Insights from Patients with Dendritic Cell Immunodeficiency

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Highlights

- Human dendritic cell immunodeficiency offers a unique opportunity to dissect the \textit{in vivo} function of dendritic cells
- Developmental pathways have been mapped in patients with dendritic cell deficiencies
- Heterozygous mutations in immune-related super-enhancer-regulated transcription factors result in clinical phenotypes

Abstract

Dendritic Cells (DCs), derived from haematopoietic stem cells, are critical to the dynamic and balanced functioning of the intact immune system and are of great interest as vehicles of immunotherapy. Genetically modified mouse models have proved powerful tools to map DC development and function \textit{in vivo} but human studies have previously relied heavily on \textit{in vitro} systems. Human dendritic cell immunodeficiency, resulting from single gene mutations, offers new opportunities to dissect the role of human DCs \textit{in vivo}, determine the genetic requirements for their development and map their haematopoietic differentiation pathways. This review will summarise the clinical phenotypes of mutations in \textit{GATA2}, \textit{IRF8} and \textit{IKZF1} genes which result in global or subset specific dendritic cell deficiencies, discuss the functional consequences of these cytopenias and how these syndromes have informed our knowledge of DC differentiation and human haematopoiesis.
Introduction

Dendritic cells (DCs) are the most potent antigen presenting cell (APC), able to activate and polarise naïve T cells to shape immunogenic or tolerogenic responses. Present in lymphoid and nearly all peripheral tissues they initiate and regulate adaptive immune responses and participate in innate immunity through their elaboration of cytokines. Polarity of response is determined by recognition of pathogen-associated molecular patterns (PAMPs), including Toll-like receptors (TLRs), NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs), while plasticity is mediated by phenotypic and functional heterogeneity, tissue-specific population architecture and microenvironmental cues (Alcántara-Hernández et al., 2017; Granot et al., 2017). The latter is particularly pertinent in disease when DC function may be modulated by inflammatory cytokines and chemokines from stromal or immune cells, or suppressed by tumour-related factors (Blanco et al., 2008; Motta and Rumjanek, 2016; Wculek et al., 2020).

In human, primary immunodeficiency, single gene germline mutations resulting in an immunodeficient or autoinflammatory phenotype, offer a unique opportunity to dissect the contribution of cell lineages or genes to immune function in vivo. A number of genetic mutations have been associated with defects in DC number, function or both. This review will focus on genetic disorders affecting DC development and the insights gained from the study of these rare patient groups.

DC heterogeneity

Phenotypic heterogeneity within the DC population has been classified by cross-species gene expression studies, linked to surface antigen expression and refined by anatomical location (Guilliams et al., 2014). Together these define plasmacytoid DC (pDC) and two subsets of myeloid or conventional/classical DC (cDC).

In humans, pDC express CD123, CD303 and CD304 but are negative for cDC markers CD11c and CD33. They produce large quantities of type I interferon, are able to prime CD4 T cells and induce Tregs through expression of inducible T cell costimulatory ligand (ICOS-L) and IDO (Ito et al., 2007; Chen et al., 2008). They also support humoral immunity, including B cell activation and proliferation, class-switching and immunoglobulin secretion and, in mouse, play an important role in the response to respiratory pathogens (Dubois et al., 1999; Crother et al., 2012). CD33-AXL-Siglec6+ cells, termed AS DC or pre-DC, were recently identified within the CD123-CD303/4- population (Villani et al., 2017; See et al., 2017). These cells showed little type I interferon elaboration, were able to upregulate cDC2 markers, and readily induced proliferation of naïve CD4 T cells in vitro, delineating them from the CD33-CD123-pDC population and necessitating re-evaluation of pDC function.

