dependent	+	+	-	-
Clec9A	+	-	-	-	-	-
CD163	-	-	Tissue-dependent	-	-	-
CD11b	-	Tissue-dependent	+	+	+	+
MerTK	-	-	+	-	-	-
FceRI	-	Tissue-dependent	-	+	-	-
Slan (M-DC8)	-	-	-	-	-	+

Table 2. Human tissues in which mo-DC have been identified.

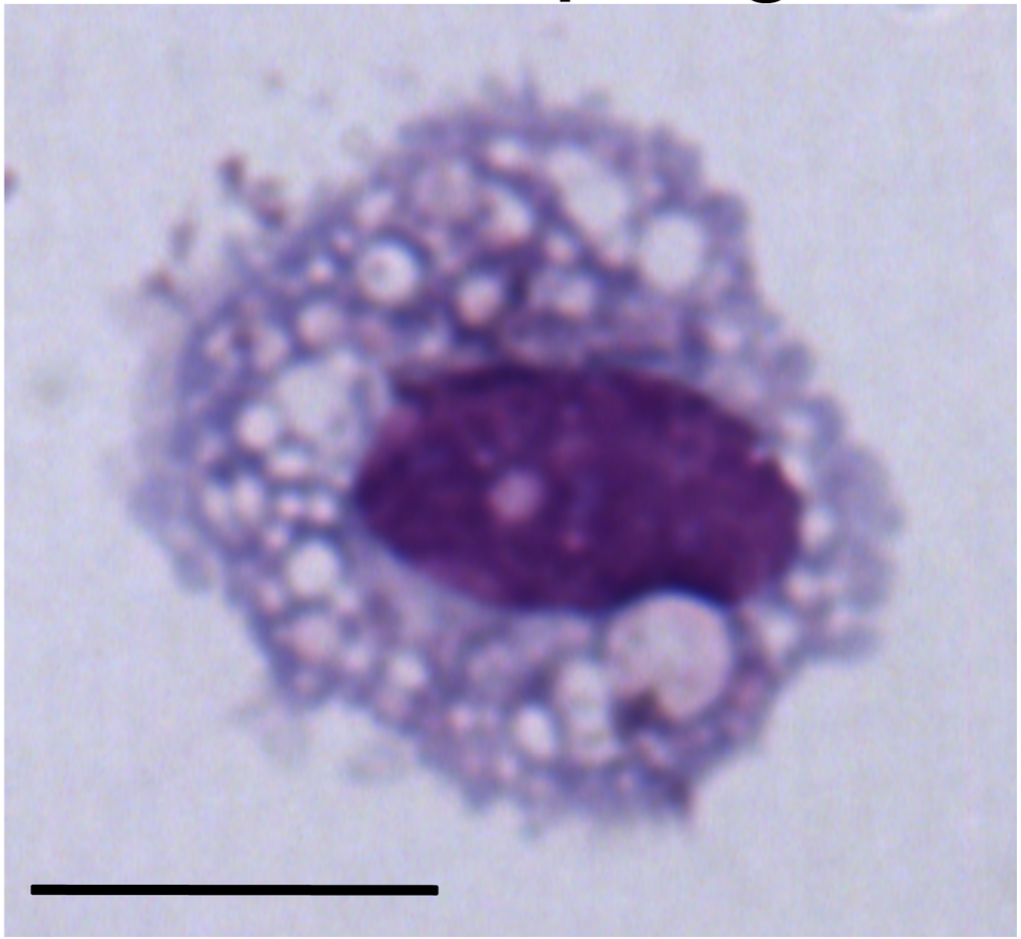
Tissue	Condition	Reference
BAL	healthy	[45]
BAL	healthy	[46]
Colorectal tumor	cancer	[28]
Intestine	healthy	[47]
Lung	healthy	[44]
Lung tumor	cancer	[28]
Lung tumor	cancer	[55]
Melanoma	cancer	[54]
Nasal mucosa	allergic rhinitis	[25]
Peritoneum	healthy	[24]
Peritoneum	peritonitis	[24]
Peritoneum	tumor ascites	[26]
Skin	atopic dermatitis	[18]
Skin	psoriasis	[19] [20]
Synovial fluid	rheumatoid arthritis	[26]

