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Impaired immune responses to herpesviruses and microbial ligands in patients with MonoMAC

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Summary
MonoMAC is a complex primary immunodeficiency caused by mutations in the myeloid transcription factor GATA2, characterized by multilineage cytopenia with malignant complications and severe infections, including mycobacteria and herpesviruses. We describe the clinical presentation, genetics and antiviral inflammatory responses in a small case series. Two patients presented in childhood with mycobacterial infection and were diagnosed with MonoMAC germline GATA2 variants; their fathers were also studied. Three patients were elderly individuals with acquired GATA2 mutations and malignant haematological conditions. Overall, this study demonstrates the heterogeneous clinical presentation and variation in immunodeficiency caused by GATA2 mutations.

Short Report
MonoMAC, also known as immunodeficiency 21 disease, is a complex primary immunodeficiency of progressive multilineage cytopenia characterized by a heterogeneous clinical presentation, incomplete penetrance and both infectious and malignant complications. The infectious phenotype includes dissemination of non-tuberculous mycobacteria, such as Mycobacterium avium complex (MAC), infections with viruses, such
as human papilloma virus (HPV), herpes simplex virus (HSV), varicella zoster virus (VZV) and Epstein-Barr virus (EBV). Whereas the infectious phenotype dominates in childhood and early adulthood, a significant fraction of patients develops myelodysplastic syndrome (MDS) with progression to acute myeloid leukaemia (AML) during the 3th or 4th decade of life (Calvo et al. 2011; Hsu et al. 2011; Spinner et al. 2013; Kazenwadel et al. 2012; Camargo et al. 2013; Dickinson et al. 2014). The immunological phenotype associated with MonoMAC is characterized by a lack of dendritic cells (DCs), profound monocytopenia, and decreased numbers of natural killer (NK) and B cells. MonoMAC originates from mutations in the myeloid transcription factor GATA2, which is pivotal for the establishment of proper gene expression during haematopoietic stem cell and progenitor cell differentiation in development and throughout adulthood. Although the disease-causing potential of variants in GATA2 is well-established, many aspects concerning the clinical penetrance, pathogenesis and basic Immunology of this disease entity remain to be clarified.

We identified a small group of patients with germline GATA2 variants and an infectious phenotype diagnosed as MonoMAC (P1 and P2), patients with somatic GATA2 mutations and haematological malignancy progressing to AML (P3-P5), as well as the healthy fathers of P1 and P2, respectively, with germline variants in GATA2 but without cytopenias, infection or malignancy (P6 and P7). Briefly P1, a 12-year-old boy, and P2, a 22-year-old woman, suffered from atypical mycobacterial infection in the lymph nodes with *Mycobacterium abscessus* and *Mycobacterium fortuitum*, respectively. Moreover, P2 suffered from extensive warts and severe vulval dysplasia, both presumably HPV-associated. Whereas P1 had hypoplastic bone marrow with marginally dysplastic signs, P2 had MDS and underwent bone marrow transplantation. P3-P5 were diagnosed with AML but did not report any notable infection history. For detailed infectious history and clinical immunological evaluation of P1-P5 see Table S1 and Data S1. Data S2 details the Materials and Methods.

GATA2 contains two Zinc Finger (ZnF) DNA binding domains, through which it interacts with DNA, other GATA family members and other transcription factors (Rodrigues et al. 2012). In patients with MonoMAC (P1 and P2) or AML (P3-P5) whole exome sequencing or gene array sequencing revealed five unique mutations in GATA2 with all mutations being localized within the conserved ZnF DNA binding regions (Figure 1A, Table SII). These findings agree with the literature, showing that the vast majority of GATA2 mutations are within these ZnF domains, although a minor fraction of patients may harbour noncoding variants within intron 4 affecting the +9.5kb regulatory elements of the GATA2 gene (Mir et al. 2015; Chong...
et al. 2018). The GATA2 mutation in P1 (MonoMAC) and mutations in P3 and P4 (AML) were located in the N-terminal ZnF DNA binding domain, whereas the mutations in P2 (MonoMAC) and P5 (AML) were located in the C-terminal ZnF DNA binding domain. P1 had a 7-nt deletion leading to a frameshift and premature stop codon, most likely associated with non-sense mediated decay of the resulting transcript. P2 had a missense mutation, R398W, previously reported in other MonoMAC patients and suggested to disrupt the binding of GATA2 to DNA (Spinner et al. 2013; Dickinson et al. 2014). The mutation in P3, N297S, was listed as a “possible oncogenic variant” in a large study on driver mutations in myelodysplastic syndromes (Papaemmanuil et al. 2016), and the R307W variant harboured by P4 was previously reported as a somatic mutation in early T-cell precursor acute lymphoblastic leukaemia and AML (Green et al. 2013). P5 had a missense GATA2 L359V mutation, which has been described as a gain-of-function mutation disturbing myelopoiesis though interference with PU.1, also termed SPI1 (Zhang et al. 2008). (Figure 1A, Table SII).