cDC1 are characterized by high expression of CD141, Clec9A, XCR1 and BTLA but lower CD11c. cDC1 are considered the most potent subset for antigen cross-presentation which, together with elaboration of IL-12, equips them for defense against intracellular pathogens, driving Th1 T cell polarization and NK cell activation as part of the IL-12/IFNγ circuit (Schreibelt et al., 2012; Széles et al., 2015; Gutiérrez-Martínez et al., 2015; Bachem et al., 2010). cDC1 also promote tolerance, through their high levels of IDO expression, ability to secrete TGFβ and induce peripheral Tregs (Yamazaki et al., 2008; Jones et al., 2016).
cDC2 have high levels of CD11c, CD1c, CD2, FCER1A and SIRPA. Functional plasticity within the human cDC2 compartment is observed, reflected in their ability to express a large repertoire of TLRs, elaborate many cytokines including IL-12 (unlike mouse cDC2), and to cross-present antigen when appropriately activated (Hemont et al., 2013; Schlitzer et al., 2013). Additional transcriptomic and phenotypic heterogeneity has recently been shown within the cDC2 compartment (Yin et al., 2017; Alcántara-Hernández et al., 2017; Korenfeld et al., 2017; Villani et al., 2017). At either end of a phenotypic continuum are found cells enriched for lymphoid antigens, CD5 and BTLA, or cells which express monocyte-related antigens, CD163, CD11b and CD14, termed DC2 and DC3, respectively (Villani et al., 2017; Dutertre et al., 2019). The developmental origin and functional consequences of this heterogeneity are beginning to be unravelling (Dutertre et al., 2019; Cytlak, 2019).

**DC Development**

DCs are derived from bone marrow haematopoietic stem cells (HSCs) independently of monocytes and macrophages, under the control of specific transcription factors (TFs). Unlike the peripheral diversification of T cell populations, DC heterogeneity is derived through lineage diversification during haematopoiesis, driven by differential TF requirement. The TFs governing subset differentiation were originally mapped largely through murine models of TF deficiency. These have revealed the dependence of pDC on Irf8, Tcf4 (E2-2) and Zeb2 while cDC1 require Irf8, Id2 and Batf3 (Cisse et al., 2008; Hacker et al., 2003; Sichien et al., 2016; Scott et al., 2016; Jaiswal et al., 2013; Grajales-Reyes et al., 2015; Ginhoux et al., 2009). Lineage specification of cDC2 requires Irf4 and Zeb2 (Scott et al., 2016; Schlitzer et al., 2013). Heterogeneity within this compartment in mouse is influenced by TF requirement including Klf4 and notch signalling and by tissue site. There is increasing recognition that the relative and absolute levels of key TF are important; development of pDC requires the suppression of Id2 and the terminal differentiation of cDC1 requires high Irf8 levels, maintained by Batf3-dependent autoactivation of Irf8 (Scott et al., 2016; Grajales-Reyes et al., 2015). In keeping with this, many TF critical in determining cell identity are regulated by super-enhancer regions for more interactive and dynamic control of transcription (Whyte et al., 2013) and haploinsufficiency is associated with a demonstrable phenotype.

Based on the traditional haematopoietic model of sequential dichotomous fate decisions, a pathway of murine DC development was described from common myeloid progenitors (CMP) through granulocyte macrophage progenitors (GMP) and macrophage and DC precursors (MDP), which gave rise to common monocyte (CMoP) and common dendritic cell (CDP) progenitors. pDC and cDC precursors arose from CDP before cDC precursors gave rise to cDC1 and cDC2 in tissues. A similar pathway has been described in human through CMP, GMP and a CD34.CD123+. CDP (Lee et al., 2015). However, these models fail to take into account the observation that DCs can also be derived from common lymphocyte progenitors (CLPs) or lymphoid primed multipotent progenitors (LMPP) (Manz et al., 2001; Doulatov et al., 2010; Helft et al., 2017).

In keeping with contemporary models of haematopoiesis in which there is early lineage priming of progenitors (Paul et al., 2015; Velten et al., 2017), heterogeneity within the phenotypically defined DC progenitor populations has been revealed by single cell culture or transcriptomic studies. In particular, a murine cDC-specific precursor has been identified within the CDP (Schlitzer et al., 2015) and, more
proximally, *in vivo* cellular barcoding revealed stable DC lineage priming within the LMPP (Naik et al., 2013). Importantly, this model defines a lympho-myeloid pathway which separates from megakaryocyte and erythroid potential at the apex of the haematopoietic hierarchy, explaining the apparent ‘dual origin’ of DC potential from both lymphoid and myeloid progenitor fractions (Notta et al., 2016; Karamitros et al., 2018; Collin and Bigley, 2018).

