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Who on IRF are you? IRF8 deficiency redirects cDC1 lineage commitment

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IRF8 has long been associated with cDC1 development. In a recent study, Lança et al., demonstrate that IRF8 is also crucial in cells already committed to the cDC1 lineage. Here, deletion of IRF8 from the XCR1-expressing pre-cDC1 stage onwards lead to a loss of commitment and reprogramming of the cells towards a cDC2-like phenotype.

Conventional dendritic cells (cDCs) are professional antigen presenting cells that bridge innate and adaptive immunity. They exist in two main subsets conserved across species termed cDC1s and cDC2s, which can be identified on the basis of the surface markers and transcription factors they express. cDC1s express XCR1, CLEC9A and the transcription factor interferon regulatory factor 8 (IRF8). Conversely, cDC2s express SIRPα, CD11b and IRF4 [1]. In mice and humans, cDCs develop in an FMS-related receptor tyrosine kinase 3 (Flt3)-dependent manner from common DC progenitors (CDPs) in the bone marrow (BM) [2] which then further develop into pre-cDCs [3]. These pre-cDCs can be further subdivided into uncommitted pre-cDCs -- which remain capable of generating both cDC1s and cDC2s -- and committed pre-cDC1s and pre-cDC2s which, as their names suggest, primarily produce cDC1s and cDC2s, respectively [3–5]. These progenitors then enter the blood from which they can subsequently access different peripheral tissues to develop into mature cDC1s and cDC2s. The development of cDCs from CDPs and pre-cDCs is tightly regulated by the concerted effort of a variety of transcriptions factors. In mice, cDC2 development and function is regulated at least in part through IRF4 (reviewed in [6]), while CEBPα/CEBPβ binding to the -165kb *Zeb2* enhancer also regulates commitment of pre-cDCs to the cDC2 lineage [7]. Conversely, cDC1 development is tightly regulated by IRF8, BATF3, ID2 and NFIL3 [6,7].

Considerable research effort has focused on the role of IRF8 in regulating the development of cDC1s across species (reviewed in [6]). However, the role of IRF8 in committed cDC1s is less

clear. To address this question, the recent study from Lança et al., utilized *Xcr1-cre.Irf8$^{fl/fl}$.R26R.EYFP* mice to selectively remove IRF8 from committed cDC1s, as XCR1 is only expressed in mature cDC1s and in pre-cDC1s that have left the BM and entered tissues [8]. The presence of the *R26R.EYFP* allele in these mice also allowed for any cells that had expressed *Cre* to be irreversibly labeled, allowing their fates to be mapped [8]. Consistent with previous studies, analysis of the spleen, mesenteric lymph node (MLN), and small intestine of these mice revealed a complete lack of XCR1$^+$ cDC1s. However, , a population of XCR1$^-$ YFP$^+$IRF8$^-$ cDCs in each of these tissues that was similar in absolute number to the XCR1$^+$YFP$^+$IRF8$^+$ cDC1s in the control animals was observed [8]. This was interesting as it suggested that loss of IRF8 might not lead to death of the committed cDC1s but rather, caused them to lose their identity. However, as some limited YFP expression was also observed in XCR1$^-$ cDCs in control mice [8], the authors next performed adoptive transfer studies of pre-cDC1s and pre-cDC2s sorted from the BM of FLT3L-treated *Xcr1-cre.R26R.EYFP* or *Xcr1-crexIrf8$^{fl/fl}$.R26R.EYFP* mice to investigate the origins of these XCR1$^-$YFP$^+$IRF8$^-$ cells. This analysis revealed that these XCR1$^-$YFP$^+$IRF8$^-$ cells arose specifically from pre-cDC1s, leading the authors to call these cells ex-cDC1s [8].

Lança and colleagues next set out to investigate the identity of the ex-cDC1s. Using a combination of RNA- and ATAC-sequencing, the authors elegantly demonstrated that ex-cDC1s shared considerable homology with cDC2s [8]. They expressed cDC2 markers such as SIRPα and CD11b, and a number of genes also expressed by *bona fide* cDC2s that are normally not expressed by cDC1s. The ex-cDC1s also downregulated a number of genes associated with cDC1s, including *Cadm1* and *Tlr3*. This was not just a change in gene expression but was also associated with a rearrangement of open and closed chromatin, whereby ex-cDC1s began to look more like cDC2s and less like cDC1s [8]. While the profiles of ex-cDC1s and cDC2s did not fully overlap, these studies highlighted that it was not just a loss of XCR1 expression that was observed upon deletion of IRF8, but rather, a process of reprogramming leading the cells to acquire a cDC2-like phenotype [8]. Given the reciprocal roles of IRF8 and IRF4 in cDC1 and cDC2 development and because IRF4 expression was increased in ex-cDC1s, the authors speculated that increased IRF4 expression might drive the conversion towards a cDC2-like phenotype. However, this possibility was excluded through the use of *Xcr1-cre.Irf8$^{fl/fl}$.Irf4$^{fl/fl}$* mice, in which ex-cDC1s developed a very similar phenotype as in the mice lacking only IRF8 from XCR1-expressing cells [8]. Alongside the transcriptomic and epigenetic differences, ex-cDC1s were also functionally distinct as they were less able to cross-present antigen or induce functional cytotoxic CD8$^+$ T lymphocytes than cDC1s. In contrast, they were as efficient as cDC2s at priming naïve CD4$^+$ T cells [8].