Due to the increased viral burden and predisposition to atypical mycobacterial infection associated with MonoMAC, we wished to examine inflammatory responses to pattern-associated molecular patterns (PAMPs) relevant for these pathogens as well as antiviral responses to herpesviruses. First, we performed immune gene expression analyses on patient peripheral blood mononuclear cells (PBMCs) stimulated with agonists of TLR2, TLR4, TLR9 and cytosolic DNA sensors. Our MonoMAC patients (P1 and P2) exhibited significantly impaired, in many cases almost abolished, antiviral and proinflammatory responses to these synthetic PAMPs compared to controls as well as P6 and P7 (healthy individuals with GATA2 variants), when measuring antiviral IFNB1, the IFN-stimulated gene CXCL10, TNF, IL6, and IFNG at the mRNA level (Figure 1 B-F) as well as at the protein level (Figure S1). Next, patient and control PBMCs were infected with HSV-1 for 6 h or co-cultured with VZV-infected MeWo cells for 48 h prior to harvest of RNA and gene expression analysis. In PBMCs from both P1 and P2, the antiviral and proinflammatory responses to HSV-1 were almost completely abolished compared to controls as well as P6 and P7 when measuring IFNB1, CXCL10, TNF, IL6, and IFNG (Figure 2A-E). The same striking cellular phenotype was found when measuring antiviral responses to VZV, in which case also IFNB1 and CXCL10 were almost absent in MonoMAC patients P1 and P2 (Figure 2F, G). These findings were supported by measurements of IFNα, IFNβ, and IFNλ protein levels in supernatants from VZV-infected PBMCs, demonstrating reduced antiviral responses in P2 as opposed to controls (Figure S2). Furthermore, T-cell responses, measured as IFNγ production in response to anti-CD3/antiCD28 stimulation, were significantly impaired in P1 and P2,
demonstrating not only impaired innate responses but also abnormal adaptive T-cell immunity in MonoMAC patients (Figure 2H, Figure S3). Finally, we found significantly reduced T-cell proliferation in vitro for P1 and P2 in response to purified protein derivative (PPD) from *Mycobacterium tuberculosis* (Table SII).

Performing a similar set of experiments in AML patients with somatic *GATA2* variants (P3-P5), affecting only a certain fraction of cell populations, we found that these patients retained a more normal, although in some cases reduced, response to most PAMPs as well as to HSV-1 and VZV (Figures S4, S5). Notably however, P3-P5 suffering from AML had all received chemotherapy; some of the impairment in antiviral and inflammatory responsiveness may be explained by secondary immunodeficiency not directly related to *GATA2* mutations in these cases.

Our main finding, that MonoMAC patients with germline *GATA2* mutations showed significantly impaired antiviral and proinflammatory responses to all innate ligands/PAMPs tested as well as to HSV-1 and VZV, is in agreement with the infectious phenotype of MonoMAC patients. (Dickinson et al. 2014). In addition to severe monocytopenia, the reduced fraction of NK cells typically described in MonoMAC also represents an immunological explanation for the increased susceptibility to herpesvirus infections, as previously described by Biron *et al* (1989) in a patient with severe NK cell deficiency, who many years later was found to have GATA2 deficiency/MonoMAC and thus be among the first patients described with this syndrome).

The results described here increase the knowledge on the association between *GATA2* mutations and increased susceptibility to infection, including mycobacteria and viruses. Importantly, the data clearly demonstrate a more pronounced immunodeficiency in patients with germline *GATA2* defect classified as MonoMAC, whereas patients with acquired somatic *GATA2* mutations leading to malignant haematological disease displayed relatively few infections and also mounted a relatively normal immune response in vitro. Given that our data did not suggest any direct association between localization of the mutation within the *GATA2* gene, we conclude that the difference in pathogenesis and degree of immune impairment must rely primarily on the fraction of haematopoietic precursor cells affected, rather than on the specific mutation. Thus, in germline *GATA2* mutations there is a profound aberration in myeloid cell development, leading in most, but not all, individuals to progressive multi-lineage monocytopenia (Dickinson et al 2014; Rodrigues et al. 2012). In contrast, only a minor fraction of the myeloid cell lineage is affected in patients with somatic
GATA2 mutations, which may provide the basis for development of malignancy without causing immunodeficiency.