In human, a CD123+CD34+ CDP was recently identified (Lee et al., 2015), comprised of cells with predominantly single DC lineage specification in single cell culture experiments (Lee et al., 2017). Subset specific precursors of cDC1 and cDC2 have also been identified in blood (Breton et al., 2015; See et al., 2017). In summary, although the populations known as GMP, MPD, CDP and pre-DC are now known to contain heterogenous populations of lineage-primed cells, the phenotypes of these populations remain useful to define compartments of haematopoiesis within the DC developmental pathway.

**Macrophage origin**

It is now well established that many populations of long-lived tissue macrophages arise and persist from fetal haematopoiesis (Schulz et al., 2012; Hoeffel et al., 2015), although replacement by monocyte-derived cells is recognised in mouse skin, gut and lung (Ginhoux and Guilliams, 2016). Some evidence for independent homeostasis of human macrophages and Langerhans cells comes from the persistence of recipient cells following allogeneic bone marrow or limb transplantation, respectively (Haniffa et al., 2009; Kanitakis et al., 2011) but much remains to be learnt about the regulation and homeostasis of human macrophage populations in the unperturbed state.

**DC immunodeficiency**

Absolute monocyte and DC deficiency has been described in the context of germline heterozygous *GATA-binding factor 2 (GATA2)* and bi-allelic *Interferon Regulatory Factor 8 (IRF8)* mutations (Bigley and Collin, 2011; Dickinson et al., 2011; Bigley et al., 2017; Hambleton et al., 2011). More subtle DC subset-specific deficiencies are associated with *IKAROS Family Zinc Finger 1 (IKZF1 or IKAROS)* and *IRF8* haploinsufficiency (Cytłak et al., 2018; Cytłak, 2019).

**Germline heterozygous GATA2 mutations**

Within the haematopoietic compartment GATA2, a member of the GATA-binding TF family, is necessary for the survival and self-renewal of HSC and for lympho-myeloid lineage specification. Homozygous GATA2 knockout is embryonically lethal when definitive haematopoiesis fails (Tsai et al., 1994) but heterozygous insufficiency in mice results in a quantitative reduction of HSC and progenitors (Rodrigues et al., 2005; Eich et al., 2018). In humans, germline haploinsufficiency has been described in a number of syndromes including Emberger's (Ostergaard et al., 2011), autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) (Hsu et al., 2011) and Dendritic Cell, Monocyte, B and NK Lymphoid (DCML) deficiency (Dickinson et al., 2014). In common, these syndromes encompass myelodysplasia with progression to AML, including the entity of ‘familial MDS/AML’ (Hahn et al., 2011). Prior to the development of overt MDS/AML, there is attrition of the mononuclear cell compartment in association with increasing levels of serum Flt3 Ligand and a resultant immunodeficiency, which may last decades. During this time,
although the presence of peripherally expanded memory lymphocytes and immunoglobulin provides some defense against previously encountered pathogens, there is a critical inability to mount an adaptive immune response to novel antigens with an associated risk of lethal infection. Additional risks during this time of dendritic cell, monocyte, B and NK cytopenias include autoimmunity (hepatitis, erythema nodosum), pulmonary alveolar proteinosis and cancer, particularly HPV/EBV-driven malignancies (Dickinson et al., 2014; Collin et al., 2015; Spinner et al., 2014).

**Germline Bi-allelic IRF8 mutations**

IRF8, a member of the Interferon Regulatory Factor Family, plays a multifaceted role in immunity, interacting with hematopoietic TFs and immune signalling molecules to direct cell development and mature cell responses, respectively. The specificity and plasticity of its functions are generated through multiple heterodimerization partners which determine its binding to differential DNA motifs where it may activate or repress gene expression. Within the granulocyte macrophage progenitor (GMP) compartment, IRF8 competes with CEBPA to determine neutrophil versus monocyte fate (Kurotaki et al., 2014; Kurotaki et al., 2013) and is necessary for the development of DCs (Tamura et al., 2015). *Irf8*−/− mice lack DCs and monocytes but develop a massively expanded neutrophil population and progress to fatal myeloblastic leukaemia (Holtschke et al., 1996). The BXH2 mouse, carrying homozygous hypomorphic *Irf8R294C* mutation in the Irf-associated domain (IAD), shows a selective loss of cDC1 and functional abnormalities of monocytes (Hu and Ivashkiv, 2009; Marquis et al., 2009). More recent models targeting IRF8 depletion to specific DC developmental stages demonstrated the requirement for IRF8 in terminal differentiation of cDC1 and the function of pDC (Sichien et al., 2016).