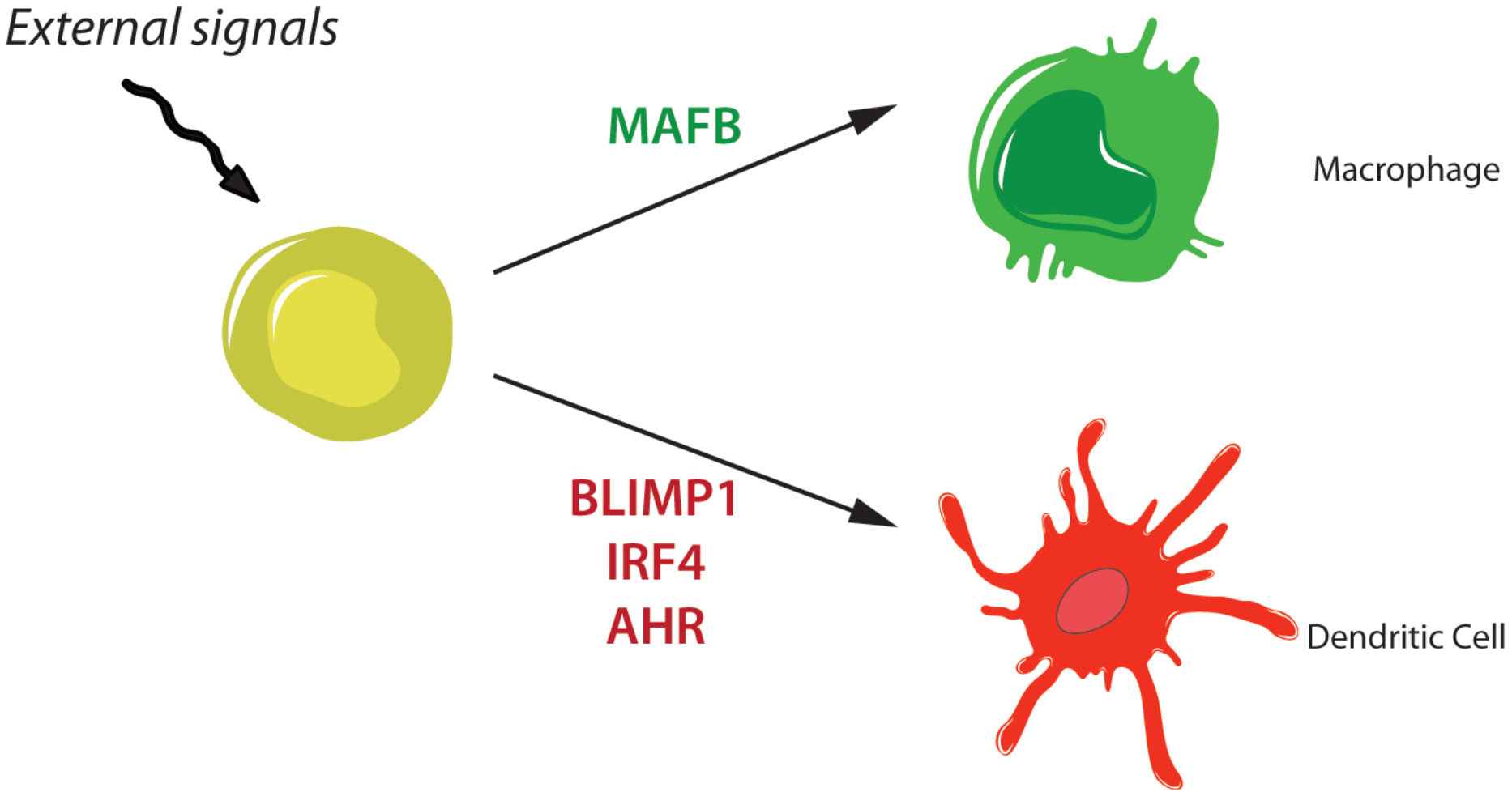

Embryonic precursor
Monocyte
pre-cDC
Resident Macrophage
Monocyte-derived Macrophage
Monocyte-derived DC
cDC2
cDC1

DC



Macrophage




External signals
MAFB
Macrophage
BLIMP1
IRF4
AHR
Dendritic Cell