While the finding that committed cDC1s could lose their identity and become cDC2-like cells upon loss of IRF8 is consistent with findings from the Murphy laboratory, which found that pre-cDC1s could convert into a cDC2-like phenotype upon loss of IRF8 [5,9], it contrasts a previous study that suggested that mature cDC1s die upon loss of IRF8 [10]. This conclusion had been based on the use of an inducible *Cag-Cre*^{ert2}.*Irf8*$^{fl/fl}$ mouse system assessing mature cDC1s and cDC2s *in vitro,* and through the use of the late *Itgax-Cre.Irf8*$^{fl/fl}$ mice, which are specific for mature cDC1s *in vivo* [10]. Lança et al. correctly point out [8], that this prior study did not fate map the cDC1s in the *in vivo* model, and hence could not rule out that the cDC1s might have also converted into a cDC2-like cell [10]; however, the *in vitro* study in which cDC1s were first purified and then IRF8 loss induced [10] could argue for an alternative explanation whereby, mature cDC1s may not be sufficiently plastic to convert into a cDC2-like cell, and thus such conversion ability would be restricted to the pre-cDC1s targeted in this new study [8] (Figure 1). Indeed, some slight upregulation of SIRPα was observed in the *in vitro* study [10] suggesting that the mature cDC1s may have tried to switch to a cDC2-like phenotype, but failed to do so and hence died. Of course, a combination of both events (conversion and death) in the mature cDC1s might also be plausible, thus, further studies will be required to examine this using appropriate fate mapping tools combined with models that only delete IRF8 from mature cDC1s.

Taken together, this recent study by the Agace laboratory further demonstrates the importance of IRF8 expression in maintaining commitment of pre-cDC1s to the cDC1 lineage. [8] While additional studies are required to definitively ascertain if these commitment issues lie within the pre-cDC1s alone or can also be extended to mature cDC1s, this study highlights the need for robust fate mapping systems to track cells upon perturbation. Another outstanding question raised by this study is what the relevance of this pathway is in IRF8-suficient individuals. Do (pre-)cDC1s downregulate IRF8 to redirect towards a cDC2-like phenotype in conditions where more cDC2s are required? And is there a reciprocal pathway for cDC2s? The presence of a minor population of YFP^{+}XCR1^{-} cells in control mice could suggest that this might occur [8], although further validation is clearly required. If yes, it will be interesting to extend this study to determine if ex-cDC1s can fulfill any functions that are not performed by cDC1s or cDC2s. In this regard, an intriguing finding is that while ex-cDC1s seem to mimic the heterogeneity found in cDC2s in the spleen (ESAM$^{+/-}$) and small intestine (CD103$^{+/-}$), they seem to preferentially generate CD103^{+} cDC2-like cells in the small intestine, which could perhaps point towards a functional skewing of ex-cDC1s [8]. Thus, while the functions of IRF8 in cDC biology have been extensively studied, we can still learn more about this essential transcription factor.

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Declaration of Interests:

The authors declare no competing interests

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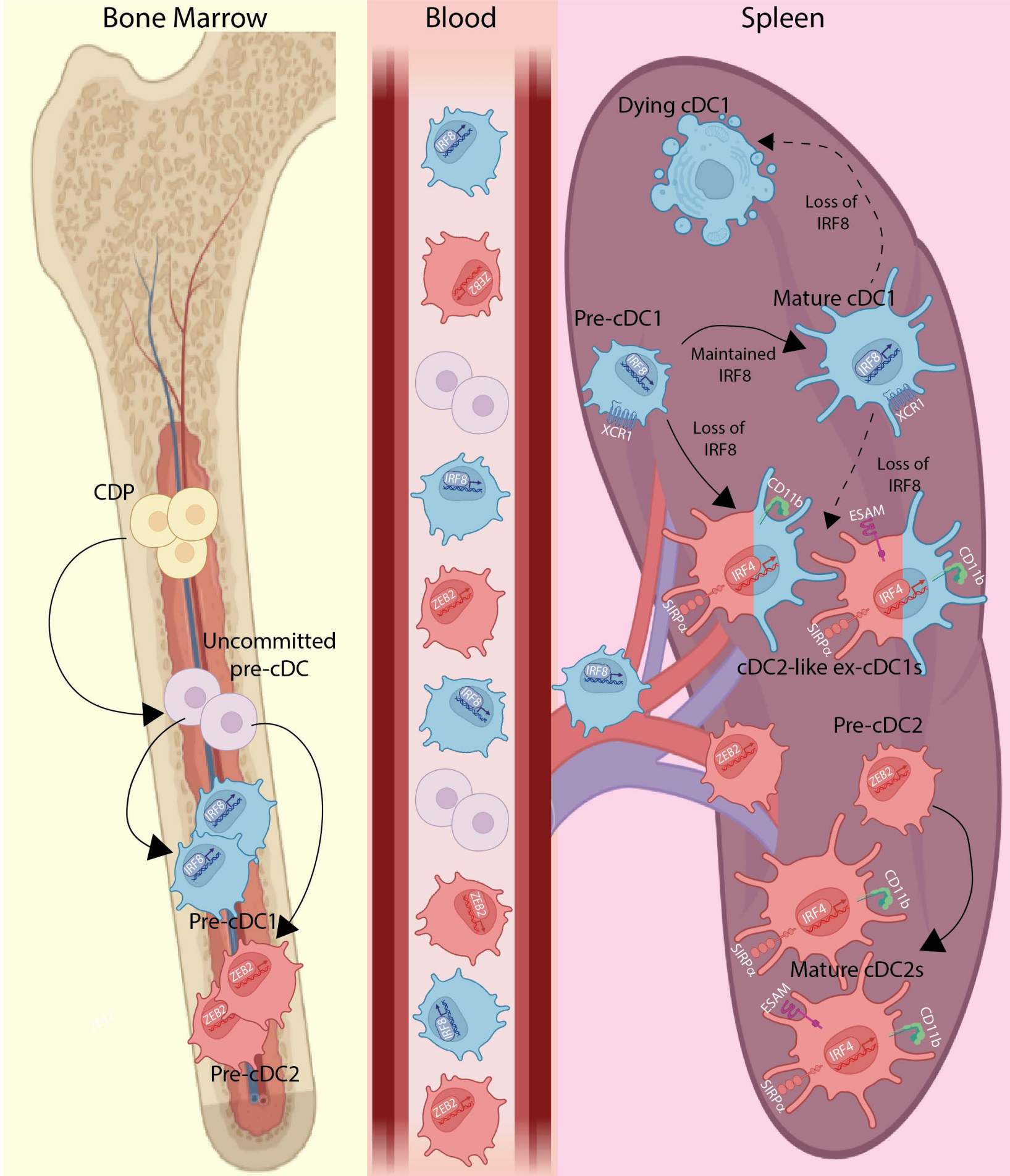
Figure 1: Loss of IRF8 from XCR1^{+} cells committed to the cDC1 lineage leads to reprogramming towards a cDC2-like cell in mice

Schematic representation of cDC1 and cDC2 development from common dendritic cell progenitors (CDPs) in bone marrow (BM) to mature cDC1s/cDC2s in tissues and the role of IRF8 in this process. CDPs give rise to uncommitted and committed pre-cDCs in the BM in a Flt3-Flt3L dependent manner, which is further regulated by a number of transcription factors [2,3]. Commitment towards the cDC1 lineage is regulated through IRF8 and BATF3 with further input from NFIL3, ID2, and TCF3/4 [5,6,9], while commitment towards the cDC2 lineage is regulated by ZEB2, IRF4, NOTCH2 and RELB [6,7]. These pre-cDCs exit the BM and enter the blood from which they can then enter peripheral tissues, including the spleen. In these tissues, pre-cDC1s acquire XCR1 expression before differentiating into mature cDC1s in an IRF8 dependent manner [8]. Loss of IRF8 from XCR1-expressing cells leads to a reprogramming of cells towards a cDC2-like phenotype [8]. Adoptive transfer experiments demonstrated this occurrence from the pre-cDC1 stage (solid line) but it remains unclear if loss of IRF8 from mature cDC1s might also lead to this reprogramming or to cell death (dotted lines) as previously proposed [10]. Alongside the development of pre-cDC1s into cDC1s, pre-cDC2s further develop into mature cDC2s, which can exist in ESAM^{+} and ESAM^{-} subtypes in the spleen [1]. ZEB2 is dispensable for this progression from pre-cDC2 to mature cDC2s [7]. While IRF4 is upregulated throughout cDC2 development, the precise role for this transcription factor in cDC2 development remains unclear [6]. Similarly, IRF4 is upregulated in ex-cDC1s; however, it is not required for their generation [8]. Figure was created with Biorender.com.


Bone Marrow
Blood
Spleen
CDP
Uncommitted pre-cDC
Pre-cDC1
Pre-cDC2
Dying cDC1
Loss of IRF8
Mature cDC1
Pre-cDC1
Maintained IRF8
Loss of IRF8
Loss of IRF8
CD11b
ESAM
CD11b
cDC2-like ex-cDC1s
Pre-cDC2
CD11b
Mature cDC2s
ESAM
CD11b
IRF8
ZEB2
IRF4
XCR1
SIRPα