Two humans with bi-allelic IRF8 mutations have been described, both presenting in infancy with infections, loss of all monocytes and DCs, marked neutrophilia and developmental delay associated with intracerebral calcification. The infant carrying homozygous IRF8*K108E* mutations received neonatal BCG vaccination and presented with BCG-osis. The patient with compound heterozygous IRF8*R291Q* (homologous to BXH2 mouse *Irf8R294C*) and IRF8*R83C* mutations presented with recurrent respiratory virus infections from the age of 7 weeks. Both required Hematopoietic Stem Cell Transplantation at the ages of 4 months and 4 years, respectively. Within the lymphocyte compartment there was a failure of T cells to elaborate Th1 or Th17 cytokines, a reduction in CD8+ T cell maturation and reduced B cell class switching with low IgA (Bigley et al., 2018). Reduced frequency and complexity of somatic hypermutation in B cells was consistent with the requirement of IRF8 for the germinal center reaction (Recaldin and Fear, 2016; Shukla and Lu, 2014).

**Germline Heterozygous IRF8 mutations**

More subtle cellular and clinical phenotypes are observed in the context of heterozygous IRF8 mutations. Given the multiplexed interactions and functions of IRF8, it is highly likely that distinct genotype-phenotype associations will emerge as heterozygous variants are identified in different domains of the gene. Unifying features associated with published heterozygous mutations, including the parents of the bi-allelic IRF8 mutation carriers, IRF8*K108E*, IRF8*R291Q* and IRF8*R83C* encompass depletion of cDC1 and a predisposition to infection with intracellular organisms (Hambleton et al., 2011; Mace et al., 2017; Cytlak et al., 2018). IRF8 variants localized to the IAD domain (*IRF8*P224L and *IRF8*A201V) result in subtle effects on
cDC1 and pDC but more pronounced effects on NK cell maturation and function (Mace et al., 2013). With state of the art phenotyping, repeat analysis of IRF8T80A, originally reported to show preservation of cDC1 but loss of CD1c+cDC2, has confirmed preservation of cDC2 and depletion of cDC1 (Kong et al., 2018), aligning the DC phenotype in all cases carrying heterozygous IRF8 mutations.

**Germline IKZF1 Mutations**

IKZF1, a zinc finger TF and founding member of the IKAROS gene family, is expressed globally in haematopoiesis (Molnar and Georgopoulos, 1994), with prominent roles in lymphocyte development and proliferative responses (Merkenschlager, 2010). In the context of IKZF1 deficiency, a gene dose-dependent effect on DC development is observed in mouse, whereby the dominant negative DNA binding domain mutant (Ikzf1DN/DN) lacks all DCs, the null allele (Ikzf1CC) prevents formation of pDC and cDC2 with preservation of some cDC1, but the hypomorph mutation (Ikzf1LL) results in specific defect of pDC with preservation of cDC1 and cDC2. In keeping with this, analysis of humans with IKZF1 haploinsufficiency, alongside a progressive loss of B cells and development of hypogammaglobulinaemia (Kuehn et al., 2016; Bogaert et al., 2017), show an invariant reduction in pDC with preservation or expansion of cDC1 (Cytlak et al., 2018). These patients show an increased susceptibility to bacterial sinopulmonary infections and autoimmunity. More recently, a cohort with dominant negative IKZF1N159 mutations, located in the DNA binding domain of the gene, have been reported. These were associated with a T, B and myeloid cell combined immunodeficiency (Boutboul et al., 2018) with susceptibility to Pneumocystis jirovecii and other bacterial and viral infections. In two patients analysed, a reduced proportion of CD11c- cells among lineage-negative HLA-DR+ CD14- CD16- non-monocytic cells was observed. The precise phenotype of these and the CD303+ cells identified remains to be determined.