Interestingly, P6 and P7, with mutations identical to the disease-causing GATA2 mutations in their son and daughter P1 and P2, respectively, are healthy and have mainly normal antiviral and inflammatory responses. These findings indicate that heterozygous GATA2 deficiency appears to be primarily a quantitative defect attributable to the presence of cytopenias, rather than a qualitative defect due GATA2 mutations per se. This question is a fundamental one, given that it remains incompletely understood as to why heterozygous GATA2 mutation often displays incomplete penetrance, implying that a given mutation may lead to severe Monomac with major haematological derangements, mycobacterial infection and, in some cases, myelodysplasia, whereas in other cases the individual retains normal immune cell populations, responds normally to microbial pathogens, and remains healthy. A role for specific epigenetic changes or the presence of other susceptibility genes with disease modifying impact remains possible.

As the number of patients described with GATA2 variants increases, we will hopefully learn more about the precise genetic and immunological basis underlying the pathogenesis of this heterogeneous disorder. These insights may teach us new lessons related to basic transcription factor biology and the signals regulating development and differentiation of stem cells and myeloid cell lineages. Moreover, such knowledge is expected to provide a stronger basis for understanding different infectious and malignant clinical phenotypes and to inform decisions on the diagnosis, management, and treatment of MonoMAC patients with mutations in the myeloid transcription factor GATA2.

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Author contribution statement

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THM and KA conceived the idea and designed experiments. MH, JV, KA, IJ, and KR identified the patients. MM, SEJ, and AS carried out experiments. MC performed genetic analysis. THM and MM drafted the first version of the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of Interest disclosure

None of the authors have any financial conflicts of interest to declare.

References


Figure legends

Figure 1. GATA2 gene and protein structure and stimulation of MonoMAC GATA2+/- patient PBMCs with synthetic PAMPs.

A: Schematic drawing of the three different GATA2 transcript variants, with black boxes and lines representing exons and introns, respectively. Arrows indicate the location of specific genetic variants identified in each patient. Below, domain structure of GATA2 protein variant NP_001139133.1, encoded by transcript variants 1 and 2. The variants found in each patient are located exclusively in the zinc finger DNA binding domains and the individual positions are indicated by arrows. B-F: Stimulation of MonoMAC GATA2+/- patient peripheral blood mononuclear cells (PBMCs) with synthetic pattern-associated molecular patterns (PAMPs). PBMCs were left untreated (UT) or stimulated with synthetic PAMPs in the following concentrations: PAM3CSK4 (200 ng/ml) (TLR2), lipopolysaccharide (LPS, 10 ng/ml) (TLR4), and ODN 2006 (10 ug/ml) (TLR9), or transfected with HSVmer60 double stranded DNA (dsDNA, 4 ug/ml) (cytosolic DNA sensors, including cGAS) for 6 h before harvesting total RNA for gene expression analysis by reverse transcription quantitative real time polymerase chain reaction. B and C: antiviral responses to each individual PAMP as measured by IFNBI and
CXCL10 mRNA expression, respectively. D, E, and F: proinflammatory responses as measured by TNF, IL6 and IFNG mRNA expression, respectively. PBMCs from 12 healthy donors were used as controls and their pooled results are shown in the black bar of each graph. Biological replicates are shown, and error bars show the mean ± standard deviation for each patient and pooled controls. Statistical comparison of each treatment between patient and controls: * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001; Two-way ANOVA with Dunnett post-tests.

Figure 2. Infection of MonoMAC GATA2+-/- patient PBMCs with HSV-1 or VZV and T cell activation assay.

A-E: Peripheral blood mononuclear cells (PBMCs) from patients and 12 controls were left untreated or infected with HSV-1 (17+ strain, multiplicity of infection 9) for 6 h, after which the cells were harvested for mRNA expression analysis of IFNB1, CXCL10, TNF, IL6 and IFNG. F and G: PBMCs from patients and 12 controls were co-cultured with MeWo cells or VZV-infected MeWo cells. The cells were left for 48 h before harvesting the cells for mRNA expression analysis of IFNB1 and CXCL10. H: PBMCs from patients and 11 controls were cultured with IL2 (40 u/ml) in untreated (UT) or anti-CD3 and anti-CD28 antibody-coated wells for 72 hs, after which the cells were harvested for isolation of total RNA and reverse transcription quantitative real time polymerase chain reaction analysis of IFNG mRNA expression as a measure of T cell activation. Data from the controls are pooled in the black bar. Biological replicates are shown, and error bars show the mean ± SD for each patient and pooled controls. Statistical comparison of each treatment between patient and controls: * p<0.05, ** p<0.01; *** p<0.001; ****; p<0.0001; Two-way ANOVA with Dunnett post-test.
1: NM_01145661.1
2: NM_032638.4
3: NM_01145662.1

P1 + P6: C319fs*5
P2 + P7: R398W
P3: N297S
P4: R307W
P5: L359V

DNA binding regions:
Zinc binding sites: