

Aryl Hydrocarbon Receptor Controls Monocyte Differentiation into Dendritic Cells versus Macrophages

Christel L Goudot, Alice Coillard, Alexandra-Chloé Villani, Paul Gueguen, Adeline Cros, Siranush Sarkizova, Tsing-Lee Tang-Huau, Mylène Bohec, Sylvain Baulande, Nir Hacohen, et al.

▶ To cite this version:

Christel L Goudot, Alice Coillard, Alexandra-Chloé Villani, Paul Gueguen, Adeline Cros, et al.. Aryl Hydrocarbon Receptor Controls Monocyte Differentiation into Dendritic Cells versus Macrophages. Immunity, 2017, 47 (3), pp.582 - 596.e6. 10.1016/j.immuni.2017.08.016 . hal-01613828

HAL Id: hal-01613828 https://hal.science/hal-01613828

Submitted on 10 Oct 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Aryl Hydrocarbon Receptor controls monocyte differentiation into dendritic cells versus macrophages

Authors: Christel Goudot¹, Alice Coillard¹, Alexandra-Chloé Villani^{2,3}, Paul Gueguen¹, Adeline Cros¹, Siranush Sarkizova⁴, Tsing-Lee Tang-Huau^{1,5}, Mylène Bohec⁶, Sylvain Baulande⁶, Nir Hacohen^{2,3}, Sebastian Amigorena¹, Elodie Segura¹*.

Affiliations:

¹ Institut Curie, PSL Research University, INSERM, U932, 26 rue d'Ulm, 75005 Paris, France
 ² Broad Institute of Harvard University and MIT, Cambridge, MA 02142, USA

³ Center for Cancer Research, Massachusetts General Hospital, Department of Medicine, Charlestown, MA 02129, USA

⁴ Department of Biomedical Informatics, Harvard Medical School, Boston, MA 02142, USA

⁵ Sanofi, Breakthrough Laboratory, 1 impasse des ateliers, 94400 Vitry-sur-Seine, France

⁶ Institut Curie, PSL Research University, NGS platform, 26 rue d'Ulm, 75005 Paris, France

*Correspondence to: elodie.segura@curie.fr

Summary

After entering tissues, monocytes differentiate into cells that share functional features with either macrophages or dendritic cells (DC). How monocyte fate is directed towards monocyte-derived macrophages (mo-Mac) or monocyte-derived DC (mo-DC) and which transcription factors control these differentiation pathways remains unknown. Using a novel *in*

vitro culture model, yielding human mo-DC and mo-Mac closely resembling those found *in vivo* in ascites, we show that IRF4 and MAFB are critical regulators of monocyte differentiation into mo-DC and mo-Mac, respectively. Activation of the aryl hydrocarbon receptor (AHR) promotes mo-DC differentiation through the induction of BLIMP-1, while impairing differentiation into mo-Mac. AhR deficiency also impairs the *in vivo* differentiation of mouse mo-DC. Finally, AHR activation correlates with mo-DC infiltration in leprosy lesions. These results establish that mo-DC and mo-Mac are controlled by distinct transcription factors, and show that AHR acts as a molecular switch for monocyte fate specification in response to micro-environmental factors.

Introduction

Mononuclear phagocytes are divided into three groups: macrophages, monocytes and DC. Macrophages derive from embryonic precursors whose differentiation is strongly imprinted by the micro-environment (Gosselin et al., 2014; Haldar et al., 2014; Lavin et al., 2014; Okabe and Medzhitov, 2014). By contrast, classical DC derive from pre-committed precursors that follow a pre-determined developmental program primed at an early stage, independently of their tissue of residence (Breton et al., 2016; Schlitzer et al., 2015). When entering tissues, monocytes can differentiate into either macrophages or DC (Mildner et al., 2013; Segura and Amigorena, 2013). Whether mo-DC and mo-Mac represent variations of one highly plastic cell type or distinct *bona fide* lineages remains unclear (Guilliams et al., 2014). In addition, what environmental cues drive monocyte fate towards mo-Mac versus mo-DC and what molecular regulators orchestrate this process remains to be established.

In mouse models, mo-DC and mo-Mac appear during inflammation but are also found in the steady-state at mucosal sites such as intestine and skin (Bain et al., 2014; Mildner et al., 2013; Segura and Amigorena, 2013; Tamoutounour et al., 2013). There is strong experimental evidence that the same scheme applies to humans. Monocyte-derived cells are found in the steady-state in human skin (McGovern et al., 2014). In inflammatory conditions, monocyte recruitment was observed in the gut of inflammatory bowel disease patients (Grimm et al., 1995), in cantharidin-induced skin blisters (Jenner et al., 2014) and in the nasal mucosa of subjects with induced allergic rhinitis (Eguiluz-Gracia et al., 2016). Inflammatory macrophages and DC have been described in atopic dermatitis (Wollenberg et al., 1996), Crohn's disease (Bain et al., 2013; Kamada et al., 2008), psoriasis (Zaba et al., 2009), allergic rhinitis (Eguiluz-Gracia et al., 2016), rheumatoid arthritis and tumor ascites (Segura et al., 2013). Transcriptomic analysis showed that ascites DC share gene signatures with *in vitro*-generated monocyte-derived cells, supporting the idea that these DC represent tissue mo-DC (Segura et al., 2013).

We hypothesized that transcription factors differentially expressed between human mo-DC and mo-Mac may be involved in their differentiation from monocytes. We identified candidate transcription factors by comparative transcriptomic analysis, and established a new model of human monocyte differentiation to test the role of these candidates. We found that *IRF4* and *MAFB* are required for mo-DC and mo-Mac differentiation, respectively. We also show that AHR is essential for driving monocyte differentiation towards mo-DC. Finally, we validated the role of AHR in mo-DC differentiation *in vivo* in a mouse model and by analyzing clinical data from leprosy patients.

Results

Establishment of a culture model for human monocyte differentiation

To address the ontogeny of human monocyte-derived cells, we first searched for transcription factors that are differentially expressed between monocytes, ascites mo-DC and ascites mo-Mac. Using our transcriptomic data (Segura et al., 2013), we established a list of candidates (table S1). To test their role, we turned to an *in vitro* model of monocyte differentiation. Current culture models yield either mo-DC or mo-Mac depending on cytokines used. By contrast, we needed a model that would mimic the differentiation of monocytes into mo-DC and mo-Mac in the same environment. We therefore established a new *in vitro* system enabling the differentiation of both mo-DC and mo-Mac in the same culture.

M-CSF and its receptor are essential for mo-DC and mo-Mac differentiation in vivo during inflammation in mice (Davies et al., 2013; Greter et al., 2012), and M-CSFR is highly expressed on human mo-DC and mo-Mac found in vivo (McGovern et al., 2014; Segura et al., 2013). Therefore, we designed a cytokine cocktail based on M-CSF or IL-34, the two ligands of M-CSFR. We also included IL-4, a cytokine known to induce the expression by cultured monocytes of CD1 molecules, which are highly expressed on ascites mo-DC (Segura et al., 2013). Finally, we added TNFa, a major mediator of inflammation. Culturing human blood CD14⁺ monocytes with M-CSF, IL-4 and TNFa or IL-34, IL-4 and TNFa yielded in the same culture two main populations expressing CD16 or CD1a, and displaying a typical macrophage or DC morphology, respectively (fig.1A and fig.S1A). Only CD1a⁺ cells could efficiently induce allogeneic naive CD4⁺ T cell proliferation (fig.1B and fig.S1B), confirming that they were *bona* fide DC. We then characterized the properties of cultured mo-DC and mo-Mac compared to that of ascites mo-DC and mo-Mac. Both mo-DC and mo-Mac secreted IL-6 after stimulation with CD40L, but only mo-DC secreted IL-23 (fig.1C and fig.S1C), similar to what we observed with ascites cells (Segura et al., 2013). Phenotypic comparison of mo-DC and mo-Mac differentiated

with M-CSF, or IL-34, IL-4 and TNFa with ascites mo-DC and mo-Mac showed similar expression for various surface markers (fig.1D and fig.S1D), except for CD14 which is downregulated upon culture. CD14⁺ monocytes were routinely isolated by positive selection using magnetic beads with 90-95% purity, contaminating cells being CD14⁺CD16⁺ monocytes. To address whether the presence of CD14⁺CD16⁺ monocytes could influence the culture outcome, we isolated highly pure CD14⁺ monocytes by cell sorting. The absence of contaminating CD14⁺CD16⁺ monocytes did not impact monocyte differentiation into both mo-DC and mo-Mac (fig.S1E). In addition, CD16⁺ monocytes (isolated using magnetic beads) had a low survival rate and did not maintain CD16 expression in culture (fig.S1F), suggesting that potential contaminating CD16⁺ monocytes had a negligeable effect on the final proportions of mo-DC and mo-Mac. Of note, monocytes differentiated with GM-CSF and IL-4, a widely used culture system, yielded only CD1a⁺ mo-DC (fig.S1G). The phenotype of mo-DC derived with GM-CSF and IL-4, with or without TNFa (fig.1D and fig.S1H), was less similar to that of ascites mo-DC. When stimulated with a TLR7/8 ligand (R848) and an endogenous danger signal (uric acid cristals), mo-DC differentiated with M-CSF, or IL-34, IL-4 and TNFa secreted high levels of inflammatory cytokines (IL-1ß and IL-6) (fig.S1I), consistent with the secretory ability of ascites mo-DC (Segura et al., 2013). Of note, mo-DC differentiated with GM-CSF and IL-4 were less efficient for the secretion of IL-1B and IL-6, although they secreted TNFa and CXCL10 at similar levels as mo-DC differentiated with M-CSF, or IL-34, IL-4 and TNFa (fig.S1I).

To determine whether these populations had a stable phenotype over time, we sorted CD1a⁻CD16⁻ cells, mo-DC and mo-Mac after 5 days of culture and re-cultured them separately with M-CSF, IL-4 and TNFa (fig.1E). After 2 days of re-culture, the phenotype of CD1a⁻CD16⁻ cells, mo-DC or mo-Mac remained stable based on the expression of CD16, CD163 and CD1a.

After 4 days of re-culture, only CD1a⁻CD16⁻ cells and mo-Mac were still viable, and their phenotype was largely unchanged (fig.S1J). These results show that mo-DC and mo-Mac are maintained as stable populations over the course of the culture, and that mo-DC or mo-Mac do not emerge from CD1a⁻CD16⁻ cells at later time points. To address whether a single monocyte could give rise to both mo-DC and mo-Mac in our culture model, we stained monocytes with a proliferation dye and analyzed cell proliferation after 5 days. As a positive control, we stimulated monocytes with the mitogen phytohaemagglutin-L (PHA-L), which induced the proliferation of a portion of monocytes. Monocytes did not proliferate in the culture (fig.1F), suggesting that there is a precursor-product relationship between a single monocyte and a single mo-DC, or mo-Mac, progeny.

Finally, to complete the characterization of our culture model, we compared the transcriptome of cell-sorted mo-DC and mo-Mac differentiated with M-CSF, or IL-34, IL-4 and TNFa with that of cell-sorted ascites mo-DC and mo-Mac, blood CD14⁺ monocytes, blood CD1c⁺ DC and mo-DC differentiated with GM-CSF and IL-4. Supervised analysis of the micro-array data showed differential expression for selected phenotypic markers as expected (fig.S2A). Comparative transcriptomic analysis showed that mo-Mac and mo-DC differentiated with M-CSF, IL-4 and TNFa were highly similar to those differentiated with IL-34, IL-4 and TNFa (fig.1G). In addition, these *in vitro*-generated mo-Mac and mo-DC clustered close to ascites mo-Mac and mo-DC respectively, while the transcriptome of mo-DC differentiated with GM-CSF and IL-4 was closer to that of blood CD1c⁺ DC (fig.1G). These results show that our culture system yields mo-DC and mo-Mac populations that closely resemble mo-DC and mo-Mac present in human tumor ascites. For the rest of the study, we used monocytes cultured with M-CSF, IL-4 and TNFa as a model to analyze monocyte-derived cell differentiation.

IRF4 and MAFB are essential for the development of mo-DC and mo-Mac

To refine our list of candidates for the differentiation of monocyte-derived cells (table S1), we removed transcription factors that are not expressed or not differentially expressed in *in vitro*-derived mo-DC versus *in vitro*-derived mo-Mac (fig.2A). We selected for further validation two candidates, *IRF4* and *MAFB*, previously proposed to be involved in the development of a subset of mouse classical DC (Murphy et al., 2015), and in macrophage differentiation (Kelly et al., 2000) respectively. *IRF4* and *MAFB* were differentially expressed in mo-DC versus mo-Mac, both *in vitro* and *in vivo* (fig.S2B). In addition, we confirmed the differential expression of IRF4 and MAFB in blood monocytes, ascites mo-DC and ascites mo-Mac at the protein level (fig.2B).

IRF4 and MAFB were expressed early during the culture both at the mRNA (fig.2C) and protein levels (fig.2D), consistent with their possible role as master regulator transcription factors. To address the role of *IRF4*, we silenced its expression by infecting monocytes at the start of the culture with lentiviral vectors containing shRNA against *IRF4*, or control shRNA (fig.2E). Inhibition of *IRF4* expression induced a dramatic reduction of mo-DC while maintaining the mo-Mac population (fig.2F). We used a similar strategy to analyze the role of *MAFB* (fig.2G). Silencing of *MAFB* resulted in a strong decrease in mo-Mac and an increase in mo-DC differentiation (fig.2H). Analysis of additional phenotypic markers confirmed the disappearance of mo-DC or mo-Mac from the culture, rather than the mere down-regulation of CD1a or CD16 expression (fig.S2C-D). These results show that *IRF4* and *MAFB* are essential for mo-DC and mo-Mac development, respectively.

Monocytes are not heterogeneous for the expression of mo-DC gene signatures

These results could be explained either by the presence of two distinct precursor populations or by the existence among blood CD14⁺ monocytes of two transcriptionnally primed populations that would be pre-committed to become mo-DC or mo-Mac (Schlitzer et al., 2015). To address the heterogeneity of CD14⁺ monocytes, we performed single-cell RNA-seq on CD14⁺ monocytes from 2 donors isolated using magnetic beads. Cell purity as assessed by flow cytometry was 93% and 95% respectively (fig.S3A). We generated single-cell transcriptomes using a droplet-based method enabling 3' mRNA counting (Zheng et al., 2017). To evaluate the heterogeneity of CD14⁺ monocytes, we clustered cells using a graph-based approach with the Seurat package, which combines dimensionality reduction and graph-based partitioning algorithms for unsupervised clustering (Satija et al., 2015). For vizualisation of the cell clusters, we used t-Distributed Stochastic Neighbor Embedding (t-SNE) (fig.3A and fig.S3B). We found two clusters, one of which represents around 25% of total cells and corresponds to cells expressing FCGR3A (encoding CD16), higher levels of MHC class II molecules and several genes preferentially expressed in CD14⁺CD16⁺ and CD16⁺ monocytes including *IFITM2* and IFITM3 (fig.S3C) (Villani et al., 2017; Wong et al., 2011). In addition to contaminating CD14⁺CD16⁺ monocytes, some of these cells may correspond to CD14⁺ monocytes *en route* to differentiating into CD14⁺CD16⁺ monocytes (Patel et al., 2017). To address the potential heterogeneity of the CD16⁻ cluster, we performed a second analysis excluding cells from the CD16⁺ cluster (fig.3B and fig.S3B). We did not detect subgroups of transcriptionally distinct cells within the CD16⁻ cluster, confirming that the CD14⁺ monocyte population is likely homogeneous, as previously reported (Villani et al., 2017). To confirm these results, we sought to address whether subpopulations of monocytes displayed transcriptional similarity with mo-DC or mo-Mac. Because of the limited number of genes detected per cell in our analysis (average of

1185), we interrogated published single-cell RNA-seq data obtained using the Smart-seq2 approach (Villani et al., 2017), characterized by a lower number of cells analyzed but a higher number of genes per cell (average of 5326). We defined gene signatures for mo-DC and mo-Mac using our transcriptomic data by identifying genes that were (i) more expressed in ascites mo-DC than in blood monocytes, (ii) more expressed in ascites mo-DC than in ascites mo-Mac, and (iii) more expressed in in vitro-derived mo-DC than in in vitro-derived mo-Mac (and vice-versa for mo-Mac) (Table S2). We then queried genes with at least a 2-fold change. Among these, 35 genes for mo-DC and 35 genes for mo-Mac were expressed in the single-cell RNA-seq data set. While none of the monocytes expressed the mo-DC signature, the mo-Mac signature was partially expressed by all monocyte subsets (fig.3C for the classical subset definition and fig.S3D for the revised subset definition (Villani et al., 2017)). We also assessed the expression of selected genes that could be involved in determining monocyte fate, including receptors for the cytokines used in our model (CSF1R, IL4R, TNFRSF1A), candidate transcription factors (IRF4, MAFB, AHR) or genes recently proposed to distinguish "DC-biased" monocytes in the mouse (FLT3, SPI1) (Menezes et al., 2016) (fig.3D for the classical subset definition and fig.S3E for the revised subset definition). While IRF4 was not expressed by monocytes, MAFB was detected in all monocyte subsets. We conclude that human CD14⁺ monocytes are not heterogeneous in their expression of mo-DC transcriptional signature. While monocytes do not contain a subpopulation that would be pre-committed towards mo-DC differentiation, they all express a partial mo-Mac gene signature, including MAFB, suggesting a default differentiation pathway towards mo-Mac if no other environmental triggers are encountered.

AHR is a molecular switch for monocyte fate

Given that monocytes do not seem to be transcriptionally primed, we then hypothesized that environmental signals play a major role in driving monocyte fate. Among candidate transcription factors (fig.2A), we identified *AHR*, a ligand-activated transcription factor sensing tryptophan catabolites and metabolites generated by dietary intake, UV exposure, or microbiota (Stockinger et al., 2014). AHR was differentially expressed by mo-DC and mo-Mac at the mRNA (fig.S2B) and protein levels (fig.2B).

To address the role of *AHR*, we first inhibited its expression by targeted knock-down using lentiviral vectors (fig.4A). *AHR* silencing reduced mo-DC differentiation while slightly increasing mo-Mac (fig.4B). Because culture medium contains small amounts of AHR ligands (Veldhoen et al., 2009) and *AHR* knock-down was incomplete (fig.4A), we sought to confirm these results using a different approach. We cultured monocytes in the presence of various doses of a natural AHR agonist (6-Formylindolo(3,2-b)carbazole, FICZ) or an AHR inhibitor (stemregenin-1, SR1) and assessed mo-DC and mo-Mac differentiation. AHR activation by FICZ increased mo-DC while decreasing mo-Mac development (fig.4C). Conversely, AHR inhibition by SR1 increased mo-Mac while decreasing mo-DC proportions (fig.4C). Of note, the phenotype of FICZ-treated mo-DC or SR1-treated mo-Mac was similar to that of untreated cells (fig.S4A-B), while the phenotype of CD1a⁻CD16⁻ cells remained unchanged (fig.S4C). These results suggest that AHR is a molecular switch for mo-DC versus mo-Mac differentiation.

To decipher how AHR shapes monocyte fate, we assessed *IRF4* and *MAFB* expression in monocytes after 3h of culture with various combinations of M-CSF, IL-4, TNFa, SR1 and FICZ (fig.4D and fig.S5A). We also analyzed the expression of *CYP1A1*, a known direct target of AHR (Stockinger et al., 2014), as a control for AHR activation (fig.S5A). IL-4 induced *IRF4* expression, as previously reported (Lehtonen et al., 2005). This induction was inhibited in the

presence of SR1, indicating that *IRF4* expression is dependent on AHR signaling, presumably in response to small amounts of AHR ligand from the culture medium. However, IRF4 is not induced in the presence of FICZ alone. The expression of IL-4-induced IRF4 was further increased in the presence of TNFa and with FICZ. By contrast, MAFB expression was induced by culture medium alone, and further increased by M-CSF (fig.4D). AHR signaling had no significant impact on MAFB expression at this time point. To address whether quantities of mRNA expressed per cell or proportions of expressing cells were regulated by these signals, we analyzed the expression of IRF4 and MAFB mRNA at the single-cell level using fluorescent in situ hybridization coupled to flow cytometry (fig.4E). After 3h of culture, monocytes upregulated MAFB in response to M-CSF, but in the presence of IL-4 and TNF-a, the proportion of MAFB-expressing monocytes was dramatically reduced while a distinct population of IRF4expressing monocytes appeared, which was further increased in the presence of FICZ. These results show that external signals polarize monocytes towards mo-DC versus mo-Mac differentiation. In line with this, increasing concentrations in the culture of IL-4 (fig.S5B) or TNFa (fig.S5C) increased the proportion of mo-DC while reducing that of mo-Mac. In addition, the effect of FICZ on the culture outcome was strongly diminished in the absence of TNFa (fig.S5D). These results indicate that IL-4, TNFa and AHR signaling synergize for IRF4 induction and mo-DC differentiation. Collectively, these results suggest the existence of a default differentiation pathway into mo-Mac, which can be switched to mo-DC differentiation in response to IL-4, TNFa and AHR ligands.

AHR acts through BLIMP-1

AHR activation triggers an autoregulatory feedback loop that restricts AHR signaling to a short timeframe (Stockinger et al., 2014). Therefore, we hypothesized that the effect of AHR activation on monocyte differentiation may be mediated by additional molecular regulators. In our differential transcriptomic analysis, we looked for transcription factors that could be induced by AHR activation based on literature (table S1). We identified *PRDM1* (encoding BLIMP-1) as a candidate. Studies on cell lines have suggested that *PRDM1* is a target of AHR (De Abrew et al., 2010; Ikuta et al., 2010). To analyze whether PRDM1 was induced by AHR signaling in monocytes, we measured *PRDM1* expression during the early stages of monocyte culture in the presence or absence of FICZ or SR1 (fig.5A). We found that *PRDM1* was rapidly induced upon AHR activation within 3 hours, suggesting that *PRDM1* is a target of AHR. At the protein level, BLIMP-1 expression peaked during the first 24h of the culture then decreased (fig.5B). To address whether PRDM1 was involved in monocyte differentiation, we knocked-down PRDM1 expression using shRNA (fig.5C). PRDM1 silencing significantly decreased mo-DC differentiation, while increasing the proportion of mo-Mac (fig.5D). To confirm that the effect of AHR was mediated by PRDM1, we silenced its expression in monocytes cultured in the presence or absence of FICZ (fig.5E). PRDM1 silencing abolished the promotion of mo-DC differentiation by FICZ, while the proportion of mo-Mac was not fully restored to the level observed in control cells in the absence of FICZ. These results suggest that BLIMP-1 is essential for AHR-induced mo-DC differentiation.

AHR is involved in mo-DC differentiation in vivo in the mouse

To address the physiological relevance of our findings, we analyzed the role of AhR in mo-DC and mo-Mac differentiation *in vivo* in the mouse. In the steady-state dermis, mo-DC and

mo-Mac continuously differentiate in situ from monocytes recruited to the skin (Tamoutounour et al., 2013). Five populations of macrophage/monocyte-related cells have been described in mouse skin: dermal monocytes, mo-DC at an early stage of differentiation, fully differentiated mo-DC, MHC class II⁺ macrophages (which contain a majority of mo-Mac) and resident MHC class II⁻ macrophages (Tamoutounour et al., 2013). Transcriptomic analysis showed that AhR and Irf4 are more expressed in mo-DC than in macrophages, while MafB was more expressed in macrophages (fig.S6A, GEO accession code GSE49358), consistent with our findings in human monocyte-derived cells. In AhR-deficient mice, the proportion of skin mo-DC was decreased as compared to wild type (WT) littermates, while the proportion of MHC class II⁺ macrophages were increased (fig.6A-B and fig.S6B). Proportions of other skin DC subsets and of monocyte subsets in the spleen were similar between AhR-deficient and WT mice (fig.S6C-D). To confirm these findings, we increased AhR ligand availability in vivo by feeding mice with an experimental diet enriched for indole-3-carbinole (I3C), which is converted into an AhR ligand by stomach acids (Bjeldanes et al., 1991). In mice fed with I3C-supplemented diet, mo-DC were significantly increased and MHC class II⁺ macrophages were decreased in the skin compared to mice fed with a control diet (fig.6C). These results show that AhR is involved in the in vivo differentiation of dermal mo-DC.

Recently, Irf4 was shown in the mouse to be involved in the differentiation of a population of peritoneal and pleural MHC II⁺CD226⁺ monocyte-derived cells, proposed to be mo-Mac (Kim et al., 2016). However, our results identify CD226 as a marker of human mo-DC, both *in vitro* and in ascites (fig.1D). To determine the identity of mouse MHC II⁺CD226⁺ monocyte-derived cells, we isolated them from the peritoneal lavage of C57BL/6 mice, and compared their morphology to that of ICAM2⁺ peritoneal macrophages (fig.6D). MHC

II⁺CD226⁺ cells display a typical DC morphology, distinct from that of *bona fide* ICAM2⁺ macrophages. Consistent with this, MHC II⁺CD226⁺ cells do not express the macrophage marker MerTK (fig.S6E) and CD226 is highly expressed by dermal mo-DC, but not by dermal macrophages (fig.S6A). These results identify Irf4-dependent MHC II⁺CD226⁺ cells as mo-DC. As previously reported (Kim et al., 2016), this population of peritoneal mo-DC is decreased upon antibiotics treatment (fig.6E). Antibiotics induce the loss of intestinal bacteria species that are a major source of endogenous AhR ligand (Zelante et al., 2013). To address whether the decrease of peritoneal mo-DC upon antibiotics treatment was AhR-dependent, we fed antibiotics-treated mice with a I3C-supplemented or control diet, and analyzed cells from the peritoneal lavage (fig.6E). Supplementation in AhR ligand restored mo-DC differentiation in antibiotics-treated mice almost up to normal levels.

Collectively, these results show that AhR activation in response to environmental stimuli has a key role in driving monocyte fate towards mo-DC *in vivo*.

AHR activation correlates with the presence of mo-DC in leprosy lesions

Finally, to put these findings in the context of human disease, we assessed AHR signaling and monocyte-derived cells presence in leprosy lesions, that contain granulomas in which monocytes are constantly recruited (Russell et al., 2009; Schreiber and Sandor, 2010). We analyzed published micro-array data from lepromatous (L-lep) and tuberculoid (T-lep) leprosy lesions (GEO accession code GSE17763). Patients with L-lep lesions display poor immunological responses against *Mycobacterium leprae*, the causative agent of leprosy, while patients with T-lep lesions have strong anti-*M. leprae* T cell responses. We first defined "AHR agonist" and "AHR antagonist" gene signatures based on publicly available transcriptomic data (Di Meglio et al., 2014) (table S3). To assess whether AHR signaling was active in leprosy lesions, we performed Gene Set Enrichment Analysis (GSEA) (fig.7A and fig.S7A). We found that the "AHR agonist" signature was enriched in T-lep lesions, while the "AHR antagonist" signature was enriched in L-lep lesions. Consistent with this, AHRR and CYP1A1, which are upregulated upon AHR activation (Stockinger et al., 2014), were highly expressed in T-lep lesions while IFIT1, which is down-regulated by AHR (Di Meglio et al., 2014), was more expressed in L-lep lesions (fig.S7B). These results suggest that the AHR pathway was preferentially activated in T-lep lesions. To address the presence of mo-Mac and mo-DC, we performed GSEA using our gene signatures (table S2). The mo-DC signature was enriched in Tlep lesions, while the mo-Mac signature was enriched in L-lep lesions (fig.7A-B). By contrast, gene signatures of human Langerhans cells, skin CD1c⁺ DC or dermal macrophages (from (Carpentier et al., 2016)) were not enriched in either dataset (fig.S7A). Consistent with these results, MERTK, CD163 and FCGR3A (encoding CD16) were more expressed in L-lep lesions and CD1A, CD1B and FCER1A were more expressed in T-lep lesions (fig.S7B). These results suggest that mo-DC are more abundant in T-lep lesions while mo-Mac are more numerous in Llep lesions. This is in line with previous work showing the absence of CD1a⁺CD1b⁺CD1c⁺ DC in L-lep lesions (Sieling et al., 1999) and the increased presence of CD163⁺ macrophages in L-lep lesions (Montoya et al., 2009). Collectively, these results show that AHR activation correlates with the selective presence of mo-DC in L-lep lesions.

Discussion

In this work, we identify transcription factors involved in the differentiation of monocytes either into mo-Mac (MAFB) or into mo-DC (IRF4, AHR, BLIMP-1). These results

show that mo-DC and mo-Mac do not represent different states of polarized monocytes, but rather are distinct lineages controlled by two different sets of molecular regulators. Several studies have evidenced that monocyte differentiation into mo-DC or mo-Mac is context-dependent (Bain et al., 2013; Zigmond et al., 2012). Here we identify micro-environmental cues that shape monocyte fate. Our results suggest that, in the presence of M-CSF, monocytes differentiate into mo-Mac by default, unless they are exposed to certain cytokines (IL-4 and TNFa) in conjunction with AHR ligands, promoting mo-DC differentiation.

Our conclusions are primarily based on the use of an *in vitro* human monocyte differentiation model, but are reinforced by the validation of our main finding *in vivo* in a mouse model and the correlation between AHR activation and mo-DC presence in leprosy lesions. In addition, BLIMP-1 and IRF4 were identified in both mouse and human as preferentially expressed in intestinal CD103⁺CD11b⁺ DC (a proposed mo-DC population), and mice deficient for Blimp-1 in DC displayed a strongly reduced population of CD103⁺CD11b⁺ DC in the intestine (Watchmaker et al., 2014), further supporting the physiological relevance of our findings.

It was suggested that mouse monocytes can be separated into two subpopulations that are pre-committed to become mo-Mac in response to pathogens or mo-DC in response to GM-CSF (Menezes et al., 2016). Using two different datasets of single-cell RNA-seq, we could not identify distinct subpopulations of mo-DC and mo-Mac precursors within human CD14⁺ monocytes. This is consistent with a recent single-cell RNA-seq analysis showing that mouse Ly6C⁺ and Ly6C⁻ monocytes are not heterogeneous at the transcriptomic level (Mildner et al., 2017). Our results show that all monocyte subsets express a partial mo-Mac transcriptomic signature, but cues from the micro-environment can drive monocyte fate towards mo-DC or mo-

16

Mac. One hypothesis to explain how the same signals can induce different outcomes within a transcriptionally homogeneous population could be the stochastic heterogeneity in chromatin accessibility (Buenrostro et al., 2015).

MafB is highly expressed by all mouse macrophage populations except for lung macrophages (Gautier et al., 2012). Based on *in vitro* over-expression in myeloid progenitor cells, MafB has been proposed to induce macrophage differentiation (Bakri et al., 2005; Kelly et al., 2000). However, subsequent work showed that MafB is dispensable both *in vivo* and *in vitro* for murine macrophage differentiation from fetal progenitors (Aziz et al., 2006), suggesting that MafB is not essential for the initial stages of differentiation of embryonic-derived macrophages. MafB is rather involved in their terminal differentiation by repressing self-renewal genes (Aziz et al., 2009). Whether MafB is important for the differentiation of mouse macrophages in an inflammatory setting remains to be addressed.

Irf4 is preferentially expressed by mouse $CD11b^+$ DC. Whether it is required for their development, or rather their migration and survival, remains unclear (Murphy et al., 2015). We show that *IRF4* is essential for human mo-DC differentiation, and its expression in human monocytes is induced by IL-4 in an AHR-dependent way. This is consistent with previous work showing *IRF4* expression upon culture with IL-4 in human and mouse monocytes(Briseno et al., 2016; Lehtonen et al., 2005). In addition, Irf4^{-/-} mouse monocytes cultured with GM-CSF and IL-4 fail to differentiate into mo-DC, but rather become mo-Mac (Briseno et al., 2016), supporting the idea of a default differentiation pathway into mo-Mac. We also show that mouse Irf4-dependent peritoneal monocyte-derived cells, initially described as mo-Mac (Kim et al., 2016), actually correspond to mo-DC, based on their morphology and phenotype.

Previous evidence suggests a major role for AhR in the control of inflammatory responses, in particular through its action on Th17 cell development and innate lymphoid cells homeostasis (Stockinger et al., 2014). Our work highlights an additional level of control by AhR ligands, by switching monocyte differentiation towards mo-DC, which are major producers of IL-23 and inducers of Th17 cells (Segura et al., 2013). Natural AhR ligands are derived from dietary intake (Bjeldanes et al., 1991) or produced through tryptophan catabolism at mucosal barriers (Fritsche et al., 2007; Zelante et al., 2013). AhR ligands can circulate throughout the body as evidenced by the regulation of astrocyte activity by microbiota-derived AhR ligands (Zelante et al., 2013), or the presence in milk of AhR ligands derived from the maternal microbiota (Gomez de Aguero et al., 2016).

Several studies have evidenced a deleterious role of monocyte-derived cells in pathological situations. mo-DC induce pathogenic T cells that mediate tissue damage in mice models of autoimmune or inflammatory diseases such as experimental autoimmune encephalomyelitis (Croxford et al., 2015) and colitis (Zigmond et al., 2012). Human "inflammatory" mo-DC likely contribute to the pathogenesis in Crohn's disease, rheumatoid arthritis and psoriasis through the secretion of high levels of IL-23 and the induction of Th17 cells (Kamada et al., 2008; Segura et al., 2013; Zaba et al., 2009), two major players in the pathogenesis of these diseases. In tumors, mo-Mac play a central role in the suppression of anti-tumoral immune responses (Noy and Pollard, 2014). Monocyte-derived cells have therefore emerged in the past few years as attractive therapeutic targets (Getts et al., 2014; Leuschner et al., 2011). By enabling a better understanding of the molecular ontogeny of human monocyte-derived cells, our results should provide new opportunities for the therapeutic manipulation of monocyte differentiation.

Author contributions. CG, ACV, NH, SA and ES designed experiments. ACo, ACV, ACr, TLTH, MB and ES performed experiments. CG, ACo, ACV, PG, SS and ES analyzed the data and prepared the figures. SB provided expertise on sequencing. CG, ACV, SA and ES wrote the manuscript, with input from PG, SS, MB, SB and NH. ES supervized the project. The authors declare no competing financial interest.

Acknowledgments

This work was supported by the program «Investissements d'Avenir» from Agence Nationale de la Recherche (ANR-10-LABX-0043 and ANR-10-IDEX-0001-02 PSL), the European Research Council (2013-AdG N° 340046 DCBIOX) (SA), La Ligue Nationale contre le Cancer (EL2014.LNCC/SA) (SA), Fondation Bristol-Myers Squibb pour la Recherche en Immuno-Oncologie (ES), the National Human Genome Research Institute Centers of Excellence in Genomics Science P50 HG006193 (NH), the National Human Genome Research Institute NIH T32 HG002295 (SS) and the Banting Postdoctoral Fellowship (ACV). T-L Tang-Huau is supported by an industry-funded PhD fellowship at Université Paris Descartes. High-throughput sequencing performed by the ICGex NGS platform of the Institut Curie was supported by grants ANR-10-EQPX-03 (Equipex) and ANR-10-INBS-09-08 (France Génomique Consortium), by Cancéropôle Ile-de-France, by SESAME program from Région Ile-de-France and by SiRIC-Curie program (SiRIC Grant «INCa-DGOS- 4654»).

The authors wish to thank S.Dogniaux, M.Sieweke, S.Henri and B.Malissen for helpful discussions, X.Coumoul and L.Juricek for providing AhR^{-/-} mice and WT littermates, and the Flow Cytometry Platform of Institut Curie for cell sorting.

References

Aziz, A., Soucie, E., Sarrazin, S., and Sieweke, M.H. (2009). MafB/c-Maf deficiency enables self-renewal of differentiated functional macrophages. Science *326*, 867-871.

Aziz, A., Vanhille, L., Mohideen, P., Kelly, L.M., Otto, C., Bakri, Y., Mossadegh, N., Sarrazin, S., and Sieweke, M.H. (2006). Development of macrophages with altered actin organization in the absence of MafB. Mol Cell Biol *26*, 6808-6818.

Bain, C.C., Bravo-Blas, A., Scott, C.L., Gomez Perdiguero, E., Geissmann, F., Henri, S.,

Malissen, B., Osborne, L.C., Artis, D., and Mowat, A.M. (2014). Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. Nat Immunol *15*, 929-937.

Bain, C.C., Scott, C.L., Uronen-Hansson, H., Gudjonsson, S., Jansson, O., Grip, O., Guilliams,
M., Malissen, B., Agace, W.W., and Mowat, A.M. (2013). Resident and pro-inflammatory
macrophages in the colon represent alternative context-dependent fates of the same Ly6C(hi)
monocyte precursors. Mucosal immunology *6*, 498-510.

Bakri, Y., Sarrazin, S., Mayer, U.P., Tillmanns, S., Nerlov, C., Boned, A., and Sieweke, M.H. (2005). Balance of MafB and PU.1 specifies alternative macrophage or dendritic cell fate. Blood *105*, 2707-2716.

Bjeldanes, L.F., Kim, J.Y., Grose, K.R., Bartholomew, J.C., and Bradfield, C.A. (1991). Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Proc Natl Acad Sci U S A *88*, 9543-9547. Breton, G., Zheng, S., Valieris, R., Tojal da Silva, I., Satija, R., and Nussenzweig, M.C. (2016). Human dendritic cells (DCs) are derived from distinct circulating precursors that are precommitted to become CD1c+ or CD141+ DCs. J Exp Med.

Briseno, C.G., Haldar, M., Kretzer, N.M., Wu, X., Theisen, D.J., Kc, W., Durai, V., Grajales-

Reyes, G.E., Iwata, A., Bagadia, P., et al. (2016). Distinct Transcriptional Programs Control

Cross-Priming in Classical and Monocyte-Derived Dendritic Cells. Cell Rep 15, 2462-2474.

Buenrostro, J.D., Wu, B., Litzenburger, U.M., Ruff, D., Gonzales, M.L., Snyder, M.P., Chang,

H.Y., and Greenleaf, W.J. (2015). Single-cell chromatin accessibility reveals principles of regulatory variation. Nature *523*, 486-490.

Carpentier, S., Vu Manh, T.P., Chelbi, R., Henri, S., Malissen, B., Haniffa, M., Ginhoux, F., and Dalod, M. (2016). Comparative genomics analysis of mononuclear phagocyte subsets confirms homology between lymphoid tissue-resident and dermal XCR1(+) DCs in mouse and human and distinguishes them from Langerhans cells. Journal of immunological methods *432*, 35-49. Carvalho, B.S., and Irizarry, R.A. (2010). A framework for oligonucleotide microarray preprocessing. Bioinformatics *26*, 2363-2367.

Chessel, D., Dufour, A.B., and Thioulouse, J. (2004). The ade4 package-I- One-table methods. R News *4*.

Croxford, A.L., Lanzinger, M., Hartmann, F.J., Schreiner, B., Mair, F., Pelczar, P., Clausen,

B.E., Jung, S., Greter, M., and Becher, B. (2015). The Cytokine GM-CSF Drives the

Inflammatory Signature of CCR2 Monocytes and Licenses Autoimmunity. Immunity.

Davies, L.C., Rosas, M., Jenkins, S.J., Liao, C.T., Scurr, M.J., Brombacher, F., Fraser, D.J.,

Allen, J.E., Jones, S.A., and Taylor, P.R. (2013). Distinct bone marrow-derived and tissue-

resident macrophage lineages proliferate at key stages during inflammation. Nature communications *4*, 1886.

De Abrew, K.N., Kaminski, N.E., and Thomas, R.S. (2010). An integrated genomic analysis of aryl hydrocarbon receptor-mediated inhibition of B-cell differentiation. Toxicol Sci *118*, 454-469.

Di Meglio, P., Duarte, J.H., Ahlfors, H., Owens, N.D., Li, Y., Villanova, F., Tosi, I., Hirota, K., Nestle, F.O., Mrowietz, U., *et al.* (2014). Activation of the aryl hydrocarbon receptor dampens the severity of inflammatory skin conditions. Immunity *40*, 989-1001.

Eguiluz-Gracia, I., Bosco, A., Dollner, R., Melum, G.R., Lexberg, M.H., Jones, A.C.,

Dheyauldeen, S.A., Holt, P.G., Baekkevold, E.S., and Jahnsen, F.L. (2016). Rapid recruitment of CD14(+) monocytes in experimentally induced allergic rhinitis in human subjects. J Allergy Clin Immunol *137*, 1872-1881 e1812.

Fernandez-Salguero, P., Pineau, T., Hilbert, D.M., McPhail, T., Lee, S.S., Kimura, S., Nebert, D.W., Rudikoff, S., Ward, J.M., and Gonzalez, F.J. (1995). Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. Science *268*, 722-726.
Fritsche, E., Schafer, C., Calles, C., Bernsmann, T., Bernshausen, T., Wurm, M., Hubenthal, U., Cline, J.E., Hajimiragha, H., Schroeder, P., *et al.* (2007). Lightening up the UV response by identification of the arylhydrocarbon receptor as a cytoplasmatic target for ultraviolet B radiation. Proc Natl Acad Sci U S A *104*, 8851-8856.

Gautier, E.L., Shay, T., Miller, J., Greter, M., Jakubzick, C., Ivanov, S., Helft, J., Chow, A., Elpek, K.G., Gordonov, S., *et al.* (2012). Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. Nat Immunol *13*, 1118-1128.

Getts, D.R., Terry, R.L., Getts, M.T., Deffrasnes, C., Muller, M., van Vreden, C., Ashhurst,
T.M., Chami, B., McCarthy, D., Wu, H., *et al.* (2014). Therapeutic inflammatory monocyte
modulation using immune-modifying microparticles. Science translational medicine *6*, 219ra217.
Gomez de Aguero, M., Ganal-Vonarburg, S.C., Fuhrer, T., Rupp, S., Uchimura, Y., Li, H.,
Steinert, A., Heikenwalder, M., Hapfelmeier, S., Sauer, U., *et al.* (2016). The maternal
microbiota drives early postnatal innate immune development. Science *351*, 1296-1302.
Gosselin, D., Link, V.M., Romanoski, C.E., Fonseca, G.J., Eichenfield, D.Z., Spann, N.J.,
Stender, J.D., Chun, H.B., Garner, H., Geissmann, F., and Glass, C.K. (2014). Environment
drives selection and function of enhancers controlling tissue-specific macrophage identities. Cell

Greter, M., Helft, J., Chow, A., Hashimoto, D., Mortha, A., Agudo-Cantero, J., Bogunovic, M., Gautier, E.L., Miller, J., Leboeuf, M., *et al.* (2012). GM-CSF Controls Nonlymphoid Tissue Dendritic Cell Homeostasis but Is Dispensable for the Differentiation of Inflammatory Dendritic Cells. Immunity *36*, 1031-1046.

Grimm, M.C., Pullman, W.E., Bennett, G.M., Sullivan, P.J., Pavli, P., and Doe, W.F. (1995). Direct evidence of monocyte recruitment to inflammatory bowel disease mucosa. J Gastroenterol Hepatol *10*, 387-395.

Guilliams, M., Ginhoux, F., Jakubzick, C., Naik, S.H., Onai, N., Schraml, B.U., Segura, E., Tussiwand, R., and Yona, S. (2014). Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. Nat Rev Immunol *14*, 571-578.

Haldar, M., Kohyama, M., So, A.Y., Kc, W., Wu, X., Briseno, C.G., Satpathy, A.T., Kretzer, N.M., Arase, H., Rajasekaran, N.S., *et al.* (2014). Heme-mediated SPI-C induction promotes monocyte differentiation into iron-recycling macrophages. Cell *156*, 1223-1234.

Ikuta, T., Ohba, M., Zouboulis, C.C., Fujii-Kuriyama, Y., and Kawajiri, K. (2010). B lymphocyte-induced maturation protein 1 is a novel target gene of aryl hydrocarbon receptor. J Dermatol Sci *58*, 211-216.

Jenner, W., Motwani, M., Veighey, K., Newson, J., Audzevich, T., Nicolaou, A., Murphy, S., Macallister, R., and Gilroy, D.W. (2014). Characterisation of leukocytes in a human skin blister model of acute inflammation and resolution. PLoS One *9*, e89375.

Kamada, N., Hisamatsu, T., Okamoto, S., Chinen, H., Kobayashi, T., Sato, T., Sakuraba, A., Kitazume, M.T., Sugita, A., Koganei, K., *et al.* (2008). Unique CD14 intestinal macrophages contribute to the pathogenesis of Crohn disease via IL-23/IFN-gamma axis. J Clin Invest *118*, 2269-2280.

Kauffmann, A., Gentleman, R., and Huber, W. (2009). arrayQualityMetrics--a bioconductor package for quality assessment of microarray data. Bioinformatics *25*, 415-416.

Kelly, L.M., Englmeier, U., Lafon, I., Sieweke, M.H., and Graf, T. (2000). MafB is an inducer of monocytic differentiation. The EMBO journal *19*, 1987-1997.

Kim, K.W., Williams, J.W., Wang, Y.T., Ivanov, S., Gilfillan, S., Colonna, M., Virgin, H.W.,

Gautier, E.L., and Randolph, G.J. (2016). MHC II+ resident peritoneal and pleural macrophages

rely on IRF4 for development from circulating monocytes. J Exp Med 213, 1951-1959.

Lavin, Y., Winter, D., Blecher-Gonen, R., David, E., Keren-Shaul, H., Merad, M., Jung, S., and Amit, I. (2014). Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. Cell *159*, 1312-1326.

Lehtonen, A., Veckman, V., Nikula, T., Lahesmaa, R., Kinnunen, L., Matikainen, S., and Julkunen, I. (2005). Differential expression of IFN regulatory factor 4 gene in human monocytederived dendritic cells and macrophages. J Immunol *175*, 6570-6579. Leuschner, F., Dutta, P., Gorbatov, R., Novobrantseva, T.I., Donahoe, J.S., Courties, G., Lee, K.M., Kim, J.I., Markmann, J.F., Marinelli, B., *et al.* (2011). Therapeutic siRNA silencing in inflammatory monocytes in mice. Nat Biotechnol *29*, 1005-1010.

McGovern, N., Schlitzer, A., Gunawan, M., Jardine, L., Shin, A., Poyner, E., Green, K.,

Dickinson, R., Wang, X.N., Low, D., et al. (2014). Human Dermal CD14(+) Cells Are a

Transient Population of Monocyte-Derived Macrophages. Immunity 41, 465-477.

Menezes, S., Melandri, D., Anselmi, G., Perchet, T., Loschko, J., Dubrot, J., Patel, R., Gautier,

E.L., Hugues, S., Longhi, M.P., et al. (2016). The Heterogeneity of Ly6Chi Monocytes Controls

Their Differentiation into iNOS+ Macrophages or Monocyte-Derived Dendritic Cells. Immunity *45*, 1205-1218.

Mildner, A., Schonheit, J., Giladi, A., David, E., Lara-Astiaso, D., Lorenzo-Vivas, E., Paul, F., Chappell-Maor, L., Priller, J., Leutz, A., *et al.* (2017). Genomic Characterization of Murine Monocytes Reveals C/EBPbeta Transcription Factor Dependence of Ly6C- Cells. Immunity *46*, 849-862 e847.

Mildner, A., Yona, S., and Jung, S. (2013). A close encounter of the third kind: monocytederived cells. Advances in immunology *120*, 69-103.

Montoya, D., Cruz, D., Teles, R.M., Lee, D.J., Ochoa, M.T., Krutzik, S.R., Chun, R., Schenk, M., Zhang, X., Ferguson, B.G., *et al.* (2009). Divergence of macrophage phagocytic and antimicrobial programs in leprosy. Cell Host Microbe *6*, 343-353.

Murphy, T.L., Grajales-Reyes, G.E., Wu, X., Tussiwand, R., Briseno, C.G., Iwata, A., Kretzer, N.M., Durai, V., and Murphy, K.M. (2015). Transcriptional Control of Dendritic Cell Development. Annu Rev Immunol.

Noy, R., and Pollard, J.W. (2014). Tumor-associated macrophages: from mechanisms to therapy. Immunity *41*, 49-61.

Okabe, Y., and Medzhitov, R. (2014). Tissue-specific signals control reversible program of localization and functional polarization of macrophages. Cell *157*, 832-844.

Patel, A.A., Zhang, Y., Fullerton, J.N., Boelen, L., Rongvaux, A., Maini, A.A., Bigley, V.,

Flavell, R.A., Gilroy, D.W., Asquith, B., *et al.* (2017). The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. J Exp Med.

Russell, D.G., Cardona, P.J., Kim, M.J., Allain, S., and Altare, F. (2009). Foamy macrophages and the progression of the human tuberculosis granuloma. Nat Immunol *10*, 943-948.

Satija, R., Farrell, J.A., Gennert, D., Schier, A.F., and Regev, A. (2015). Spatial reconstruction of single-cell gene expression data. Nat Biotechnol *33*, 495-502.

Schlitzer, A., Sivakamasundari, V., Chen, J., Sumatoh, H.R., Schreuder, J., Lum, J., Malleret, B., Zhang, S., Larbi, A., Zolezzi, F., *et al.* (2015). Identification of cDC1- and cDC2-committed DC progenitors reveals early lineage priming at the common DC progenitor stage in the bone marrow. Nat Immunol *16*, 718-728.

Schreiber, H.A., and Sandor, M. (2010). The role of dendritic cells in mycobacterium-induced granulomas. Immunology letters *130*, 26-31.

Segura, E., and Amigorena, S. (2013). Inflammatory dendritic cells in mice and humans. Trends Immunol *34*, 440-445.

Segura, E., Touzot, M., Bohineust, A., Cappuccio, A., Chiocchia, G., Hosmalin, A., Dalod, M., Soumelis, V., and Amigorena, S. (2013). Human Inflammatory Dendritic Cells Induce Th17 Cell Differentiation. Immunity *38*, 336-348. Servant, N., Gravier, E., Gestraud, P., Laurent, C., Paccard, C., Biton, A., Brito, I., Mandel, J., Asselain, B., Barillot, E., and Hupe, P. (2010). EMA - A R package for Easy Microarray data analysis. BMC Res Notes *3*, 277.

Sieling, P.A., Jullien, D., Dahlem, M., Tedder, T.F., Rea, T.H., Modlin, R.L., and Porcelli, S.A. (1999). CD1 expression by dendritic cells in human leprosy lesions: correlation with effective host immunity. J Immunol *162*, 1851-1858.

Smyth, G.K. (2004). Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol *3*, Article3.

Stockinger, B., Di Meglio, P., Gialitakis, M., and Duarte, J.H. (2014). The aryl hydrocarbon receptor: multitasking in the immune system. Annu Rev Immunol *32*, 403-432.

Tamoutounour, S., Guilliams, M., Montanana Sanchis, F., Liu, H., Terhorst, D., Malosse, C., Pollet, E., Ardouin, L., Luche, H., Sanchez, C., *et al.* (2013). Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. Immunity *39*, 925-938.

van der Maaten, L. (2013). Barnes-Hut-SNE. Proceedings of the International Conference on Learning Representations.

Veldhoen, M., Hirota, K., Christensen, J., O'Garra, A., and Stockinger, B. (2009). Natural agonists for aryl hydrocarbon receptor in culture medium are essential for optimal differentiation of Th17 T cells. J Exp Med *206*, 43-49.

Villani, A.C., Satija, R., Reynolds, G., Sarkizova, S., Shekhar, K., Fletcher, J., Griesbeck, M., Butler, A., Zheng, S., Lazo, S., *et al.* (2017). Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. Science *356*. Watchmaker, P.B., Lahl, K., Lee, M., Baumjohann, D., Morton, J., Kim, S.J., Zeng, R., Dent, A., Ansel, K.M., Diamond, B., *et al.* (2014). Comparative transcriptional and functional profiling defines conserved programs of intestinal DC differentiation in humans and mice. Nat Immunol *15*, 98-108.

Wollenberg, A., Kraft, S., Hanau, D., and Bieber, T. (1996). Immunomorphological and ultrastructural characterization of Langerhans cells and a novel, inflammatory dendritic epidermal cell (IDEC) population in lesional skin of atopic eczema. J Invest Dermatol *106*, 446-453.

Wong, K.L., Tai, J.J., Wong, W.C., Han, H., Sem, X., Yeap, W.H., Kourilsky, P., and Wong, S.C. (2011). Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. Blood *118*, e16-31.

Zaba, L.C., Fuentes-Duculan, J., Eungdamrong, N.J., Abello, M.V., Novitskaya, I., Pierson, K.C., Gonzalez, J., Krueger, J.G., and Lowes, M.A. (2009). Psoriasis is characterized by accumulation of immunostimulatory and Th1/Th17 cell-polarizing myeloid dendritic cells. J Invest Dermatol *129*, 79-88.

Zelante, T., Iannitti, R.G., Cunha, C., De Luca, A., Giovannini, G., Pieraccini, G., Zecchi, R., D'Angelo, C., Massi-Benedetti, C., Fallarino, F., *et al.* (2013). Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity *39*, 372-385.

Zheng, G.X., Terry, J.M., Belgrader, P., Ryvkin, P., Bent, Z.W., Wilson, R., Ziraldo, S.B., Wheeler, T.D., McDermott, G.P., Zhu, J., *et al.* (2017). Massively parallel digital transcriptional profiling of single cells. Nature communications *8*, 14049.

28

Zigmond, E., Varol, C., Farache, J., Elmaliah, E., Satpathy, A.T., Friedlander, G., Mack, M., Shpigel, N., Boneca, I.G., Murphy, K.M., *et al.* (2012). Ly6C(hi) Monocytes in the Inflamed Colon Give Rise to Proinflammatory Effector Cells and Migratory Antigen-Presenting Cells. Immunity *37*, 1076-1090.

Figure legends

Fig. 1. Monocytes cultured with MCSF, IL-4 and TNFa yield DC and macrophages that closely resemble inflammatory DC and macrophages found *in vivo*. (A-F) CD14⁺ monocytes isolated by positive selection using magnetic beads were cultured with MCSF, IL-4 and TNFa for 5 days. (A) Cell-sorted CD1a⁺ and CD16⁺ cells were analyzed after cytospin and Giemsa/May-Grünwald staining. Bar=10 µm. N=5. (B) Cell-sorted CD1a⁺ and CD16⁺ cells were cultured with allogeneic naive CD4 T cells for 6 days. Number of T cells having divided is shown. Mean +/- SEM (n=5). (C) Cell-sorted mo-DC (CD1a⁺ cells) and mo-Mac (CD16⁺ cells) were cultured for 24 hours with or without dimerized CD40-L. IL-23 secretion was analyzed by ELISA and IL-6 secretion by CBA. Each symbol represents an individual donor (n=14). (D) CD14⁺ monocytes were cultured with MCSF, IL-4 and TNFa, or GM-CSF and IL-4. Cells were analyzed by flow cytometry and compared to ascites mo-DC and mo-Mac. Grey shaded histograms represent isotype control stainings. N=6. (E) Cell-sorted CD1a⁻CD16⁻ cells, mo-DC and mo-Mac were re-cultured with MCSF, IL-4 and TNFa, and analyzed by flow cytometry after 2 days. Grey shaded histograms represent isotype control stainings. N=8. (F) Monocytes were stained with a proliferation dye and cultured with or without phytohaemagglutin-L (PHA-L). Percentage of cells having divided. N=6. (G) Transcriptomic analysis of cell-sorted DC and macrophages differentiated in MCSF, IL-4 and TNFa (n=6), in IL-34, IL-4 and TNFa (n=6), DC differentiated in GM-CSF and IL-4 (n=6), DC and macrophages from tumor ascites (n=5), blood $CD14^+$ monocytes (n=4) and blood $CD1c^+$ DC (n=4). Principal component analysis on the 1000 most variant genes. See also Figure S1.

Fig. 2. IRF4 and MafB are essential for mo-DC and mo-Mac differentiation. (A) Volcano plot showing the fold change and significance of transcription factor genes between ascites mo-DC and mo-Mac. Genes not expressed in in vitro-generated mo-DC or mo-Mac were filtered out. Adjusted p values determined by differential expression analysis. (B) Cell-sorted ascites mo-DC and mo-Mac, and blood CD14⁺ monocytes (Mono) were analyzed by Western Blot. N=4. (C) CD14⁺ monocytes were cultured with MCSF, IL-4 and TNFa for 3h, 6h, 12h or 24h, or processed directly after isolation (0). IRF4 and MAFB expression were analyzed by RT-qPCR in total cells. Each color represents an individual donor (n=5). (D) CD14⁺ monocytes were cultured with MCSF, IL-4 and TNFa for 5 days. Total cells were lyzed at different days and analyzed by Western Blot. N=5. (E-H) Monocytes were infected at day 0 with lentivirus containing sh RNA against IRF4 (E-F), or MAFB (G-H), or control sh RNA. After 5 days of culture, cells were analyzed by Western Blot (E and G), or by flow cytometry (F and H). (E and G) Silencing quantified based on Western Blot stainings. (F and H) Proportions of DC and macrophages in the culture at day 5. Each symbol represents an individual donor (F n=6, H n=8). *p<0.05, **p<0.01. See also Figure S2.

Fig. 3. Monocytes are not heterogeneous for the expression of mo-DC and mo-Mac gene signatures. (A-B) CD14⁺ monocytes isolated by positive selection using magnetic beads were analyzed by single-cell RNA-seq using a drop-seq approach. Colors represent unbiased clustering from graph-based clustering. Each dot represents an individual cell from one donor. *T*-SNE analysis of individual cells for (A) total cells (n=424) or (B) CD16⁻ cells only (n=320). (C-D) Cell-sorted monocyte and DC populations from blood were analyzed by single-cell RNA-seq using a Smart-seq2 approach. (C-D) Heat map of scaled expression (log values of transcripts per

million) of signature genes for mo-DC and mo-Mac (C) and selected genes (D). *See also Figure S3*.

Fig. 4. AHR is a molecular switch for mo-DC versus mo-Mac differentiation. (A-B) CD14⁺ monocytes were infected at day 0 with lentivirus containing sh RNA against AHR, or control sh RNA. After 5 days of culture, cells were analyzed by Western Blot (A) or by flow cytometry (B). (A) Silencing quantified based on Western Blot stainings. (B) Proportions of DC and macrophages in the culture at day 5. Each symbol represents an individual donor (n=6). *p<0.05. (C) Monocytes were cultured with MCSF, IL-4 and TNFa for 5 days, in the presence of various concentrations of FICZ (AHR agonist) or SR1 (AHR inhibitor). Proportions of DC and macrophages at day 5. Each symbol represents an individual donor (n=10 for FICZ and n=8 for SR1). (D-E) CD14⁺ monocytes were analyzed directly after isolation, or were cultured for 3h in medium alone or with various combinations of MCSF, IL-4, TNFa, FICZ or SR1. (D) Relative expression of IRF4 and MAFB measured by RT-qPCR. Box plots representing the 5-95 percentile (n=6). (E) Expression of *IRF4* and *MAFB* at the single cell level was measured by fluorescent in situ hybridization coupled to flow cytometry. Proportions of IRF4⁺ and MAFB⁺ cells. Each symbol represents an individual donor (n=6). *p<0.05, **p<0.01, ***p<0.001. See also Figure S4 and S5.

Fig. 5. AHR acts on mo-DC differentiation through BLIMP-1. (A) $CD14^+$ monocytes were cultured with MCSF, IL-4 and TNFa for 3h, 6h, 12h or 24h in the presence or absence of FICZ or SR1, or processed directly after isolation (0). *PRDM1* expression measured by RT-qPCR. Each color represents an individual donor (n=5). (B) Monocytes were cultured with MCSF, IL-4

and TNFa for 5 days. Total cells were lyzed at different days and analyzed by Western Blot. N=4. (C-E) Monocytes were infected at day 0 with lentivirus containing sh RNA against *PRDM1*, or control sh RNA. After 5 days of culture, cells were analyzed by Western Blot (C) or by flow cytometry (D-E). (C) Silencing quantified based on Western Blot stainings. (D) Proportions of DC and macrophages in the culture at day 5. Each symbol represents an individual donor (n=8). *p<0.05. **p<0.01. (E) Cells were cultured in presence or absence of 62 pM FICZ. Proportions of DC and macrophages in the culture at day 5. Each symbol represents an individual donor (n=6). *p<0.05.

Fig. 6. AhR is involved in mo-DC differentiation *in vivo* **in the mouse.** (A-C) Ear skin from individual AhR^{-/-} mice or WT littermates (A-B), or C57BL/6 mice fed with an experimental diet supplemented or not with indole-3-carbinol (I3C) (C), was digested to prepare single-cell suspensions. After gating on live CD45⁺CD3⁻NK1.1⁺CD19⁻Ly6G⁻CD24⁺CD11b⁺ cells, cells were separated into 5 subsets based on the expression of Ly6C, CD64, MHC II and CCR2. (A) One representative AhR^{-/-} and WT mouse is shown. (B-C) Proportions of monocytes, early mo-DC, mo-DC, MHC II⁺ macrophages and resident MHC II⁻ macrophages among Ly6C⁺ or CD64⁺ cells. Each symbol represents an individual mouse (B n=9 in 2 independent experiments, C n=12 in 2 independent experiments).*p<0.05, **p<0.01. (D-E) Cells from the peritoneal lavage were analyzed by flow cytometry. After gating on live CD115⁺CD11b⁺ cells, cells were separated into MHCII⁺CD226⁺ cells and ICAM2⁺MHCII⁻ cells. (D) Cell-sorted cells were analyzed after cytospin and Giemsa/May-Grünwald staining. Bar=10 µm. Representative of 2 independent experiments. (E) C57BL/6 mice fed with an experimental diet supplemented or not with I3C, and treated or not with a cocktail of antibiotics. Percentage of MHCII⁺CD226⁺ mo-DC among

 $CD115^+CD11b^+$ cells is shown. Each symbol represents an individual mouse (n=6 in 2 independent experiments).*p<0.05, **p<0.01, ****p<0.0001. See also Figure S6.

Fig. 7. AHR activation correlates with high expression of mo-DC signature genes in leprosy lesions. Gene expression data from lepromatous (L-lep, n=7) or tuberculoid (T-lep, n=10) leprosy lesions was extracted from publicly available micro-array data (GSE17763). Gene set enrichment analysis (GSEA) analysis was performed on comparisons of L-lep versus T-lep for selected gene signatures. (A) Schematic BubbleGUM map of GSEA analysis. Red bubbles indicate enrichment in L-lep and blue bubbles enrichment in T-lep data sets. Strength of enrichment is represented by normalized enrichment score (NES). Significance of enrichment is represented by the false discovery rate (FDR). ns=not significant. (B) Gene set enrichment plot for mo-DC and mo-Mac gene signatures. *See also Figure S7.*

STAR Methods

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Elodie Segura (elodie.segura@curie.fr).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human samples. Samples of ovarian or breast tumor ascites from untreated patients were obtained from Hôpital de l'Institut Curie (Paris) in accordance with hospital guidelines. Ovarian cancer ascites were used for transcriptomic analysis, and both ovarian and breast cancer ascites were used for flow cytometry. Buffy coats from healthy donors were obtained from Etablissement Français du Sang (Paris) in accordance with INSERM ethical guidelines. According to French Public Health Law (art L 1121-1-1, art L 1121-1-2), written consent and IRB approval are not required for human non-interventional studies.

Mice. AhR^{-/-} mice (B6.129-Ahr^{tm1Gonz}/Nci) have been described previously (Fernandez-Salguero et al., 1995), and were kindly provided by X.Coumoul (INSERM UMR_S1124, Université René Descartes, Paris, France). AhR^{-/-} mice and WT littermates were maintained under specific pathogen-free conditions at the animal facility of INSERM UMR_S1124 in accordance with institutional guidelines. AhR^{-/-} female mice and WT female littermates were sacrificed at age 7-9 weeks. C57BL/6 female mice were maintained under specific pathogen-free conditions at the animal facility of Institut Curie in accordance with institutional guidelines. In some experiments, C57BL/6 mice were maintained on a purified diet (AIN-93M, Safe diets) supplemented or not with 200 p.p.m. indole-3-carbinol (Sigma) for at least 4 weeks, starting when the mice were 3 weeks-old. In some experiments, C57BL/6 mice were treated orally during 2-3 weeks with

antibiotics (Sigma) in drinking water: vancomycin (0.5 g/L), neomycin (1 g/L), ampicillin (1 g/L) and metronidazole (1 g/L). Antibiotics activity was confirmed by macroscopic changes observed at the level of caecum. All animal procedures were in accordance with the guidelines and regulations of the French Veterinary Department.

METHOD DETAILS

Monocyte isolation and culture. Peripheral Blood Mononuclear Cells (PBMC) were prepared by centrifugation on a Ficoll gradient (Lymphoprep, Greiner Bio-One). Blood CD14⁺ monocytes were isolated from healthy donors' PBMC by positive selection using magnetic beads (Miltenyi). Monocytes were 95-98% CD14⁺CD16⁻ as assessed by flow cytometry. In some experiments, monocytes were isolated on a cell sorter (Aria, BD Biosciences) after staining PBMC with APCeFluor780 anti-HLA-DR (eBioscience, clone LN3), Pe/Cy7 anti-CD11c (Biolegend, clone Bu15), PE anti-CD14 (BD Biosciences, clone M5E2) and APC anti-CD16 (Biolegend, clone 3G8). In some experiments, $CD16^+$ monocytes were isolated from PBMC using $CD16^+$ monocytes selection kit (Miltenvi). Monocytes $(1 \times 10^6 \text{ cells/mL})$ were cultured for 5 days in RPMI-Glutamax medium (Gibco) supplemented with antibiotics (penicillin and streptomicin) and 10% Fetal Calf Serum in the presence or absence of 100 ng/mL M-CSF (Miltenyi), 100 ng/mL IL-34 (R&D Biotechne), 100 ng/mL GM-CSF (Miltenyi), 40 ng/mL IL-4 (Miltenyi) and 5 ng/mL TNF- α (R&D Biotechne). Cytokines were added only at the start of the culture, and medium was not refreshed during the course of the culture. Cell populations were isolated by cell sorting on a FACSAria instrument (BD Biosciences). In some experiments, monocytes were cultured in the presence of various concentrations of Stemregenin-1 (Cayman chemicals) or 6-Formylindolo(3,2-b)carbazole (FICZ) (Enzo Life Sciences). In some experiments, monocytes

were stained with Cell Trace Violet (Thermo Fisher Scientific) prior to culture, and cultured in the presence or absence of 2 µg/mL PHA-L (Sigma).

Flow cytometry. Cells were stained in PBS containing 0.5% human serum and 2mM EDTA with APC anti-CD1a (Biolegend, clone HI149) or APC-Vio770 anti-CD1a (Miltenyi, clone HI149) or PE-Vio770 anti-CD1a (Miltenyi, clone HI149), FITC or APC anti-CD16 (Biolegend, clone 3G8), APC-eFluor780 anti-HLA-DR (eBioscience, clone LN3), Pe/Cy7 anti-CD11c (Biolegend, clone Bu15), PerCP-eFluor710 anti-CD1c (eBioscience, clone L161) or APC anti-CD1c (Biolegend, clone L161), PE anti-CD11b (BD Biosciences, clone M1/70), PE anti-FccRI (eBioscience, clone AER-37), Alexa647 anti-CD206 (Biolegend, clone 15-2), PE anti-CD163 (Biolegend, clone GHI/61), APC anti-CD226 (Miltenyi, clone DX11), APC anti-MerTK (R&D Biotechne, clone 125518), PE anti-CD141 (Miltenyi, clone AD5-14H12), PE anti-CD64 (Biolegend, clone S5/1), APC anti-CD14 (BD Biosciences, clone M5E2), biotinylated anti-CD172a (Biolegend, clone SE5A5) followed by staining with APC streptavidin (eBioscience), or isotype-matched control antibodies. Cells were analyzed on a FACSVerse (BD Biosciences) or MACSQuant (Miltenyi) instrument. Data was analyzed with FlowJo (FlowJo LLC).

Morphological analysis. Cells were subjected to cytospin and colored with May-Grunwald/Giemsa staining (Sigma). Pictures were taken with a CFW-1308C color digital camera (Scion Corporation) on a Leica DM 4000 B microscope.

MLR. Allogeneic CD4⁺ T cells were isolated from healthy donors' PBMC by negative selection using a naive CD4⁺ T cell isolation kit (Miltenyi). CD4⁺ T cells were stained with Cell Trace CFSE (Thermo Fisher Scientific) and cultured (5×10^4 cells/well) with different numbers of antigen-presenting cells. After 6 days, T cell proliferation was assessed by flow cytometry.

37

Affymetrix micro-array hybridization. RNA was extracted using the RNAeasy Micro Kit (Qiagen) according to the manufacturer's protocol. For each condition, 100 ng of polysomalbound RNA were employed to synthesize double-stranded cDNA using two successive reversetranscription reactions according to the standard Affymetrix protocol. Labelled DNA was hybridized on the Affymetrix human Gene ST1.1, an oligonucleotide 28,000-gene microarray processed on an Affymetrix GeneTitan device.

Affymetrix Microarray Data Analysis. Microarray data were carried out with R (version 3.1.0) using packages from the Bioconductor. Raw data CEL files were used and the quality control analysis was performed using *ArrayQualityMetrics* package (Kauffmann et al., 2009). The raw data were preprocessed using the RMA (Robust Multi-array Average) method available in *oligo* package (Carvalho and Irizarry, 2010). Gene expression levels were analyzed on a base-2 logarithmic scale. Moderated t-tests were performed using the *limma* package (Smyth, 2004) and the p-values were corrected for multiple testing with the Benjamini Hochberg method. Probeset were considered as statistically differentially expressed if associated adjusted p-value was lower than 5%.

PCA. The analysis was performed in two steps. First, samples were grouped in "in vitro" (IL34 mo-Mac, IL34 mo-DC, M-CSF mo-Mac, M-CSF mo-DC, GM-CSF mo-DC) or "ex vivo" (monocytes, ascites mo-Mac, ascites mo-DC, CD1c+ DC) datasets, and genes which were only expressed in one group were excluded from the analysis. In the second step, the Principal Component Analysis (PCA) was performed using *Ade4* package (Chessel et al., 2004) of the R software (version 3.1.0) using 1000 genes expression profiles with the highest interquartile range (IQR) values obtained with EMA package (Servant et al., 2010). The two first components of the PCA represent 41% of the total variance. The barycenters were calculated in each condition and

indicated into the PCA plot. The 95% confidence ellipses were also drawn around the barycenters.

Identification of transcription factors. To identify genes involved in monocyte differentation into mo-DC, we have implemented a strategy of analysis that identified the genes that are concomitantly (i) up-regulated genes in ascites mo-DC compared to monocytes, (ii) up-regulated genes in ascites mo-DC compared to ascites mo-Mac, (iii) up-regulated genes in IL34 mo-DC compared to IL34 mo-Mac and (iv) up-regulated genes in M-CSF mo-DC compared to M-CSF mo-Mac. After running this pipeline, a list of genes was obtained and we extracted only genes coding for transcription factors. The list of transcription factors was downloaded from AnimalTFDB website (http://bioinfo.life.hust.edu.cn/AnimalTFDB/). The exact same approach was used to identify candidate genes involved in differentation into mo-Mac.

Single cell RNA-seq library preparation. Cellular suspensions (1700 cells) were loaded on a 10X Chromium instrument (10X Genomics) according to manufacturer's protocol based on the 10X GEMCode proprietary technology. Single-cell RNA-Seq libraries were prepared using Chromium Single Cell 3' v2 Reagent Kit (10X Genomics) according to manufacturer's protocol. Briefly, the initial step consisted in performing an emulsion where individual cells were isolated into droplets together with gel beads coated with unique primers bearing 10X cell barcodes, UMI (unique molecular identifiers) and poly(dT) sequences. Reverse transcription reactions were engaged to generate barcoded full-length cDNA followed by the disruption of emulsions using the recovery agent and cDNA clean up with DynaBeads MyOne Silane Beads (Thermo Fisher Scientific). Bulk cDNA was amplified using a GeneAmp PCR System 9700 with 96-Well Gold Sample Block Module (Applied Biosystems) (98 °C for 3 min; cycled $14 \times : 98$ °C for 15 s, 67 °C for 20 s, and 72 °C for 1 min; 72 °C for 1 min; held at 4 °C). Amplified cDNA product was

cleaned up with the SPRI select Reagent Kit (Beckman Coulter). Indexed sequencing libraries were constructed using the reagents from the Chromium Single Cell 3' v2 Reagent Kit, following these steps: (1) fragmentation, end repair and A-tailing; (2) size selection with SPRI select; (3) adaptor ligation; (4) post ligation cleanup with SPRI select; (5) sample index PCR and cleanup with SPRI select beads. Library quantification and quality assessment was performed using Qubit fluorometric assay (Invitrogen) with dsDNA HS (High Sensitivity) Assay Kit and Bioanalyzer Agilent 2100 using a High Sensitivity DNA chip (Agilent Genomics). Indexed libraries were equimolarly pooled and sequenced on an Illumina HiSeq2500 using paired-end 26x98bp as sequencing mode. Using a full Rapid flow cell, a coverage around 100M reads per sample (around 1000 cells) were obtained corresponding to 100,000 reads/cell.

Single-cell RNA-seq data analysis. Single-cell expression data used in figures 3 A-B and S3 A-C was analyzed using the Cell Ranger Single Cell Software Suite (v1.3.1) to perform quality control, sample de-multiplexing, barcode processing, and single-cell 3' gene counting (Zheng et al., 2017). Sequencing reads were aligned to the UCSC hg19 transcriptome using the Cell Ranger suite with default parameters. A total of 856 single cells were analyzed, from two different donors. The samples consisted of 425 and 431 cells. Each sample was normalized separately. Mean raw reads per cell were 289,807 and 320,866 respectively. Further analysis was performed in R (v3.3) using the Seurat package (v1.4.0.14) (Satija et al., 2015). The gene-cell-barcode matrix of the samples was log-transformed and filtered based on the number of genes detected per cell (any cell with less than 400 genes per cell was filtered out). Regression in gene expression was performed based on the number of unique molecular identifiers (UMI) and the percentage of mitochondrial genes. Only genes detected in at least 10 cells were included. Cells were then scaled to a total of 1^{e4} molecules. Any cell with more than 10% of mitochondrial UMI

counts was filtered out. Altogether, 424 and 429 cells were kept for statistical analysis. To reduce data dimensionality, 2089 and 2127 variable genes were selected based on their expression and dispersion (expression cut-off=0.0125, and dispersion cut-off=0.5). PCA was run on the normalized gene-barcode matrix. Barnes-hut approximation to *t*-SNE (van der Maaten, 2013) was then performed on the first 30 principal components to visualize cells in a two-dimensional space. The first 30 principal components were used for the *t*-SNE projection and clustering analysis using the Elbow Plot approach. Clusters were identified using the "Find_Clusters" function in Seurat with a resolution parameter of 0.6. This graph-based clustering method relies on a clustering algorithm based on shared nearest neighbor (SNN) modularity optimization. Unique cluster-specific genes were identified by running the Seurat « Find_All_Markers » function using default parameters. Names for Cluster 1 and 2 were assigned based on *FCGR3A* (encoding CD16) expression levels. Heatmaps were plotted using Seurat.

Single-cell expression data used in figures 3 C-D and S3 D-E was obtained from GEO GSE94820 (supplementary file:

GSE94820_raw.expMatrix_DCnMono.discovery.set.submission.txt.gz) and analysis was performed with the Seurat R package following the methodology described in (Villani et al., 2017). Briefly, (1) low-quality cells (requiring a minimum of 3000 expressed genes, where a gene is considered expressed if it has non-zero read count in at least 3 cells) and contaminating cells (basophils detected as in (Villani et al., 2017)) were filtered out, and DoubleNeg cells were excluded from the analysis in 3 C-D; (2) heatmaps were used to visualize scaled (*z*-score) gene expression level (log₂(TPM+1)) of gene sets of interest, either grouping cells according to the classical subset definitions (fig.3C-D) or according to the revised subset definition (fig.S4 D-E) (Villani et al., 2017), where the purple-yellow color scheme corresponds to *z*-score distribution, from -2.5 to 2.5.

GSEA analysis. GSEA analysis were performed using published gene signatures for Langerhans cells, skin CD1c⁺ DC and dermal macrophages (Carpentier et al., 2016), AHR agonist and antagonist signatures from (Di Meglio et al., 2014), and has been performed with the default parameters except for the number of permutations that we fixed at n = 10000 and the number of min gene at n = 8.

Western blot. Cells were lysed in RIPA buffer (Thermo Scientific) supplemented with cOmplete Mini EDTA-free protease inhibitor cocktail (Roche). Post-nuclear lysates were resolved by SDS-PAGE using 4-12% BisTris NuPAGE gels (Invitrogen) and proteins were transferred to membranes (Immunoblot PVDF membranes, Bio-Rad). Membranes were stained with primary antibodies against IRF4 (Cell Signaling, #4964), AhR (Enzo Life Science, BML-SA210), Blimp-1 (NovusBio, clone 3H2-E8), MafB (NovusBio, NBP1-81342), hsp70 (Enzo Life Science, clone C92F3A-5) or actin (Millipore, clone C4), followed by HRP-conjugated secondary antibodies (Jackson Immunoresearch). Some membranes were incubated with "Reblot Plus" buffer (Millipore).

Cytokine secretion. Cells $(2.5 \times 10^4 \text{ cells/well})$ were incubated during 24 hours in Yssel medium in the presence or absence of 1 µg/mL R848 (Invivogen), 1 µg/mL dimerized CD40-ligand (Alexis) and 100 µg/mL Uric Acid (Invivogen). Supernatants were collected and kept at -20°C. Cytokine secretion was assessed by CBA (BD Biosciences) or ELISA (for IL-23, eBioscience).

Fluorescent *in situ* hybridization coupled to flow cytometry. Purified monocytes were kept on ice or cultured during 3 hours in the presence or absence of M-CSF, IL-4, TNF-a or 62 pM

FICZ. Dead cells were identified using LIVE/DEAD fixable Aqua dye (Thermo Fischer Scientific). Cells were analyzed using PrimeFlow RNA Assay (eBioscience) according to the manufacturer's protocol with Alexa647-IRF4 and Alexa488-MAFB probes. Cells were analyzed on a FACS Verse instrument (BD Biosciences).

shRNA interference. shRNA (all from Sigma) against IRF4 (sh1: NM 002460sh2: TRCN0000014764, NM 002460-TRCN0000014767), AHR NM 001621-(sh1: TRCN0000021254, sh2: NM 001621-TRCN0000021256), MAFB NM 005461-(sh1: TRCN0000017680, sh2: NM 005461-TRCN0000017681), PRDM1 (sh1: NM 001198-TRCN0000013608, sh2: NM 001198-TRCN0000013609) or nontargeting control shRNA (MISSION shRNA SHC002) were in the LKO.1-puro vector (Sigma). Viral particles were produced by transfection of 293FT cells in 6-well plates with 3 µg DNA and 8 µl TransIT-293 (Mirus Bio) per well: for VSV-G pseudotyped SIVmac VLPs, 0.4 µg CMV-VSVG and 2.6 µg pSIV3⁺; for shRNA vectors, 0.4 µg CMV-VSV-G, 1 µg psPAX2 and 1.6 µg LKO1puro-derived shRNA vector. One day after 293FT cells transfection, culture medium was replaced by fresh medium. Viral supernatants were harvested 1 day later and filtered through 0.45 µm filters. Freshly isolated CD14⁺ monocytes were infected with viral particles containing the control vector or individual shRNA vectors, and cultured as above. Puromycin (InvivoGen) was added 2 days later (2 μ g/mL). At day 5, cells were harvested for analysis.

PCR. Cells were harvested and lysed in RLT buffer (Qiagen). RNA extraction was carried out using the RNAeasy micro kit (Qiagen) according to manufacturer's instructions. Total RNA was retro-transcribed using the superscript II polymerase (Invitrogen), in combination with random hexamers, oligo dT and dNTPs (Promega). Transcripts were quantified by real time PCR on a 480 LightCycler instrument (Roche). Reactions were carried out in 10 μ L, using a master mix

(Eurogentec), with the following Taqman Assays primers (Thermo Fisher Scientific): B2M (Hs99999907 m1), RPL34 (Hs00241560 m1), HPRT1 (Hs02800695 m1), IRF4 (Hs01056533 m1), CYP1A1 (Hs01054797 g1), MAFB (Hs00271378 s1), PRMD1 (Hs00153357 m1). Cp from each analyte were obtained using the second derivative maximum method, and the transcripts were quantified as fold changes in comparison to the mean of the three housekeeping genes (B2M, HPRT1 and RPL34).

Analysis of mouse skin cells. Skin cells were isolated from ear skin. Ears were split into ventral and dorsal parts, and incubated overnight at 4°C in PBS containing 2.5 mg/mL dispase II (Roche). Ears were then cut into small pieces using scalpels, and incubated for 90 min at 37°C in RPMI-Glutamax medium containing 0.5 mg/mL DNAse (Roche) and 1 mg/mL collagenase 4 (Roche). Cell suspensions were filtered through a 40 µm cell strainer and washed in PBS containing 2% Fetal Calf Serum and 2mM EDTA. Cells were stained with FITC anti-Ly6C (BD Biosciences, clone AL-21), PE anti-CCR2 (R&D Biotechne, clone 475301), PeCy5 anti-CD24 (Biolegend, clone M1/69), PerCPCy5.5 anti-CD45 (eBioscience, clone 30-F11), PeCy7 anti-CD11b (BD Biosciences, clone M1/70), Alexa647 anti-CD64 (BD Biosciences, clone X54-5/7.1), Alexa700 anti-IA^b (eBioscience, clone M5/114.15.2), APCCy7 anti-CD45R (clone RA3-6B2), APCCy7 anti-NK1.1 (clone PK136), APCCy7 anti-CD3 (clone 145-2C11), APCCy7 anti-Ly6G (clone 1a8) (all from Biolegend). Cells were analysed on a LSR II instrument (BD Biosciences).

Analysis of mouse peritoneal cells. Peritoneal lavage was obtained after injection of 5 mL of PBS into the peritoneal space of sacrificed mice. Cell suspensions were filtered through a 40 μ m cell strainer and washed in PBS containing 2% Fetal Calf Serum and 2mM EDTA. Cells were stained with FITC anti-ICAM2 (Biolegend, clone MIC2/4), PE anti-CD226 (Biolegend, clone

10E5), APC anti-CD115 (eBioscience, clone AFS98), PerCPCy5.5 anti CD11b (BD Biosciences, clone M1/70), APCCy7 anti-MHCII (Biolegend, clone M5/114.15.2) and PeCy7 anti-MerTK (eBioscience, clone DS5MMER) or PeCy7 isotype control. Cells were analyzed on a FACS Verse instrument (BD Biosciences) or sorted on an Astrios cell sorter (Beckman Coulter).

Analysis of mouse spleen cells. Mouse spleens were cut into small pieces using scalpels, and incubated for 30 min at room temperature in RPMI-Glutamax medium containing 0.5 mg/mL DNAse (Roche) and 1 mg/mL collagenase 4 (Roche). Cell suspensions were filtered through a 40 μm cell strainer and washed in PBS containing 2% Fetal Calf Serum and 2mM EDTA. Red blood cells were lyzed using Reb Blood Cells Lysis Buffer (Sigma). Cells were stained with FITC anti-CD8a (BD Biosciences, clone 53-6.7), PE anti-CD3 (BD Biosciences, clone 145-2C11) and anti-CD19 (BD Biosciences, clone 1D3), PerCPCy5.5 anti-CD11b (BD Biosciences, clone M1/70), PeCy anti-CD11c (BD Biosciences, clone N418), APC anti-Ly6C (BD Biosciences, clone AL-21), APC-Cy7 anti-IA^b (Biolegend, clone M5/114.15.2). Cells were analysed on a FACS Verse instrument (BD Biosciences).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis. Wilcoxon matched paired test (fig.1, 2, 3, 4, 5, S1, S5), Mann-Whitney test (fig. S7) and unpaired t-test (fig. 6 and S6) were performed using Prism (GraphPad Software). Statistical details for each experiment can be found in the corresponding figure legend. N corresponds to the number of individual donors or the number of individual mice analyzed.

DATA AND SOFTWARE AVAILABILITY

The Affymetrix data has been deposited in GEO (Accession number XXX). The sequencing data from single-cell RNA-seq has been deposited in GEO (Accession number XXX).

Supplemental item titles

Fig.S1 related to Fig.1. Characterisation of the monocyte differentiation model.

Fig.S2 related to Fig.1. Analysis of monocyte-derived cells.

Fig.S3 related to Fig.3. Single-cell RNA-seq analysis of monocytes.

Fig.S4 related to Fig.4. AHR inhibition or activation does not alter cell phenotype.

Fig.S5 related to Fig.5. AHR synergizes with IL-4 and TNFa to induce mo-DC differentiation.

Fig.S6 related to Fig.6. Analysis of mouse monocyte-derived cells.

Fig.S7 related to Fig.7. Analysis of gene expression in leprosy lesions.

Table S1 related to Fig.2. List of transcription factor genes upregulated in ascites mo-DC versus ascites mo-Mac.

Table S2 related to Fig.3. List of genes for mo-DC and mo-Mac signatures.

Table S3 related to Fig.7. List of genes for "AHR agonist" and "AHR antagonist" signatures.















Color intensity = FDR

mo-Mac are cross at 1109 ativaly correlated 10 000 20 000 Rank in Ordered Dataset Enrichment profile — Hits Ranking metric scores mo-DC (positively correlated Zero cross at 11082 **Panked list** T' (negatively correlated 5 000 7 500 10 000 12 500 15 000 17 500 20.000 Rank in Ordered Dataset Enrichment profile — Hits Ranking metric scores

Supplemental information



Fig.S1 related to Fig.1. Characterisation of the monocyte differentiation model. (A-D) CD14⁺ monocytes isolated by positive selection using magnetic beads were cultured with IL-34, IL-4 and TNF- α for 5 days. (A) Sorted CD1a⁺ and CD16⁺ cells were analyzed after cytospin and Giemsa and May-Grünwald staining. Bar=10 µm. Representative of 3 independent experiments. (B) Cell-sorted CD1a⁺ and CD16⁺ cells were cultured with allogeneic naive CD4 T cells for 6 days and CD4 T cell proliferation was assessed by flow cytometry. Mean +/- SEM of 5 independent experiments. (C) Cell-sorted DC (CD1a⁺ cells) and macrophages (CD16⁺ cells) were cultured for 24 hours with or without dimerized CD40-L. IL-23 secretion was analyzed by ELISA and IL-6 secretion by CBA. Each symbol represents an individual donor (n=12). (D) Cells were analyzed by flow cytometry. Grey shaded histograms represent isotype control stainings. Representative of 6 independent experiments. (E) Blood CD14⁺ monocytes were isolated by cell sorting and cultured with M-CSF, IL-4 and TNF- α for 5 days. Representative of 5 independent experiments. (F) Blood CD16⁺ monocytes were isolated using magnetic beads and cultured with M-CSF, IL-4 and TNF- α for 5 days. Representative of 5 independent experiments. (G) CD16 and CD1a expression. Representative of 3 independent experiments. (G) CD14⁺ monocytes were cultured with GM-CSF and IL-4 or M-CSF, IL-4 and TNF- α for 5 days. Representative of

10 independent experiments. (H) CD14⁺ monocytes were cultured with GM-CSF, IL-4 and TNF- α for 5 days. Cells were analyzed by flow cytometry. Grey shaded histograms represent isotype control stainings. Representative of 6 independent experiments. (I) CD14⁺ monocytes were cultured for 5 days with MCSF, IL-4 and TNF- α , or IL-34, IL-4 and TNF- α , or GM-CSF and IL-4. Cell-sorted DC were cultured for 24 hours with or without R848 and uric acid crystals (UA). Cytokine secretion was analyzed by CBA. Each symbol represents an individual donor (n=9). **p<0.01. (J) Cell-sorted CD1a⁻CD16⁻ cells, mo-DC and mo-Mac were re-cultured with MCSF, IL-4 and TNF- α , and analyzed by flow cytometry after 2 or 5 days. Percentage of viable cells is shown (n=8). Grey shaded histograms represent isotype control stainings. Representative of 8 independent experiments.