**Insights from DC deficiency**

The relatively small numbers of patients identified with DC immunodeficiency thus far relates to a lack of routine immunology/haematological testing, DC being invisible by light scatter properties in automated blood counting and too rare or unrecognisable to enumerate by manual counting. Although classical, but not non-classical, monocytes can be identified by both techniques, monocytopenia may be masked by the appearance of immature myeloid cells which frequently fall in the monocyte gate of automated counters. Flow cytometry represents a powerful tool for enumeration and phenotyping of cells from small volumes of blood, although more complex antigen panels are required to cover the increasing heterogeneity being unmasked by single cell analysis techniques within the monocyte and DC populations (Villani et al., 2017; Dutertre et al., 2019; Cytlak, 2019).

Those patients that are known, thoroughly demonstrate that PID offers a unique opportunity to dissect the important non-redundant functions of DC in vivo, unpick their developmental pathways, understand the role of immune genes in the intact human and the genotype-phenotype effects of mutations.
DC function in vivo
Deficiency of monocytes and cDCs results in disruption of the IL-12/IFNγ axis at a cellular level. In keeping with this, both DCML deficiency in GATA2 mutation and loss of monocytes and DCs in bi-allelic IRF8 mutation are associated with a predisposition to infection with tuberculous and non-tuberculous mycobacteria (Bigley and Collin, 2011; Hsu et al., 2011; Hambleton et al., 2011). In the context of heterozygous IRF8 mutations, cDC1 deficiency coupled with reduced IL-12 elaboration by cDCs appears sufficient for increased susceptibility to these pathogens (Hambleton et al., 2011; Cytlak, 2019).

Cell intrinsic effects of IRF8 deficiency are seen in murine Irf8−/− T cells including defective integration of γ-chain and T cell receptor signalling pathways, secretion of IFNγ and cytotoxicity (Miyagawa et al., 2012). However, in Itgax conditional Irf8 knockouts, the observed T cell dysfunction was attributed to cell extrinsic mechanisms due to loss of cDC1 (Luda et al., 2016). In human IRF8 mutation, reduced Th1 and Th17 polarisation and defective CD8+ effector memory differentiation would at least in part be explained by compromised T cell receptor signalling strength in the absence of DCs.

In the B cell compartment, lineage-specific knockout in mice demonstrates that IRF8 is required at several stages of B-cell development with similar effects seen in the context of human bi-allelic mutations where there was reduction in the extent and complexity of somatic hypermutations and restricted VH gene repertoire (Bigley et al., 2018). A cell extrinsic effect of DC deficiency may be reflected in the defective IgA class switching which has been reported to require DC-B cell interactions in Peyer patches (Reboldi et al., 2016).

Defects in NK cell maturation and function, reported in bi-allelic IRF8 mutations, may have both cell intrinsic and extrinsic mechanisms, where reciprocal cross talk between NK cells and DCs has been shown to be critical in the regulation of innate immune responses (Harizi, 2013; Mace et al., 2017; Bigley et al., 2018).

pDC deficiency in IKZF1 haploinsufficiency, may contribute to the increased risk of bacterial respiratory infection, commensurate with the role of pDC in prompt bacterial clearance and limitation of inflammation in the lung. Lack of pDC support for B cell function and humoral immunity may exacerbate the B cell attrition and progressive hypogammaglobulinemia, despite the presence of plasma cells in tissues. Reduction of the tolerogenic influence of pDC may permit the development of autoimmunity.

Human DC development
Analysis of the bone marrow progenitor and precursor compartments in DC immunodeficiency has been critical in establishing the TF requirement for human DC development and subset specification, and the stage of differentiation at which a TF becomes non-redundant. In the context of specific DC subset deficiencies, identifying missing progenitor or precursor components, in comparison with healthy controls, has allowed the mapping of differentiation pathways (Figure 1A).

GATA2 mutation results not only in reduction of HSC but also in an early block in mononuclear cell differentiation, distal to the loss of megakaryocyte and erythroid potential, resulting in near complete depletion of LMPP and specific segments of
GMP, leaving a small segment of CD123low cells sufficient to to sustain neutrophil generation (Figure 1B). Bi-allelic \textit{IRF8} mutation results in a later block of DC and monocyte differentiation within the GMP compartment, downstream of lymphocyte fate decisions (Figure 1C). Heterozygous null or hypomorphic \textit{IRF8} mutations affect distal, subset-specific DC development, consistently in the cDC1 pathway. pDC may be deficient or numerically unaffected but in both cases show an abnormal phenotype with increased proportions of CD2-pDC which may represent failure of terminal differentiation (Cytlak, 2019) (Figure 1C).

Most recently, analysis of the progenitor and precursor compartments in an allelic series of \textit{IRF8} mutations, including a kindred with a dominant negative mutation, allowed the dissection of cDC2 lineage specification: cDC2 expressing lymphocyte antigens CD5 and BTLA require a high level of IRF8 expression during development, in parallel with cDC1 and pDC, and are reduced in mono-allelic mutations. Monocyte-related cDC2 monocyte antigens such as CD14 and CD163 require lower levels of IRF8 for their differentiation and are only depleted when both alleles of IRF8 are defective (Cytlak, 2019).

Human LC and Macrophage homeostasis
The observation of preserved LCs and tissue macrophages in the context of cytopenias due to \textit{GATA2} or bi-allelic \textit{IRF8} mutations demonstrates, not only their independence from this transcription factor but also their ability to survive in the absence of circulating monocyte precursors. Preservation of tissue macrophages in patients presenting with \textit{GATA2} mutation may reflect either the longevity or self-renewal capacity of these cells, or both. It is not possible to conclude that they had a fetal origin from observations in \textit{GATA2} mutation, as circulating monocytes were likely present in early childhood, prior to the development of the cellular deficiency. However, no evidence of circulating definitive haematopoietic monocytes was found in young infants presenting with bi-allelic \textit{IRF8} mutations, in keeping with the derivation of their preserved tissue macrophages from fetal precursors. The contribution of macrophage dysfunction to the phenotypes of \textit{GATA2} and bi-allelic \textit{IRF8} mutations remains to be determined. Patients with pulmonary alveolar proteinosis do have alveolar macrophages but their function is presumably impaired. In particular, further investigation is required to determine whether the developmental delay and intracerebral calcification seen in patients bearing \textit{IRF8K108E/K108E} and \textit{IRF8R83C/R291Q} is related to defective microglial development and function, as reported in mouse (Kierdorf et al., 2013; Masuda et al., 2012; Masuda et al., 2014), or to defective interferon responses.

Transcription Factor mutations in immunodeficiency
It is unsurprising that complete loss of a critical TF will result in a disease phenotype. What is strikingly revealed by patients with \textit{IRF8} mutation is the complexity of IRF8 function, resulting in multi-lineage and multi-functional deficits. In addition both \textit{IRF8} and \textit{GATA2} cause detectable phenotypic anomalies in the heterozygous or haplo-insufficient state. This association of monogenic heterozygous mutations with disease is consistent with the mutation of TFs controlled by super-enhancer (SE) regions (Afzali et al., 2017). Indeed \textit{GATA2} is regulated by a super-enhancer region at -110kb from the transcription start site and the murine \textit{IRF8} locus has a SE structure with different elements active in cDC1 and pDC lineages, consistent with the requirement for precise control of IRF8 levels to determine DC lineage specification (Gröschel et al.,
2014; Grajales-Reyes et al., 2015; Ginhoux et al., 2009; Lee et al., 2017). IKZF1 is critical for the activity of super-enhancers at genes required for pre-B cell receptor signalling and differentiation, working with B cell master regulators including EBF1 and PAX5 (Hu et al., 2017). It is now recognised that SE structures identify TF with strict transcriptional regulation that may confer an autosomal dominant pattern of inheritance in the heterozygous or haploinsufficient state, including many TF mutations associated with PID (Afzali et al., 2017).

Finally, it is intriguing to observe that TF functions within the immune system often control both the early development of specific lineages and their function as mature cells. Evolutionarily, this may have been driven by the selection of ‘immune modules’ to combat specific pathogenic challenges; IRF8 is critical for the differentiation and function of cell components necessary for defence against intracellular pathogens while IKZF1 strongly supports both developmental and functional pathways required for robust humoral immunity. The continual generation of DC by haematopoiesis, and their complex patterns of maturation, provide many examples of the dual roles of key immune-related TF.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutations Described</th>
<th>Cellular phenotype</th>
<th>Clinical Phenotype</th>
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<tr>
<td>Heterozygous GATA2</td>
<td>Many, concentrated in exons 3, 4, 5 and zinc finger domains. Splice site and enhancer mutations and gene deletions also described (Collin 2015)</td>
<td>Progressive DC, monocyte, B and NK lymphocyte (DCML) deficiency</td>
<td>Susceptible to infections, HPV warts, viral-driven malignancies, autoimmunity, MDS/AML, primary lymphodema, sensori-neural deafness</td>
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<tr>
<td>Bi-allelic IRF8</td>
<td>K108E/K108E R83C/R291Q (Hambleton 2011; Bigley 2018)</td>
<td>Complex immunodeficiency with myeloproliferation, absence of all monocytes and DCs, B and T cell defects</td>
<td>Susceptibility to viral and intracellular infections (including BCG). Required bone marrow transplant in infancy/early childhood</td>
</tr>
<tr>
<td>Heterozygous IRF8</td>
<td>K108E, R83C, R291Q, T80A, P224L, A201V (Bigley 2018; Hambleton 2011; Mace 2017)</td>
<td>cDC1 and often pDC deficiency. Mild-moderate CD14+ monocytosis. NK cell defects with increased CD56bright NKs</td>
<td>Possible susceptibility to EBV and intracellular organisms including mycobacteria</td>
</tr>
<tr>
<td>Heterozygous IKZF1</td>
<td>S46Afs, R162L, R162Q, H167R, R184Q, deletions in chrom 7 (Bogaert 2017; Kuehn 2016)</td>
<td>Progressive hypogammaglobulin-aemia, attrition of B cells, pDC deficiency with normal or expanded cDC1</td>
<td>Susceptibility to sinopulmonary infections and possibly B-cell ALL</td>
</tr>
<tr>
<td>Dominant Negative IKZF1</td>
<td>N159S, N159T (Boutboul 2018)</td>
<td>T, B and myeloid cell combined immunodeficiency. Reduced proportion of HLA-DR+CD14, CD16, CD11c+ cells</td>
<td>Susceptibility to <em>Pneumocystis jirovecii</em> and other bacterial and viral pathogens</td>
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HPV, Human Papilloma Virus; MDS, myelodysplastic syndrome; AML, acute myeloid leukaemia; BCG, Bacillus Calmette-Guerin; EBV, Epstein Barr Virus; ALL, acute lymphoblastic leukaemia
Figure 1: Consequences of *GATA2* and *IRF8* mutations on DC developmental pathways

A. The revised model of haematopoiesis encompasses early lineage priming so that phenotypically similar populations of progenitors are composed of cells biased towards single lineage potentials. In this model, lymphoid and myeloid potential run together originating as the lymphoid primed multi-potent progenitor (LMPP) that separates from megakaryocyte and erythroid potential (MkE) at the apex. Thus the gates defined by CD38 (blue borders) and CD45RA (red borders) contain phenotypically related cells but with restricted potentials, indicated by bands of colour each corresponding to a discrete lineage. From LMPP arise multi-lymphoid and BNK progenitors, and a CD38+CD45RA+ ‘Granulocyte Macrophage Progenitor’ within which significant heterogeneity in cell phenotype, transcriptome and potential is identified. pDC, cDC1 and CD5+cDC2 (DC2) arise from an IRF8-expressing CD123+GMP while CD163+cDC2 (DC3) and monocyte potential is restricted to an IRF8-low CD33+GMP population (Cytlok, 2019). Dark red and turquoise shading indicate the cell developmental requirement for GATA2 or IRF8, respectively, with colour intensity representing expression level.

B. Heterozygous *GATA2* mutation resulted in attrition of mononuclear cells, loss of LMPP and reduction of GMP, within the progenitor compartment. Granulo-, megakaryo- and erythro-poiesis were relatively preserved until the onset of acute myeloid leukaemia. Peripheral T cell populations were maintained through proliferative expansion and showed a largely terminally differentiated phenotype.

C. Bi-allelic *IRF8* mutations resulted in a selective depletion of Monocytes and all DC subsets with a reciprocal expansion of neutrophils. Development of erythrocytes, megakaryocytes, lymphocytes and granulocytes was preserved.

D. Heterozygous *IRF8* mutation caused a more selective DC deficiency of lineages dependent on high IRF8 for development, with depletion of cDC1, reduction in CD5+DC2 and increased proportion of CD2+pDC which may represent failure of terminal differentiation of the pDC lineage. DC3 and monocytes, reliant on a low level of IRF8 for development, were relatively or absolutely expanded.
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References


