Altered chemotactic response to CXCL12 in patients carrying GATA2 mutations

Anna Maciejewska-Duval,* Floriane Meuris,*1 Alexandre Bignon,*1 Marie-Laure Aknin,†
Karl Balabanian,* Laurence Faivre,† Marlène Pasquet,‡ Vincent Barlogis,* Claire Fieschi,§
Christine Bellanne-Chantelois,† Jean Donadieu,** Géraldine Schlecht-Louf,*
Viviana Marin-Esteban,*2,3 and Françoise Bachelerie*.2,3

ABSTRACT

GATA2 deficiency—formerly described as MonoMAC syndrome; dendritic cells, monocytes, B cells, and natural killer cell deficiency; familial myelodysplastic syndrome/acute myeloid leukemia; or Emberger syndrome—encompasses a range of hematologic and nonhematologic anomalies, mainly characterized by monocytopenia, B lymphopenia, natural killer cell cytopenia, neutropenia, immunodeficiency, and a high risk of developing acute myeloid leukemia. Herein, we present 7 patients with GATA2 deficiency recruited into the French Severe Chronic Neutropenia Registry, which enrolls patients with all kinds of congenital neutropenia. We performed extended immunophenotyping of their whole blood lymphocyte populations, together with the analysis of their chemotactic responses. Lymphopenia was recorded for B and CD4+ T cells in 6 patients. Although only 3 patients displayed natural killer cell cytopenia, the CD56bright natural killer cell subpopulation was nearly absent in all 7 patients. Natural killer cells from 6 patients showed decreased CXCL12/CXCR4-dependent chemotaxis, whereas other lymphocytes, and most significantly B lymphocytes, displayed enhanced CXCL12-induced chemotaxis compared with healthy volunteers. Surface expression of CXCR4 was significantly diminished in the patients’ natural killer cells, although the total expression of the receptor was found to be equivalent to that of natural killer cells from healthy individual controls. Together, these data reveal that GATA2 deficiency is associated with impaired membrane expression and chemotactic dysfunctions of CXCR4. These dysfunctions may contribute to the physiopathology of this deficiency by affecting the normal distribution of lymphocytes and thus potentially affecting the susceptibility of patients to associated infections. J. Leukoc. Biol. 99: 1065–1076; 2016.

Introduction

Mutations in the transcription factor GATA2 are associated with diverse clinical phenotypes, now collectively called GATA2 deficiency. This encompasses large syndromes, such as familial MDS and AML; primary lymphedema with predisposition to AML (Emberger syndrome); dendritic cells, monocytes, B cell, and natural killer cell deficiency; MonoMAC; aplastic anemia; and pediatric MDS [1–10]. Recently, we reported [11] a high frequency of GATA2 mutations in patients with mild chronic neutropenia evolving to MonoMAC syndrome, MDS, and AML. Despite a diverse clinical presentation of GATA2 deficiency, most patients experienced severe infections (81% of patients suffered from viral infections, 53% from disseminated nontuberculous mycobacterium, and 48% other bacterial infections) and displayed an overall reduction of monocytes, B lymphocytes, and NK cells [12].

Two subsets of NK cells can be distinguished according to their CD56 cell-surface expression level, namely CD56bright and CD56dim, identifying, respectively, the more immature and the fully mature circulating NK cells. Independent of the clinical presentation of GATA2 deficiency, many of these patients (82%) displayed circulating NK lymphopenia [12] characterized by an almost complete absence of the CD56bright NK subset [12–14]. The underlying mechanism of this NK cytopenia directly depends on the GATA2 dysfunction, because the in vitro differentiation of...
CD34+ cells from patients resulted in fewer NK cells with a near-total absence of the CD56bright subset [14]. The precise role of GATA2 in the development of NK cells remains to be described. NK cells develop mainly in the BM and are thought to reach the blood via venous sinusoids, similar to other lymphocytes [15, 16]. They are considered highly motile cells that patrol between lymphoid and nonlymphoid organs, and several different GPCR have been reported to have a role in their trafficking [17]. Among them, CXCR4, a widely distributed GPCR specific for the CXCL12 chemokine, was shown to critically contribute to the BM homing of NK cells in mice [15]. Other reports have extended this notion to human NK cells, thus highlighting the critical contribution of the CXCL12/CXCR4 axis in the proper distribution of NK cells between the BM and the peripheral blood [18, 19].

Disfunctions of the CXCL12/CXCR4 signaling axis (e.g., increased chemotactic responses) from a dominant gain-of-function, inherited mutation in CXCR4 prevent leukocytes from egressing into blood circulation, thus contributing to the peripheral panleukopenia that characterizes the WHIM syndrome [20, 21]. This rare combined immunodeficiency disorder gets its name from the acronym of its main manifestations: warts, hypogammaglobulinemia, infections, and myelokathexis [22–24]. A knock-in mouse model of this syndrome, harboring a WHIM-associated heterozygous mutation of the CXCR4 gene, exhibits dysfunctions of the CXCL12/CXCR4 axis and peripheral leukopenia as reported in patients with WHIM [20, 25], together with a deficit in circulating NK cells [15]. This peripheral leukopenia can be transiently reversed by treating mice with the specific CXCR4 antagonist AMD-3100 [25], as observed in patients with WHIM [26, 27]. We reported similar dysfunctions of CXCR4 (i.e., impaired CXCL12-induced internalization and increased chemotaxis) in patients suffering from life-threatening diseases related to the WHIM syndrome but without the germline mutation in the CXCR4 gene [20, 28]. Later on, these patients were diagnosed with GATA2 deficiency [11]. Patients suffering from GATA2 deficiency also display a high susceptibility to HPV infections, as do patients with WHIM syndrome [11–13, 22, 23, 29]. Interestingly, a role for CXCR4 dysfunctions in HPV-associated disease infections has been suggested recently [27, 30, 31]. Collectively, these observations are highly suggestive of a possible interplay between GATA2 deficiency and CXCR4 dysfunctions. In the present work, this hypothesis was investigated by studying the CXCR4-dependent, chemotactic response of the different lymphocyte populations in the peripheral blood from 7 patients suffering from GATA2 deficiency.

**MATERIALS AND METHODS**

**Individuals**

Patients with GATA2-deficiency were recruited from the French Severe Chronic Neutropenia Registry. Patients provided their written, informed consent for genetic testing and inclusion in the register approved by the Commission Nationale de l’Informatique et des Libertés. Healthy donor volunteers, matched whenever possible for age and sex, were included as controls. Clinical presentations of the patients are presented in Table 1.

**Blood samples**

Peripheral blood samples were collected from patients in heparin tubes. Blood from a donor declared healthy was collected at the same time and place. Samples were manipulated in the 24 h following collection to avoid possible modification of leukocyte phenotype and activation. Absolute cell counts were determined with automated analyzer at clinical laboratory services. Leukocyte counts from the patients are provided in Supplemental Table 1.

**GATA2 mutations screening**

Genomic DNA was extracted from blood using standard procedures. PCR and Sanger sequencing were used to search for GATA2 mutations in coding exons 2–6 and in the minimal region of intron 4 (c.1017+500_700) containing a regulatory element. Sequences were analyzed with Seqcape software (version 2.5, Life Technologies, Saint Aubin, France). Mutations are numbered following the recommendations of the Human Genome Variation Society using the reference sequence NM_032638.4.

**Antibodies and CD1d tetramers**

For immunophenotyping, the following fluoroochrome-conjugated antibodies were used: FITC anti-CD3 (clone HIT3a), PE or PE-Cy5 anti-CD4 (RPA-T4), pacific blue anti-CD8 (RPA-T8), PerCP-Cy5.5 anti-CD19 (HIB19), PE-Cy5 anti-CD56 (B159), Alexa Fluor 700 anti-CD45RA (H100), PE-Cy7 anti-CCR7 (3D12), PE or APC anti-CXCR4 (12G5), PE anti-CXCR2 (6G8), Alexa Fluor 700 anti-CXCR3 (1C6), and Alexa Fluor 647 anti-CXCR5 (RF8B2). All antibodies were purchased from BD Bioscience (Le Pont-de-Clair, France). The chemokines CXCL8, CXCL12, CXCL13, and CCL19 were purchased from R&D Systems (Lille, France). CD1d tetramers loaded with α-GalCer and conjugated with APC were kindly provided by Dr A. Herbelin (INSERM 1082, Poitiers, France).

**Lymphocytes phenotyping**

To study the phenotype of different lymphocyte populations, 100 μl of total blood were mixed with appropriate antibodies or isotype controls and incubated for 30 min at 4°C. Then, red blood cells were lysed by adding 500 μl OptiLyse C (Beckman Coulter, Marseille, France) and were incubated for 15 min at room temperature. After washing, cells were analyzed using an LSR-Fortessa cytometer (BD Biosciences). Morphologic and phenotypic analysis of cell populations was performed on single cell events. The main lymphocyte populations analyzed were B cells (CD19+), T lymphocytes (CD3+), and NK cells (CD3–CD56–). T lymphocytes subclasses were segregated as a function of CD4, CD8, or CD56 expression. Naive/memory T lymphocyte subclasses were analyzed according to CD45RA and CCR7 expression. The iNKT lymphocytes were identified with α-GalCer/CD1d tetramers. When indicated, cryopreserved PBMCs were stained after cell fixation and permeabilization with the cytokine/cytoskeleton kit from BD Biosciences. When indicated, flow cytometry data were presented as relative mean fluorescence, corresponding to the ratio between MFI of the specific labeling and that of the isotype control.

**Transwell-based chemotaxis assay**

Chemotactic migration assays were performed on whole blood samples using transwell inserts with a 5-μm pore polycarbonate membrane (Corning Life Sciences, New York, NY, USA), as previously described [34]. RPMI 1640 medium with 1% human serum (600 μl) without or with a chemokine (CXCL12, CXCL13, CCL19, or CXCL8) at the indicated concentrations was placed in the lower chamber. Blood was diluted in RPMI 1640/1% human serum in a 1:4 ratio. Diluted blood (150 μl) was added to the upper chamber. When used, AMD-3100 was at 20 μM and placed in both upper and lower chambers. Cells having migrated to the lower chamber after 4 h were collected and stained with anti-CD3, anti-CD4, anti-CD8, anti-CD19, and anti-CD56.
TABLE 1. Clinical presentation of patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender (age, yr)</th>
<th>GATA2 mutation</th>
<th>Overall phenotype</th>
<th>Initial manifestation (age, yr)</th>
<th>Infections</th>
<th>Karyotype</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (7108)</td>
<td>F (16)</td>
<td>3q21.1–3, interstitial deletion</td>
<td>Multiple congenital malformations, including dysmorphism with asymmetric skull and face, pancytopenia, MDS</td>
<td>MDS (11)</td>
<td>Gingivitis, staphylococcal skin infections, CMV–</td>
<td>3q21.1-q21.3, chr 7 monosomy</td>
<td>Antistaphylococcal and antistreptococcal treatment</td>
</tr>
<tr>
<td>2 (7015)</td>
<td>M (19)</td>
<td>c.670G&gt;T</td>
<td>Neutropenia</td>
<td>HPV warts (4) Neutropenia (11)</td>
<td>HPV warts, CMV–</td>
<td>ND</td>
<td>GCSF corticosteroids</td>
</tr>
<tr>
<td>3 (5964)</td>
<td>M (7)</td>
<td>c.944T&gt;C</td>
<td>Neutropenia, moderate macrocytosis and NK, MDS</td>
<td>Pseudomonas aeruginosa skin infection (1.3)</td>
<td>CMV–</td>
<td>ND</td>
<td>No specific treatment</td>
</tr>
<tr>
<td>4 (6836)</td>
<td>F (72)</td>
<td>c.1114G&gt;A</td>
<td>Aplasticity B and NK, MDS</td>
<td>MDS (66)</td>
<td>NI</td>
<td>ND</td>
<td>No specific treatment</td>
</tr>
<tr>
<td>5 (6739)</td>
<td>F (17)</td>
<td>c.1143+5G&gt;C</td>
<td>Macrocystosis, monocytopenia, aplasticity B and NK, MDS</td>
<td>Pneumonia (mycoplasma) Multiple pneumonia episodes (mycoplasma)</td>
<td>Normal</td>
<td>Normal</td>
<td>No specific treatment</td>
</tr>
<tr>
<td>6 (6165)</td>
<td>M (18)</td>
<td>c.1187G&gt;A</td>
<td>Neutropenia, MDS</td>
<td>Severe pneumonia and MDS (16)</td>
<td>Severe pneumonia, chronic EBV replication in gut pre-HSCT</td>
<td>Chr 8 trisomy</td>
<td>HSCT (18)</td>
</tr>
<tr>
<td>7 (6861)</td>
<td>M (30)</td>
<td>c.1193G&gt;A</td>
<td>Neutropenia, monocytopenia, MDS</td>
<td>Pneumonia (atypical mycobacteria) and MDS (25)</td>
<td>Pneumonia (atypical mycobacteria) Isolated chr 8 trisomy</td>
<td>GCSF antimycobacterial treatment</td>
<td></td>
</tr>
</tbody>
</table>

Any myelogram reported the presence of large granular lymphocytes. chr, chromosome; CMV– and CMV+, results from serology for Cytomegalovirus negative or positive, respectively; EBV, Epstein–Barr virus; F, female; GCSF, granulocyte colony-stimulating factor; M, male; ND, not defined; NI, not informed. *Patient number in parentheses is from the French Severe Chronic Neutropenia Registry. †Patient 7108 described in Callier et al. (2009) [32] and Kazenwadel et al. (2012) [33]. ‡Patient 7108 deceased. Constitutive 3q21.1-q21.3 interstitial deletion and somatic chromosome 7 monosomy. Patient 7015 described in Pasquet et al. (2015) [11]. Patient 6165 described in Pasquet et al. 2013 [11].

RESULTS

Clinical and genetic features of patients

Here, we describe 7 patients with GATA2 deficiency included in the French Severe Chronic Neutropenia Registry. Three of the patients (patients 1, 2, and 6) were reported earlier [11, 32], and the remaining 4 were newly diagnosed. Five patients were suffering from MDS, and the most common early complications were MDS and respiratory and skin infections (mostly HPV-related) (Table 1).

The 7 patients carried 7 different heterozygous and germline GATA2 mutations affecting coding and noncoding sequences of GATA2 Mutations included a large heterozygous deletion of the long arm of chromosome 3 (patient 1), where the GATA2 locus is located; a nonsense mutation in exon 3 (patient 2); a missense mutation in the region encoding for the N-terminal ZF domain (patient 3); 4 missense mutations in the region encoding for the C-terminal ZF domain (patients 4, 6, and 7); and 1 splice-site mutation in the 5’ consensus site of exon 5 (patient 5) also affecting the region encoding for the C-terminal ZF domain (Supplemental Figure 1). Five of these 7 mutations have already been described (patients 1, 2, 4, 6, and 7) [11, 13, 32] and 2 correspond to newly described mutations (c.944T>C in patient 3 and c.1143+5G>C in patient 5).

Immunologic features of patients

Patients displayed at least a mild neutropenia (5 patients), severe monocytopenia (5 patients), or mild lymphopenia (5 patients) or a combination of the 3 (Supplemental Table 1), which are hematologic anomalies usually associated with
GATA2 deficiency. Thus, among the 7 patients, 3 patients combined the 3 hematologic anomalies (patients 1, 5, and 6) and 2 patients displayed lymphopenia combined with either neutropenia (patient 2) or monocytopenia (patient 7). The 2 remaining patients had either neutropenia (patient 3) or monocytopenia (patient 4) but no lymphopenia; 1 displayed, however, abnormal lymphocyte subpopulation counts (patient 4). Median cell counts for neutrophils, monocytes, and lymphocytes for all patients (n = 7) were 1.6 Gl (range, 1.1–8.46 Gl), 0.07 Gl (range, 0.01–0.42 Gl), and 1.2 Gl (range, 0.49–2.9 Gl), respectively.

As shown in Fig. 1A for a representative patient (patient 5), among the patients with lymphopenia, a strongly reduced frequency of B lymphocytes was observed (1.2 vs. 8.8% in the control). This decrease was accompanied by an altered ratio of the CD4+:CD8+ T lymphocytes, as shown for patient 5 in Fig. 1B (ratio 28:59.4% vs. 65:31% in the control). The CD4+:CD8+ ratio of T lymphocytes was close to 1:2 in patient 5 and to 1:1 in patients 2, 6, and 7 (Supplemental Table 1). Patient 4, who was not lymphopenic, was also found to display a CD4+:CD8+ T lymphocyte ratio close to 1:1 and a reduced frequency of B cells (Supplemental Table 1). More specifically, Fig. 1C shows that not only the frequency but also the absolute cell counts of B lymphocytes were significantly reduced in patients, evaluated as a whole, compared with controls (respectively, 1.2 ± 1.55 vs. 9.2 ± 1.4% and 61.77 ± 47.44 vs. 285.33 ± 37.75 cells/μl; n = 7). Most patients were suffering from severe B lymphopenia, with the exception of patient 3. The frequency of CD4+ T lymphocytes was also decreased compared with the controls (Fig. 1D), and conversely, the frequency of CD8+ T lymphocytes was significantly increased (Fig. 1E). We further identified that the CD4+ T lymphocyte absolute cell counts were significantly reduced in patients as compared with controls, (Fig. 1D) (392.52 ± 101.47 vs. 1334.07 ± 95.79 cells/μl, n = 7). In contrast, the absolute cell counts of CD8+ T lymphocytes were not significantly different in patients and controls (Fig. 1E) (341.11 ± 74.39 vs. 583.81 ± 70.90 cells/μl, n = 7). Thus, the CD4+ T lymphopenia observed in patients might account for the altered CD4+:CD8+ T cell ratio.

**Selective deficiency of the CD56bright NK cells in patients**

Analysis of NK cell populations, based on the expression level of CD56, indicated a dramatic reduction of the percentage of CD56bright cells, as illustrated in Fig. 2A for a representative patient (patient 6). Absolute NK cell counts were significantly reduced in patients evaluated as a whole, as compared with controls, (Fig. 2B) (means 75.97 ± 23.77 vs. 179.88 ± 38.06 cells/μl, n = 7). These counts remained within the reference range in 4 patients, and NK cell cytopenia was evidenced in only 3 patients (see also Supplemental Table 1). For most patients, the frequency of NK cells among lymphocytes was slightly reduced compared with controls although that did not reach statistical significance (Fig. 2B, left panel). However, as mentioned earlier, for patient 6 in Fig. 2A, the frequency of the CD56bright NK cell subset was strongly decreased in patients compared with healthy volunteers (Fig. 2C) (0.11 ± 0.05 vs. 0.64 ± 0.11%). In terms of absolute cell counts, this CD56bright NK population was notably and significantly reduced in all patients (Fig. 2C) (1.65 ± 0.76 vs. 17.34 ± 2.92 cells/μl in controls). In contrast, the frequency of CD56dim NK cells was not affected in patients, yet their absolute counts were significantly reduced (Fig. 2D) (74.32 ± 23.57 vs. 162.7 ± 36.31 cells/μl in controls). Together, these results show that in patients, both NK cell subpopulations were affected with a more dramatic effect on the CD56bright subset.

**Increased frequency of CD8+CD56+ effector T lymphocytes in patients**

In contrast to the reduction in NK cells (Fig. 2B), we found a significant, abnormal increase in CD56+ T lymphocytes in

---

**Figure 1. Analyses of B and T lymphocyte populations.** (A and B) Gating strategy to identify B and T lymphocytes is shown for 1 representative lymphopenic patient (patient 5) and the corresponding control. Populations: 1) lymphocytes, 2) B lymphocytes, and 3) T lymphocytes. (A) Lymphocytes, identified in a forward scatter/side scatter dot plot of total blood leukocytes, were analyzed for their CD19 and CD3 expression, (B) CD3 T lymphocytes were further analyzed for CD4 and CD8 expression (populations 4 and 5, respectively). (C) Comparison of frequencies and absolute counts of B lymphocytes and CD4+ (D), and CD8+ (E) T lymphocytes between patients and controls. Each point represents an individual, and lines indicate the means ± sem (n = 7). *P < 0.05 and **P < 0.0005, as determined by unpaired 2-tailed Student’s t test. The gray zones indicate the reference range from clinical service.
patients, with the mean frequency of these cells being 10 times higher than it was in controls (Fig. 2E, left panel) (14.53 ± 6.39 vs. 1.74 ± 0.64%, n = 7), and their absolute cell counts being 3 times greater (Fig. 2E, right panel) (165.6 ± 49.9 vs. 38.4 ± 12.66 cells/µL, n = 7). In 2 patients (patients 5 and 6), it was possible to characterize, within these CD56+ T lymphocytes, a population expressing high levels of CD56, here, referred as CD56high T lymphocytes. As shown for patient 5 in Fig. 3A, CD56high T lymphocytes represented 1.48% of patient lymphocytes, whereas for control individual that population was almost absent (0.0138% of lymphocytes). CD56high T lymphocytes homogeneously expressed CD8 (Fig. 3B) (81.6%) and corresponded to a terminal effector population, according to their CCR7−CD45RA+ phenotype (Fig. 3C) (94.3%). Similarly, CD8 cells were abnormally overrepresented among the CD56low T lymphocytes in the patients (Fig. 3D) (74.2%), and they predominantly displayed a terminal effector phenotype (Fig. 3E) (82%). The increased frequency of CD3+CD56+CD8+ cells was extended to 2 others patients (patients 2 and 3) and not explored in the other patients. These results confirm the increased frequency of CD8+CD56+ T lymphocytes already described in GATA2-deficient patients [12, 13] and identify the occasional presence of an abnormal subpopulation of T lymphocytes expressing very high levels of CD56 in patients.

Considering the increased CD56+ T lymphocyte counts, we searched for the presence of iNKT lymphocytes, in particular, those expressing the invariant TCR Vα24-Jα18, using tetramers of their specific ligand α-GalCer presented in the context of CD1d. We were able to explore this cell population in 2 patients. In patient 5, iNKT lymphocytes were found to represent 0.17% of the patient lymphocytes (Fig. 3F). Although they were 20 times more abundant than the lymphocytes in control subjects, these cells remain a minority population among the lymphocytes. These iNKT lymphocytes predominantly displayed a CD56low phenotype (Fig. 3G) (75.4%). However, in the second patient investigated (patient 4), the frequency of α-GalCer specific These iNKT was almost unchanged compared with the control (data not shown).

**Figure 2. Virtual lack of blood CD56bright NK cells and expansion of the CD3+CD56+ lymphocyte population in patients.** (A) Lymphocytes were analyzed for CD56 and CD3 expression. Populations: 1) CD56bright NK cells, 2) CD56dim NK cells, and 3) CD56+ T lymphocytes are shown for representative patient 6 and the control. Frequency of total NK cells (B), CD56bright NK cells (C), CD56dim NK cells (D), and CD56+ T lymphocytes (E) among lymphocytes (gated as in Fig. 1A) and their absolute cell counts are shown for the 7 patients and controls. Each point represents an individual, and lines indicate the means ± SEM (n = 7). *P < 0.05, **P < 0.005, and ***P < 0.0005, as determined by unpaired 2-tailed Student's t test. The gray zones indicate the reference range.

**NK cells from patients displayed a reduced CXCL12/CXCR4-dependent chemotaxis**

We performed a whole blood chemotaxis assay to measure the migratory responses of lymphocytes to the CXCL12, CXCL8, CXCL13, and CCL19 chemokines, known to be implicated in lymphocyte trafficking [35]. As shown in Fig. 4A for patient 6, NK cells from patients displayed reduced CXCL12-induced chemotaxis. NK cells represented 5.62% of the lymphocytes for patient 6 before whole blood chemotaxis and were drastically reduced to 0.87% of the recovered lymphocytes upon chemotaxis. As a comparison using the blood of a healthy volunteer, NK cells before and upon chemotaxis corresponded, respectively, to 9.27% and to 8.7% of lymphocytes. When analyzed as a percentage of input cells, NK cells from patients 2 and 6 poorly responded to CXCL12 (Fig. 4B and C, respectively). This impairment was observed for all the chemokine concentrations used from 10 to 100 nM, a range found to be effective in promoting a dose-dependent migration of control NK cells (Fig. 4B and C). Specificity of the CXCR4 engagement by CXCL12 was established by the substantial blockade of the CXCL12-induced cell migration in the presence of AMD-3100, a selective CXCR4 antagonist [36]. In contrast, other chemokine receptors remained fully
sensitive to their cognate ligand, such as CXCL8 and CCL19 chemokines, as evidenced by the responsiveness of the patients’ NK cells, which remained in the same range as that of control NK cells (Fig. 4B and C). Considering the low frequency of the CD56\textsuperscript{high} NK population among blood NK cells, 5–10% of NK cells in healthy individuals, and moreover, the strong reduction of this population in patients, it can be speculated that our results are representative of the behavior of the CD56\textsuperscript{dim} NK population. B and T lymphocytes from representative patient 2 also remained normally responsive to the CXCL13 and the CCL19 chemokines, respectively (Fig. 4D–G). However, unlike NK cells, B lymphocytes from patients were responding to CXCL12 in a dose-dependent manner and displayed stronger migratory responses at the lowest concentration of CXCL12 as compared with controls (Fig. 4D), suggesting a higher potency of these cells toward CXCL12.

The same trend was observed for CD4\textsuperscript{+} and CD8\textsuperscript{+} T lymphocytes (Fig. 4E and F) but not for CD56\textsuperscript{+} T lymphocytes of patient 2 (Fig. 4G). However, these differences with control cells were not observed for CD4\textsuperscript{+} and CD8\textsuperscript{+} T lymphocytes from other patients, as illustrated in a pooled analysis of lymphocyte migration for all patients (Fig. 5, middle panels). Thus, chemotactic response of T lymphocytes, gated according to their CD4, CD8, or CD56 expression, was equivalent between patients and donors (Fig. 5, middle and lower panels; \( n = 7 \)). The diminished chemotaxis of NK cells was confirmed and found significant for 6 patients (Fig. 5, upper left panel; \( n = 6 \)). Accordingly, the higher CXCL12-dependent chemotaxis observed for B lymphocytes in patient 2 at low CXCL12 concentrations (Fig. 4D) was confirmed and extended to 5 patients (Fig. 5, upper right panel; \( n = 5 \)). Together, these results indicate that whatever the mutation in GATA2 harbored by these patients, GATA2 deficiency is associated with CXCL12/CXCR4 dysfunctioning both in NK cells and B lymphocytes but with opposing effects, illustrated by the dramatically reduced chemotactic response of NK cells and to a rather enhanced migration of B lymphocytes in response to CXCL12.

**Cell surface expression of CXCR4 is reduced in NK cells from patients**

When comparing the expression level of CXCR4 at the cell surface for all patients (\( n = 6 \); CXCR4 exploration was not performed in patient 1) and controls, a significant reduction, of \( \sim 50\% \), in the expression of CXCR4 on NK cells from patients was observed (Fig. 6A) (relative MFI, 2.78 ± 0.83 vs. 5.6 ± 0.82 in controls). Moreover, the frequency of CXCR4\textsuperscript{−} NK cells in patients was also significantly diminished, by 60%, as compared with controls (14.55 ± 4.62 vs. 34.33 ± 5.16%; \( P = 0.017 \) unpaired 2-tailed Student’s \( t \) test) (Supplemental Fig. 2). In contrast, neither the expression level of other chemokines receptors (i.e., CXCR1, CXCR2) in NK cells nor the percentage of NK cells expressing each of the 2 receptors, was significantly modified between patients and controls (Supplemental Fig. 2). Similar observations were made for the expression of the CXCR5 receptor in B lymphocytes from patients and controls (Supplemental Fig. 2A). However, for CXCR4, we noticed a trend toward reduced cell surface expression level in B lymphocytes from patients, as illustrated for patient 2 (MFI, 2914 vs. 5008 in controls; positive cells 68 vs. 85% in controls; Supplemental Fig. 2A). This reduction was not statistically significant (Fig. 6A) (relative MFI, 42.43 ± 34.01 vs. 65.55 ± 55.1; \( n = 6 \)), and the percentage of CXCR4 positive B lymphocytes remained in the same range between patients and controls (78.6 ± 8.8 vs.
79.67 ± 7.48%; n = 6). Figure 6A also shows that cell surface expression levels of CXCR4 on CD4+, CD8+, and CD56+ T lymphocytes were equivalent in patients and controls. Moreover, the percentage of CXCR4+ cells among CD4+, CD8+, and CD56+ T lymphocytes remained equivalent between patients and controls (~40%; data not shown). In conclusion, CXCR4 membrane expression level was selectively impaired in NK cells from patients and associated with a dramatic reduction in the frequency of CXCR4+ NK cells.

We performed immunostaining of CXCR4 in PBMCs from patients 2, 5, and 7 and confirmed, as shown in Fig. 6B, the reduced expression (~50%) of the receptor at the cell surface in NK cells reported above upon whole blood staining (Supplemental Fig. 2A). However, staining of CXCR4 upon permeabilization of PBMCs, which allowed detection of the cell surface and the intracellular molecules, indicated that NK cells from the 3 patients displayed a total CXCR4 pool in the same range than that of control cells (Fig. 6B). Based on these results, we propose that GATA2 deficiency is associated with dysregulation of CXCR4 function, including the expression of this receptor at the cell surface. Thus, an impaired cell-surface trafficking of CXCR4 in a patient’s NK cells might account for the reduced efficiency of CXCL12 to induce their migration, which is a function of the receptor occupancy.

**DISCUSSION**

In the present work, we analyzed the frequency and chemotactic potency of blood lymphocyte populations from 7 patients with GATA2 deficiency, recruited in the French Severe Chronic Neutropenia Registry. This registry currently includes 59 patients with identified GATA2 deficiency, of which, 15 have received HSCT, and 30 are deceased. The 7 patients studied here displayed clinical features characteristic of GATA2 deficiency. Five of them were diagnosed with MDS, and all patients were found to have reduced counts for 1, 2, or 3 leukocyte populations compared with reference ranges. The most salient anomalies regarding lymphocyte populations were a severe B lymphopenia and a CD4+ T lymphopenia observed in 6 patients. In 3 out of the 7 patients, CD8+ T lymphocyte counts were also less than reference range.

In contrast, a population of T lymphocytes expressing CD56+ was found to be overrepresented in patients, reaching up to 32.9% of total lymphocytes. These cells predominantly display a
CD8+ terminal effector phenotype, as illustrated in 1 patient. In spite of sharing the CD56 marker with NK cells, CD56+ T lymphocytes are described as being functionally distant and distinct from NK and iNKT lymphocytes [37]. Although we have not studied the expression of KIR in CD56+ T lymphocytes, Dickinson et al. [13] have shown that, in a large cohort of patients with GATA2 deficiency (n = 30), expression of different KIR markers is significantly increased in the corresponding cell population. CD56+KIR+ T lymphocytes are associated with effective clinical protection against the specific pathogen that induced their original activation [38], yet they are expected to display a cytotoxic function dependent on Fas ligand or on KIR engagement, in a TCR-independent manner [37, 39, 40]. We also identified in 2 patients a subpopulation of T lymphocytes expressing high levels of CD56, here referred to as CD56high T cells. Both patients were diagnosed with MDS and had suffered from severe or multiple pneumonia episodes. Whether the CD56high phenotype is related to the active state of T lymphocytes maintained by chronic infections remains to be answered. An enhanced CD56 expression might reflect an exacerbated cytotoxic function in NK cells [41, 42] and in CD8+ T lymphocytes [43]. Abnormal overrepresentation not only of CD56+ but also of CD57+ T lymphocytes are found in BM of patients with GATA2 deficiency [4]. Our preliminary results

Figure 5. CXCL12-promoted chemotaxis of different lymphocyte populations for all patients. CXCL12-promoted migration was analyzed for NK cells, B lymphocytes, and CD4+, CD8+, or CD56+ T lymphocytes in most patients (black columns) and their controls (white columns). Results (means ± sex) are from 7 patients and their controls, with the exception of data for NK cells from patient 5 and for B lymphocytes from patients 4 and 5. These data were not included in the analysis because of the few corresponding cells recovered in the migration assay. NK cells, n = 6; B lymphocytes, n = 5; and T lymphocytes, n = 7. *P < 0.05 and **P < 0.005 in comparison with controls, as determined by unpaired 2-tailed Student’s t test.

CD8+ terminal effector phenotype, as illustrated in 1 patient. In spite of sharing the CD56 marker with NK cells, CD56+ T lymphocytes are described as being functionally distant and distinct from NK and iNKT lymphocytes [37]. Although we have not studied the expression of KIR in CD56+ T lymphocytes, Dickinson et al. [13] have shown that, in a large cohort of patients with GATA2 deficiency (n = 30), expression of different KIR markers is significantly increased in the corresponding cell population. CD56+KIR+ T lymphocytes are associated with effective clinical protection against the specific pathogen that induced their original activation [38], yet they are expected to display a cytotoxic function dependent on Fas ligand or on KIR engagement, in a TCR-independent manner [37, 39, 40]. We also identified in 2 patients a subpopulation of T lymphocytes expressing high levels of CD56, here referred to as CD56high T cells. Both patients were diagnosed with MDS and had suffered from severe or multiple pneumonia episodes. Whether the CD56high phenotype is related to the active state of T lymphocytes maintained by chronic infections remains to be answered. An enhanced CD56 expression might reflect an exacerbated cytotoxic function in NK cells [41, 42] and in CD8+ T lymphocytes [43]. Abnormal overrepresentation not only of CD56+ but also of CD57+ T lymphocytes are found in BM of patients with GATA2 deficiency [4]. Our preliminary results

Figure 6. Expression of CXCR4 in patient cells. (A) Cell surface expression of CXCR4 evaluated as relative MFI is shown for the different lymphocyte populations (cells) for all patients (black points) and their controls (white points), except for patient 1 for whom this data was not collected (n = 6). Histograms for NK cells are shown in Supplemental Fig. 2. Bars indicate means ± SEM. *P < 0.05 in comparison with controls, as determined by unpaired 2-tailed Student’s t test. (B) Expression level of cell surface and total CXCR4 cellular pool in NK cells are shown for patients 2, 5, and 7. Staining was performed in 2 different experiments, and PBMCs from a healthy donor control was included in each one: control 1 for patient 2 and control 2 for patients 5 and 7.
extended these results to circulating blood, where we identified up to 54% of CD57+ T lymphocytes in some patients (data not shown). This marker is associated with terminal differentiation and senescence of lymphocytes [44] and also with an expansion of clonal, nonmalignant, large, granular lymphocyte populations during reactive lymphocytosis [45, 46]. Interestingly, increased populations of such large, granular lymphocytes have been found in myelograms of 16% of patients with GATA2 deficiency in a U.S. National Institutes of Health cohort [12]. Myelograms of the patients presented here did not reveal the presence of lymphocyte with this morphology. The CD57 marker also defines a T lymphocyte subset gathering oligoclonal TCR diversity [47]. Thus, an increased frequency of CD57+ T lymphocytes in patients with GATA2 deficiency might be indicative of an abnormal distribution of TCR diversity, as described in the BM of patients [4].

We also presented evidence that α-GalCer–specific iNKT lymphocytes were significantly increased in 1 patient. These cells might be related to given infection profile, such as in this patient having suffered from recurrent episodes of mycoplasma-associated pneumonia, the glycolipids of which are proposed to be processed and further presented by CD1d [48]. However, expansion of this cell population appears not to be a characteristic trait in patients with GATA2 deficiency because a broad analysis of 30 patients showed that the absolute counts of these cells, identified as TCRVα24Jα18+ cells, were not significantly increased [13].

NK cells are important effectors for eliminating virus-infected or bacteria-infected cells [17, 49]. A frequent NK cell cytopenia and functional defects on these cells, such as disturbed maturation and reduced cytotoxic function, have been reported in patients with GATA2 deficiency [2, 12–14]. Although among the 7 patients studied here, only 3 displayed a NK cell cytopenia, all 7 patients had noticeable and abnormally low CD56dim NK population, a hallmark of GATA2 deficiency [2, 12–14].

Importantly, we show that NK cells from patients, thus mostly CD56dim NK cells, express a lower level of surface CXCR4 while keeping an apparently normal total pool of the receptor and display a reduced chemotactic response to CXCL12 as compared with control cells. On one hand, this diminished cell surface expression and function of CXCR4 could be related to intrinsic NK cell factors. Development defaults in NK cells carrying GATA2 mutations have been evidenced by a partial or a complete avoidance of the development stage identified by high expression of the CD56 marker (CD56bright) [14]. Moreover, GATA2 knockdown experiences on human HSC suggest that expression of some GPCRs as well as the regulatory elements of their function can be directly perturbed under conditions of GATA2 insufficiency [50]. Strikingly, reduced GATA2 function leads to a decreased expression of filamin A and β-arrestin1 [50]. 2 proteins notably regulating CXCR4 cell surface expression and endocytosis and the chemotactic response of cells to CXCL12 [51–53], the anomalies of which might account for the down-regulation of CXCR4 at the cell surface. If a reduced function for CXCR4 is also present in NK precursors, such an anomaly is anticipated to reduce their interaction with BM stromal [54] cells and to shorten their retention time in this compartment [15, 18, 19], as suggested in wild-type and humanized mice, thus possibly impinging on the differentiation program of NK cells. In support of this hypothesis, the BM from patients with GATA2 deficiency is hypocellular and displays reduced counts of NK cells [4] and of multilymphoid progenitor [13].

On the other hand, diminished expression and function of CXCR4 in a patient’s NK cells could be related to extrinsic factors. This diminished expression and function of CXCR4 could indicate a recent activation of NK cells or their differentiation into memory-like NK cells, as demonstrated in rodents (i.e. rats and mice) [55, 56]. In line with this, cytokine-driven activation of NK cells (e.g., IL-2 or IL-15), can also down-regulate CXCR4 expression [18]. However, CXCR4 expression has also been reported to be nonaltered [57] or even up-regulated upon cytokine activation of NK cells [58]. These contrasting results thus open the issue of how CXCR4 expression can be modulated upon activation of patients-derived NK cells. For human NK cells, a reduced CXCR4 expression has also been reported in CD57+ NK cells, a population that corresponds to the final stage of NK cell peripheral maturation [59]. Our preliminary data indicated that CD57+ NK cells can be abundant among NK cells in some patients (up to ~90% of NK cells) and suggested a trend toward reduced expression of CXCR4 at the cell surface of CD57+ NK, which will be further explored in future studies (data not shown). CD57 expression designates a terminal maturation stage of NK cells with higher cytotoxic capacity and reduced capacity to proliferate, but they are long-lived [59–61] and accumulate with age [62] and during chronic infections (such as CMV infection), as a result of NK clonal expansion [63, 64]. Clonal expansion of NK cells could be heightened in GATA2 deficiency because of recurrent infections and also as a potential mechanism to compensate for the NK cell cytopenia and the gradual installation of MDS [4, 12–14].

The potential relationship between GATA2 mutations and CXCR4 dysfunctions and its association with MDS needs to be addressed in future experiments, notably including longitudinal studies to follow a patient’s evolution, and comparative analyses with sporadic MDS. Because MDS is a highly heterogeneous syndrome [65], such a study will demand a discriminative choice of the sporadic MDS cases to establish a group comparable in age and clinical manifestations with patients with GATA2 deficiency.

Lastly, we cannot exclude that dysfunction of CXCR4 on patient’s cells, diminished in NK and increased in B cells, can arise from mutations of CXCR4 or anomalies in CXCR4-regulatory proteins. That last possibility was suggested in our previous study [11] linking the gain in CXCR4 function to down-regulation of the GRK3 kinase [66] in a patient identified later as suffering from GATA2 deficiency. So far, no CXCR4-inherited mutations have been identified in patients 2 and 6 of the present study [11], or in other patients with GATA2 deficiency [11, 20, 28]. The absence of a specific genotype-phenotype correlation with GATA2 mutations [12] suggests the cooperation of other factors and additional acquired mutations for disease development [13, 67]. In this context, future studies should explore the potential presence of acquired mutations affecting genes encoding for CXCR4 or CXCR4-regulatory proteins.
Although altered CXCR4-dependent migration in NK cells correlates with reduced CXCR4 cell surface expression, this correlation is not a rule, and cells expressing low levels of CXCR4 can normally respond to CXCL12 [68], and conversely, equivalent expressions of CXCR4 can translate into disparate responsiveness directed by differences in intracellular signal transduction as observed for human B lymphocytes with their maturation [69]. This divergence is illustrated here by B lymphocytes from patients that display an enhanced CXCR4-dependent chemotactic response in spite of a reduced expression level of this GPCR in some patients. This gain in CXCR4 function in B lymphocytes extends our previous reports that also describe a gain of CXCR4 function in CD4+ and CD8+ T lymphocytes from patients suffering from WHIM-like diseases [11, 20, 28], later identified as GATA2 deficiency [11, 20, 28]. Here, although we observed a slight trend toward increased CXCR4-dependent chemotaxis in patients’ CD4+ and CD8+ T lymphocytes, that increase was not significant. Different factors could contribute to this apparent discrepancy with our former reports and must be explored. It could be related to sample treatment. In our previous reports, we used cryopreserved PBMCs, the isolation of which can enhance CXCR4 cell surface expression [70]. Nevertheless, such an effect is expected to equally influence PBMCs from patients and controls. In the present work, we performed experiments on fresh whole-blood cells, minimizing the handling of samples but introducing potential interferences from plasmatic components. Additionally, the clinical/infectious status of patients and their ongoing treatment can unevenly affect NK cells and B and T lymphocytes and need to be explored as a potential source of this discrepancy. Along this line, patients in our previous reports presented with active, life-threatening infections [20, 28].

Susceptibility to infections is a frequent clinical feature associated with GATA2 deficiency. In particular, HPV infections are frequently observed in these patients [12]. For patients with WHIM, who also suffer extensive and severe HPV infections [22, 23], the role of the CXCL12/CXCR4 pair in the susceptibility to this infection is strongly suggested by the control of viral pathogenesis in patients upon CXCR4 pharmacologic blockade or spontaneous biologic knockout of the CXCR4 mutant allele [27, 30, 31]. Moreover, in vitro expression of the WHIM-associated CXCR4 gain-of-function mutant promoted an evolution toward HPV-associated carcinogenesis [71]. However, the question as to whether the association between CXCR4 dysfunctions and HPV susceptibility can be extrapolated to GATA2 deficiency remains open. In summary, GATA2 deficiency and WHIM syndrome show significant differences, notably in term of blood and marrow leucopenia, but also display common pathologic traits, including the CXCR4 dysfunctions identified in this study although the dysfunctions manifest differently (Supplemental Table 2). Indeed, in contrast to the gain of CXCR4 function that is a hallmark of leukocytes derived from patients with WHIM, CXCR4-dependent chemotactic responses in patients with GATA2 deficiency are differently affected among lymphocytes with a marked loss of function for NK cells but an enhanced function for B lymphocytes. These results underscore the importance of delineating the mechanisms underlying CXCL12/CXCR4 dysfunctions, including their penetrance and potential role in the pathogenesis of GATA2 deficiency.


KEY WORDS: primary immunodeficiency - myelodysplastic syndrome - NK cells - cytokopenia - human papillomavirus infection - HPV
Altered chemotactic response to CXCL12 in patients carrying GATA2 mutations

Anna Maciejewski-Duval, Floriane Meuris, Alexandre Bignon, et al.

Access the most recent version at doi:10.1189/jlb.5MA0815-388R

Supplemental Material
http://www.jleukbio.org/content/suppl/2015/12/24/jlb.5MA0815-388R.DC1

References
This article cites 69 articles, 37 of which can be accessed free at:
http://www.jleukbio.org/content/99/6/1065.full.html#ref-list-1

Subscriptions
Information about subscribing to Journal of Leukocyte Biology is online at
http://www.jleukbio.org/site/misc/Librarians_Resource.xhtml

Permissions
Submit copyright permission requests at:
http://www.jleukbio.org/site/misc/Librarians_Resource.xhtml

Email Alerts
Receive free email alerts when new an article cites this article - sign up at
http://www.jleukbio.org/cgi/alerts

© Society for Leukocyte Biology
Figure S1. GATA2 mutations in patients. (A - B) Mutations in the GATA2 gene for the seven patients. Location and type of mutations are indicated above the gene structure. For most patients, mutations locate in exon coding regions (gray boxes), except for patient 5 who is carrying a splice site mutation and for patient 1 who has a large interstitial deletion in the chromosome 3 region, encompassing GATA2 gene. White boxes indicate exon non-coding regions. (C) Schematic representation of GATA2 protein indicating the relative position of the different functional domains and the location of the mutations occurring in patients (patient's number indicated in brackets). TAD: transactivation domain, NRD: negative regulatory domain, ZF1 and ZF2: N-terminal and C-terminal zinc finger domains, respectively, NLS: nuclear localization signal.
Supplemental Figure 2

Figure S2. Cell surface expression of chemokine receptors on NK cells and B lymphocytes from patients. (A) For patient 2, expression of CXCR4 and CXCR1 on NK cells and of CXCR4 and CXCR5 on B lymphocytes. (B) Expression of CXCR4 on NK cells from patients 3, 4, 5, 6 and 7 and of CXCR2 for patients 3, 4 and 7. Numbers inside histograms indicate MFI (upper right corner) and percentage of positive cells (lower right corner). Cont.: control, pat.: patient.
### Table S1. Leukocyte absolute counts in patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Neutrophils</th>
<th>Monocytes</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1330</td>
<td>70</td>
<td>490</td>
</tr>
<tr>
<td>P2</td>
<td>1100</td>
<td>280</td>
<td>1000</td>
</tr>
<tr>
<td>P3</td>
<td>1100</td>
<td>420</td>
<td>2900</td>
</tr>
<tr>
<td>P4</td>
<td><strong>8460</strong>a</td>
<td>110</td>
<td>1710</td>
</tr>
<tr>
<td>P5</td>
<td>1800</td>
<td>10</td>
<td>1200</td>
</tr>
<tr>
<td>P6</td>
<td>1600</td>
<td>20</td>
<td>1300</td>
</tr>
<tr>
<td>P7</td>
<td>5771</td>
<td>27</td>
<td>742</td>
</tr>
</tbody>
</table>

**Absolute values:** 2000 - 7500

<table>
<thead>
<tr>
<th>Patient</th>
<th>Neutrophils</th>
<th>Monocytes</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1330</td>
<td>70</td>
<td>490</td>
</tr>
<tr>
<td>P2</td>
<td>1100</td>
<td>280</td>
<td>1000</td>
</tr>
<tr>
<td>P3</td>
<td>1100</td>
<td>420</td>
<td>2900</td>
</tr>
<tr>
<td>P4</td>
<td><strong>8460</strong>a</td>
<td>110</td>
<td>1710</td>
</tr>
<tr>
<td>P5</td>
<td>1800</td>
<td>10</td>
<td>1200</td>
</tr>
<tr>
<td>P6</td>
<td>1600</td>
<td>20</td>
<td>1300</td>
</tr>
<tr>
<td>P7</td>
<td>5771</td>
<td>27</td>
<td>742</td>
</tr>
</tbody>
</table>

**Reference values:**

<table>
<thead>
<tr>
<th>Neutrophils</th>
<th>Monocytes</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>8460</strong>a</td>
<td>110</td>
<td>490</td>
</tr>
<tr>
<td>Reference values</td>
<td>1800</td>
<td>1000</td>
</tr>
<tr>
<td>1330</td>
<td>70</td>
<td>490</td>
</tr>
<tr>
<td>1100</td>
<td>280</td>
<td>1000</td>
</tr>
<tr>
<td>1100</td>
<td>420</td>
<td>2900</td>
</tr>
<tr>
<td><strong>8460</strong>a</td>
<td>110</td>
<td>1710</td>
</tr>
<tr>
<td>1800</td>
<td>10</td>
<td>1200</td>
</tr>
<tr>
<td>1600</td>
<td>20</td>
<td>1300</td>
</tr>
<tr>
<td>5771</td>
<td>27</td>
<td>742</td>
</tr>
</tbody>
</table>

**Absolute neutrophil, monocyte and lymphocyte counts were determined with automated analyzer at clinical laboratory services.** Cell counts of lymphocyte populations were calculated combining percentages obtained by flow cytometry and the absolute total lymphocyte counts. Arrows indicate subpopulations of T lymphocytes or of NK cells. Gray background marks values under normal range.

### Note

- **Reference values** are from the clinical laboratory service. ND, reference values not determined.
- **Values over normal range.**
- **Note CD4 / CD8 ratios.** Patient 1: 1.69 / 1, patient 2: 1.17 / 1, patient 3: 2.09 / 1, patient 4: 1.18 / 1, patient 5: 0.47 / 1, patient 6: 0.95 / 1, patient 7: 1.31 / 1. Normal CD4 / CD8 ratio ~ 2 / 1.
### Table S2. Genetic, clinic and immuno-hematologic features of GATA2 and WHIM disorders

<table>
<thead>
<tr>
<th></th>
<th>GATA2 deficiency</th>
<th>WHIM syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic defect</td>
<td>Heterozygous mutations in GATA2</td>
<td>Heterozygous mutations in CXCR4</td>
</tr>
<tr>
<td>Penetrance</td>
<td>Partial&lt;sup&gt;a, b, c&lt;/sup&gt;</td>
<td>Complete&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Consequence</td>
<td>GATA2 haploinsufficiency</td>
<td>Gain of CXCR4 function</td>
</tr>
<tr>
<td>Manifestation</td>
<td>From early childhood to late adulthood&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Early childhood&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical features</td>
<td>MDS / AML, immunodeficiency&lt;sup&gt;a, b, c&lt;/sup&gt;,</td>
<td>Immunodeficiency&lt;sup&gt;e, f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Widespread HPV infection (63 %)&lt;sup&gt;b&lt;/sup&gt; with high frequency of HPV-driven malignancy, disseminated NTM (53 %), miscellaneous viral, bacterial and fungal infections&lt;sup&gt;b, h&lt;/sup&gt;</td>
<td>Widespread HPV infection (58 %) with high frequency of HPV-driven malignancy (30%), pyogenic and mycobacterial infections&lt;sup&gt;e, f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Main hematologic</td>
<td>Peripheral and marrow pancytopenia mainly affecting B and NK lymphocytes and monocytes with frequent evolution to MDS and high risk to develop AML&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>Peripheral panleukocytopenia&lt;sup&gt;e, f&lt;/sup&gt;</td>
</tr>
<tr>
<td>features</td>
<td>Hypocellular BM with reticulin fibrosis (46 %) and relative increased polyclonal CD3+CD56+, CD3+CD57+ (14 %)&lt;sup&gt;c&lt;/sup&gt; and CD3+CD8+CD56+ large granular lymphocyte populations (16 %)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Hypercellular marrow with myelokatexis&lt;sup&gt;e, f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Progressive exhaustion of hematopoietic progenitors&lt;sup&gt;a, c, h, i, j&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Immune cells</td>
<td>Poor NK cell cytotoxic function&lt;sup&gt;k&lt;/sup&gt;</td>
<td>Impaired B-lymphocyte class switch and memory response&lt;sup&gt;b, q&lt;/sup&gt;</td>
</tr>
<tr>
<td>dysfunctions</td>
<td>Presumed macrophage dysfunction&lt;sup&gt;b&lt;/sup&gt;</td>
<td>CXCL12/CXCR4-driven dysfunctions (increased T-lymphocyte and neutrophil chemotaxis and decreased CXCR4 internalization)&lt;sup&gt;m, o, r&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>CXCL12/CXCR4-driven chemotaxis dysfunctions (decreased in NK cells from most patients&lt;sup&gt;l&lt;/sup&gt; and increased in B&lt;sup&gt;i&lt;/sup&gt; and T&lt;sup&gt;m, n, o&lt;/sup&gt; lymphocytes from some patients.</td>
<td></td>
</tr>
</tbody>
</table>

<sup>c</sup> Ganapathi et al. 2015, *Blood* 125, 56-70.  
<sup>e</sup> Beaussant Cohen et al. 2012, *Orphanet J. Rare Dis.* 7, 71.  
<sup>g</sup> Percentage in parentheses indicates the reported ratio of affected patients.  
<sup>i</sup> Calvo et al. 2011, *Haematologica* 96, 1221-5.  
<sup>j</sup> Bigley et al. 2011, *J. Exp. Med.* 208, 227-34.  
<sup>k</sup> Mace et al. 2013, *Blood* 121, 2669-77.  
<sup>l</sup> reported in the present work.  
<sup>o</sup> Balabanian et al., 2008, *JCI* 118,1074-84.  
<sup>q</sup> Handisurya et al. 2010, *Vaccine* 28, 4837-41.  