Fig.S2 related to Fig.2. Analysis of monocyte-derived cells. (A-B) Transcriptomic analysis. mRNA expression from Affymetrix data (arbitrary units) for selected phenotypic markers (A) and candidate transcription factors (B). Each symbol represents an individual donor. (C-D) Monocytes were infected at day 0 with lentivirus containing sh RNA against *IRF4* (C) or *MAFB* (D), or control sh RNA. After 5 days of culture, cells were analyzed by flow cytometry. Cells were gated as mo-DC (CD16⁻ CD1a⁺), CD16⁻CD1a⁻ cells or mo-Mac (CD16⁺ CD1a⁻). Grey shaded histograms represent isotype control stainings. Representative of 5 independent experiments.



Fig.S3 related to Fig.3. Single-cell RNA-seq analysis of monocytes. (A-C) Purified CD14^+ monocytes isolated by positive selection using magnetic beads were analyzed by single-cell RNA-seq using a drop-seq approach. (A) Purity of monocytes was assessed by flow cytometry. (B) tSNE analysis of individual cells for donor 2. Colors represents unbiased clustering from graph-based clustering. Each dot represents an individual cell (n=429 total cells, n=306 cells for CD16⁻ cells only). (C) Heatmap of scaled expression of top enriched genes for the two clusters. (D-E) Cell-sorted monocyte and DC populations from blood were analyzed by single-cell RNA-seq using a Smart-seq2 approach (Villani et al., 2017). (D) Heatmap of scaled expression of signature genes for mo-DC and mo-Mac. Heatmap color scheme is based on z-score distribution from -2.5 (yellow) to 2.5 (purple). (E) Heat map of scaled expression for selected genes. Heatmap color scheme is based on z-score distribution from -2.5 (yellow) to 2.5 (purple).



Fig.S4 related to Fig.4. AHR inhibition or activation does not alter cell phenotype. $CD14^+$ monocytes were cultured with MCSF, IL-4 and TNF- α for 5 days, in the presence of 8 μ M SR1 (A and C) or 62 nM FICZ (B and C). Cell populations were analyzed by flow cytometry. Grey shaded histograms represent isotype control stainings. Representative of 6 independent experiments.



Fig.S5 related to Fig.4. AHR synergizes with IL-4 and TNFa to induce mo-DC differentiation. (A) Purified blood CD14⁺ monocytes were analyzed directly after isolation, or were cultured for 3h in medium alone or with various combinations of MCSF, IL-4, TNF- α , FICZ or SR1. Relative expression of *IRF4*, *MAFB* and *CYP1A1* were measured by RT-qPCR. Each symbol represents an individual donor (n=6). (B) Monocytes were cultured for 5 days with 100 ng/mL MCSF, 5 ng/mL TNF- α and various concentrations of IL-4. Proportions of DC and macrophages at day 5 are shown. Each symbol represents an individual donor (n=6). (C) Monocytes were cultured for 5 days with 100 ng/mL MCSF, 40 ng/mL IL-4 and various concentrations of TNF- α . Proportions of DC and macrophages at day 5 are shown. Each symbol represents an individual donor (n=9). (D) Monocytes were cultured for 5 days with 100 ng/mL MCSF, 40 ng/mL IL-4 and various concentrations of FICZ in the presence or absence of 5 ng/mL TNF-a. Proportions of DC and macrophages at day 5 are shown. Each symbol represents an individual donor (n=9). (D) Monocytes were cultured for 5 days with 100 ng/mL MCSF, 40 ng/mL IL-4 and various concentrations of FICZ in the presence or absence of 5 ng/mL TNFa. Proportions of DC and macrophages at day 5 are shown. Each symbol represents at day 5 are shown. Each symbol represents at day 5 are shown. Each of 2 ng/mL TNFa. Proportions of DC and macrophages at day 5 are shown. Each symbol represents an individual donor (n=8). *p<0.05; **p<0.01; ***p<0.01.



Fig.S6 related to Fig.6. Analysis of mouse monocyte-derived cells. (A) mRNA expression from Affymetrix data (arbitrary units) for *AhR*, *Irf4*, *Mafb* and *Cd226*. Each symbol represents an individual data set. Microarray data from ((Tamoutounour et al., 2013), GEO accession code GSE49358). (B-C) Ear skin from individual mice was dissociated and digested to prepare single-cell suspensions for flow cytometry analysis. (B) Gating strategy for skin monocyte-derived cells. Live cells are gated on CD45⁺ cells, then on CD3⁻NK1.1⁻CD19⁻Ly6G⁻ cells (lineage⁻ cells), then on CD24⁻CD11b⁺ cells. (C) Gating strategy for DC skin subsets. Lineage⁻ cells are gated on MHC II⁺ cells. Langerhans cells (LC) are CD24⁻CD11b⁺, CD11b⁻DC are CD24⁺CD11b⁻ and CD11b⁺ DC are CD24⁻CD11b⁺ Ly6C⁻ CD64⁻. Proportions of Langerhans cells, CD11b⁺ DC and CD11b⁻ DC among lineage⁻MHC II⁺ cells are shown. Each symbol represents an individual mouse (n=9 in 2 independent experiments). (D) Proportions of Ly6C⁺ and Ly6C⁻ cells among spleen monocytes of AhR^{-/-} or WT littermates. Each symbol represents an individual mouse (n=9 in 2 independent experiments). (E) Peritoneal MHC II⁺ ICAM2⁻CD226⁺ cells and MHC II⁻ICAM2⁺ cells were analyzed by flow cytometry for the expression of MerTK. Grey shaded histograms represent isotype control stainings. Results representative of 9 individual mice in 3 independent experiments.



Fig.S7 related to Fig.7. Analysis of gene expression in leprosy lesions. (A) Gene set enrichment plot for gene signatures of interest. (B) Gene expression of selected genes from Affymetrix micro-arrays. Each symbol represents an individual donor. *p<0.05, **p<0.01, ***p<0.001.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
anti-CD1a APC human (clone HI149)	Biolegend	Cat: #300110
anti-CD1a APC-Vio770 human (clone HI149)	Miltenyi	Cat: #130-100-225
anti-CD1a PE-Vio770 human (clone HI149)	Miltenyi	Cat: #130-105-527
anti-CD16 FITC human (clone 3G8)	Biolegend	Cat: #302006
anti-CD16 APC human (clone 3G8)	Biolegend	Cat: #302012
anti-HLA-DR APC-eFluor780 human (clone LN3)	eBioscience	Cat: #47-9956-42
anti-CD11c Pe/Cy7 human (clone Bu15)	Biolegend	Cat: #337216
anti-CD1c PerCP-eFluor710 human (clone L161)	eBioscience	Cat: #46-0015-42
anti-CD1c APC human (clone L161)	Biolegend	Cat: #331524
anti-CD11b PE mouse/human (clone M1/70)	BD Biosciences	Cat: #553311
anti-FceRI PE human (clone AER-37)	eBioscience	Cat: #12-5899-42
anti-CD163 PE human (clone GHI/61)	Biolegend	Cat: #333605
anti-CD206 Alexa647 human (clone 15-2)	Biolegend	Cat: #321116
anti-CD226 APC human (clone DX11)	Miltenyi	Cat: #130-100-436
anti-MerTK APC human (clone 125518)	R&D Biotechne	Cat: #FAB8912A
anti-CD141 PE human (clone AD5-14H12)	Miltenyi	Cat: #130-090-514
anti-CD88 PE human (clone S5/1)	Biolegend	Cat: #344303
anti-CD1b APC human (clone eBioSN13)	eBioscience	Cat: #17-0018-42
anti-CD64 PE human (clone 10.1)	Biolegend	Cat: #305007
anti-CD14 PE human (clone M5E2)	BD Biosciences	Cat: #301806
anti-CD172a biotin human (clone SE5A5)	Biolegend	Cat: #323804
Rabbit polyclonal Anti-AhR	Enzo Life Science	Cat: #BML-SA210
Rabbit polyclonal Anti-IRF4	Cell Signaling	Cat: #4964
Mouse monoclonal Anti-Blimp1 (clone 3H2-E8)	NovusBio	Cat: #NB600-235
Rabbit polyclonal Anti-MAFB	NovusBio	Cat: #NBP1-81342
anti-ICAM2 FITC mouse (clone MIC2/4)	Biolegend	Cat: #105606
anti-CD226 PE mouse (clone 10E5)	Biolegend	Cat: #128806

anti-CD115 APC mouse (clone AFS98)	eBioscience	Cat: #17-1152-82
anti CD11b PerCPCy5.5 mouse (clone M1/70)	BD Biosciences	Cat: #550993
anti-MHCII APCCy7 mouse (clone M5/114.15.2)	Biolegend	Cat: #107628
anti-MerTK PeCy7 mouse (clone DS5MMER)	eBioscience	Cat: #25-5751-80
anti-Ly6C FITC mouse (clone AL-21)	BD Biosciences	Cat: #553104
anti-CCR2 PE mouse (clone 475301)	R&D Biotechne	Cat: #FAB5538P
anti-CD24 PeCy5 mouse (clone M1/69)	Biolegend	Cat: #101811
anti-CD45 PerCPCy5.5 mouse (clone 30-F11)	eBioscience	Cat: #45-0451-82
anti-CD11b PeCy7 mouse/human (clone M1/70)	BD Biosciences	Cat: #552850
anti-CD64 Alexa647 mouse (clone X54-5/7.1)	BD Biosciences	Cat: #558539
anti-IA ^b Alexa700 mouse (clone M5/114.15.2)	eBioscience	Cat: #56-5321-82
anti-CD45R APCCy7 mouse (clone RA3-6B2)	Biolegend	Cat: #103224
anti-NK1.1 APCCy7 mouse (clone PK136)	Biolegend	Cat: #108724
anti-CD3 APCCy7 mouse (clone 145-2C11)	Biolegend	Cat: #100330
anti-Ly6G APCCy7 mouse (clone 1a8)	Biolegend	Cat: #127624
Bacterial and Virus Strains		
Biological Samples		
Chemicals, Peptides, and Recombinant Proteins		

6-Formylindolo(3,2-b)carbazole	Enzo Life Sciences	BML-GR206-0100
Critical Commercial Assays		
PrimeFlow RNA Assay	eBioscience	Cat: # 88-18005-210
RNAeasy micro kit	Qiagen	Cat: #74004
Deposited Data		
Affymetrix data	GEO	GSE102046
Single-cell RNA-seq data	GEO	GSE103544
Experimental Models: Cell Lines		
Experimental Models: Organisms/Strains		
Mouse: B6.129-Ahr ^{tm1Gonz} /Nci	{Fernandez-Salguero, 1995 #981}	RRID:IMSR_NCIMR: 01XC3

Oligonucleotides		
Primer IRF4	Thermo Fisher Scientific	Hs01056533_m1
Primer CYP1A1	Thermo Fisher Scientific	Hs01054797_g1
Primer MAFB	Thermo Fisher Scientific	Hs00271378_s1
Primer PRDM1	Thermo Fisher Scientific	Hs00153357_m1
Recombinant DNA		
Software and Algorithms		
Cell Ranger Single Cell Software Suite (v1.3.1)	(Zheng et al., 2017)	https://support.10xge nomics.com/single- cell-gene- expression/software/ pipelines/latest/rkit
Seurat package (v1.4.0.14)	(Satija et al., 2015)	https://github.com/sa tijalab/seurat
Ade4 package	(Chessel et al., 2004)	http://pbil.univ- lyon1.fr/ade4/
EMA package	(Servant et al., 2010)	http://bioinfo- out.curie.fr/projects/e ma/
ArrayQualityMetrics package	(Kauffmann et al., 2009)	https://bioconductor. org/packages/releas e/bioc/html/arrayQua lityMetrics.html
oligo package	(Carvalho and Irizarry, 2010)	https://www.biocond uctor.org/packages/r elease/bioc/html/olig o.html

<i>limma</i> package	(Smyth, 2004)	https://bioconductor. org/packages/releas e/bioc/html/limma.ht ml
FlowJo (v.9.9.5)	FlowJo LLC	https://www.flowjo.co m/
Other		