Low-affinity immunoglobulin gamma Fc region receptor III-B (FcγRIIIB, CD16B) deficiency in patients with blood and immune system disorders

Alfredo Minguela,1,2 Eduardo J. Salido,2,3 María F. Soto-Ramírez,1,2 Montes-Ares Olga,1,2 Juan D. Leal,2,3 María C. García-Garay,2,3 Adela Periago,2,4 Mercedes Berenguer,2,5 Miguel Blanque2,3 and José A. Campillo1,2
1Immunology Service, Clinic University Hospital Virgen de la Arrixaca (HCUVA), 2Biomedical Research Institute of Murcia (IMIB), 3Hematology Service, Clinic University Hospital Virgen de la Arrixaca (HCUVA), Murcia, 4Hematology Service, Hospital Rafael Méndez, Lorca, and 5Hematology Service, General University Hospital Santa Lucía, Cartagena, Spain

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Correspondence: Alfredo Minguela, Immunology Service, Clinic University Hospital Virgen de la Arrixaca (HCUVA), El Palmar, 30120 Murcia, Spain.
E-mail: alfredo.minguela@carm.es

Introduction

The low-affinity immunoglobulin gamma Fc region receptor III-B (FCGR3B) copy number variation (CNV)1 is a special feature of this gene that encodes for the FcγRIIIB (or CD16B) mainly expressed on mature neutrophils. Whilst genotypes with a single copy of the FCGR3B gene are associated with systemic lupus erythematosus (SLE), rheumatoid arthritis and systemic autoimmunity,2-3 FCGR3Bnull genotype is not associated with autoimmunity, immune-complex-mediated diseases or immunodeficiency.4 Human neutrophils, natural killer (NK) cells, T lymphocytes and macrophages can express up to six different FcγRs: FcγRI (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIC (CD32C), FcγRIIIA (CD16A) and FcγRIIIB.5 They interact with and respond to aggregated immunoglobulins, immune-complexes and opsonised particles.5 The main function of FcγRIIIB in neutrophils is the removal of spontaneously forming immune-complexes to dampen Fc-dependent immune reactions.5 Neutrophils also express modest amounts of FcγRIIIA.4 Engagement of FcγRIIIA on neutrophils from FcγRIIIB-deficient individuals efficiently triggers cell activation and may explain why FcγRIIIB deficiency has no impact on the functional capacity of neutrophils.4 Thus, FCGR3Bnull individuals are detected randomly when immunophenotyped with anti-CD16 for diverse clinical conditions. FcγRIIIB deficiency is a rare event present in ~0.001% in the Netherlands, 0.028% in France, 0.047% in Japan or 0.08% in Spain,6 with most of the reported cases concerning healthy young mothers of newborns with neutropenia mediated by maternal anti-FcγR3 antibodies.7 Nonetheless, sporadic cases with blood disorders such as anaemia related to paroxysmal nocturnal haemoglobinuria (PNH)8 or severe idiopathic aplastic anaemia9 have also been reported.

Summary

Low-affinity immunoglobulin gamma Fc region receptor III-B (FcγRIIIB) deficiency is present in ~0.05% of the general population. Among our patients, FcγRIIIB deficiency was less frequent in those with immune-system disorders (one of 1815 patients, 0.05%) than in those with blood disorders (nine of 2147 patients, 0.42%, P = 0.023): mainly primary immune thrombocytopenia (4.34%), therapy related myeloid neoplasms (1.16%) and myelodysplastic syndrome with excess blasts (1.28%). Four of the nine (44.4%) patients with blood disorders were diagnosed with or quickly evolved to acute myeloid leukaemia (AML), suggesting that FcγRIIIB deficiency could be an adverse prognostic factor for progression to AML that should be confirmed in large multicentre studies.

Keywords: low-affinity immunoglobulin gamma Fc region receptor III-B (FcγRIIIB or CD16B) deficiency, acute myeloid leukaemia, blood diseases, immune system disorders.
Patients and methods

To assess the incidence of FcγRIIIB deficiency and its clinical implications, immunophenotypic analysis including CD16 monitoring of bone marrow (BM) and/or peripheral blood neutrophils were retrospectively reviewed in 2147 and 1815 patients with blood and immune-system disorders, respectively. The institutional review board (IRB-00005712) approved the study. Written informed consent was obtained from all patients and controls in accordance with the Declaration of Helsinki.

Samples anti-coagulated with ethylenediamine tetra-acetic acid (EDTA) were labelled with CD11b, CD13, CD14, CD16, CD34 (in BM samples), CD45, CD56 and human leucocyte antigen-DR isotype (HLA-DR) [Becton Dickinson (BD), San Diego, CA, USA] following standard procedures, acquired in an eight-colour FACSCanto-II (BD, until June 2020) or 12-colour FACSLyric (BD) flow cytometers and analysed in Diva software (BD) following the gating strategy described in Fig 1A. To evaluate the functionality of neutrophils in patients with immune-system disorders, Phagotest™ (BD) and oxidative-burst test using dihydrorhodamine (DHR; Thermo Fisher Scientific, Waltham, MA, USA) were used. Data analysis using the Statistical Package for the Social Sciences (SPSS®) version 15.0 (SPSS Inc., Chicago, IL, USA), included Pearson’s chi-square and analysis of variance with post hoc tests for categorical or continuous variables, respectively. Kaplan–Meier curves were used to evaluate progression to acute myeloid leukaemia (AML). Time to event (progression to AML) was estimated as months from the first CD16 immunophenotypic analysis. A P < 0.05 was considered statistically significant.

Results

FcγRIIIB deficiency is eight-times more frequent among patients with haematological than immunological disorders

FcγRIIIB deficiency was significantly more frequent among patients with blood [nine of 2147 patients (0.42%), P = 0.023] than with immune-system disorders [one of 1815 patients (0.05%); Table I]. In patients with blood disorders, FcγRIIIB deficiency was detected more frequently in those

Fig 1. Detection and clinical evolution of patients with FcγRIIIB (CD16B) deficiency. (A) Gating strategy of the immunophenotypic analysis to detect FcγRIIIB (CD16B) deficiency: (1) singlets were selected in a FSC-A/FSC-H dot plot; (2) lymphocytes and iRBC were gated in a FSC/SSC dot plot (FSC/SSClow); (3) iRBC (CD45 SSClow) and granulocytes (CD45 SSChigh) were selected in a CD45/SSC dot plot, logical gating was applied to differentiate lymphocytes and iRBC; (4) monocytes (CD33high HLA-DR+) were selected in a CD33/HLA-DR dot plot; (5) eosinophils (CD45 intermediate CD16+) were selected in a CD45/CD16 dot plot, logical gating was applied to differentiate neutrophil and eosinophil granulocytes; (6) CD34+ progenitor cells were selected in a CD34/SSC dot plot; and (7) NK cells were selected in a CD13/CD16 dot plot and logically combined with lymphocytes. The expression of CD16 in neutrophils to discriminate FcγRIIIB deficiency was evaluated in a CD16/SSC dot plot. Lower dot plots show two representative patients, without and with FcγRIIIB deficiency. (B) Kaplan–Meier curves for progression to AML according to the type of haematological disease. Cumulative incidence of AML is indicated in the graph. Table shows number of patients at risk. AML, acute myeloid leukaemia; FcγRIIIB, immunoglobulin gamma Fc region receptor III-B; FSC(-A)(-H), forward scatter (-area) (-height); HLA-DR, human leucocyte antigen-DR isotype; iRBC, immature red blood cells; NK, natural killer; SSC, side scatter. [Colour figure can be viewed at wileyonlinelibrary.com]
screened for immune thrombocytopenia (ITP, 4-34%), therapy related myeloid neoplasms (1-16%), myelodysplastic syndrome (MDS) with excess blasts (1-28%), AML (0-75%), idiopathic cytopenia of undetermined significance (ICUS) such as anaemia, thrombocytopenia and leucopenia (0-30%), PNH (0-26%) and chronic myeloproliferative neoplasm (cMPN) such as thrombocytopenia, polycythaemia, leucocytosis, chronic myelomonocytic leukaemia or myelofibrosis (0-22%). No cases were detected in patients screened for MDS, pancytopenia or chronic myelogenous leukaemia (CML).

The only patient seen in the immunology clinic with bronchiectasis showed neutrophils with normal phagocytic and oxidative functional studies, as previously described in patients with FcγRIIIB deficiency.4

Clinical and biological characteristics of patients with FcγRIIIB deficiency

In May 2021 a new diagnosis of AML with mutated nucleophosmin 1 (NPM1), normal FMS-like tyrosine kinase-3 (FLT3) [internal tandem duplication (ITD) and tyrosine kinase domain (TDK)] and normal karyotype was made for a 56-year-old male. The patient received standard therapy (idarubicin + cytarabine) achieving complete remission. Interestingly this patient showed a typical pattern of FcγRIIIB deficiency with CD16+ neutrophils and CD16+ NK cells. This made us wonder: what would have been the clinical evolution of other cases with this deficiency? From a total of 10 cases with FcγRIIIB deficiency detected in a 9-year retrospective study, three additional cases had developed early fatal AML transformation:

1. A 73-year-old man diagnosed with follicular lymphoma in 2010. The patient was treated with immunochemotherapy [R-CHOP (rituximab-cyclophosphamide, doxorubicin, vincristine and prednisone) and R-FC (rituximab-fludarabine, cyclophosphamide)]. In 2013, he was diagnosed with high risk MDS and treated with azacitidin. AML progression occurred and patient died in 2015.
2. An 85-year-old female diagnosed with cMPN with Janus kinase 2 (JAK2) V617F mutation (primary myelofibrosis) in 2018. The patient progressed to AML in 2019 and died in 2021.
3. A 74-year-old male diagnosed with high risk MDS in 2016 with early AML transformation who died this year.

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The remaining six patients who did not develop leukaemic transformation are:

1. A 74-year-old male and a 31-year-old female currently diagnosed with primary ITP.
2. Two females aged 62 and 64 years currently diagnosed with ICUS without progression.
3. A 25-year-old patient with Budd-Chiari syndrome who was studied to rule out PNH clone.
4. A 24-year-old patient with immune-system disorders and bronchiectasis.

High cumulative incidence of AML was observed in patients with FcγRIIIB-deficiency

A growing cumulative incidence of AML was found between patients with PNH (0%), ITP (0%), ICUS (1-8%), CML (2-73%), pancytopenia (5-9%), MDS (14-3%), therapy related myeloid neoplasms (18-0%), MDS with excess blasts (36-4%) and finally, with the highest rate in our present series, patients with FcγRIIIB deficiency (44-4%). Kaplan–Meier curves and cumulative incidence of AML are shown for each pathology group in Fig 1B.

Discussion

In the present study, nine cases with FcγRIIIB deficiency treated in our haematology unit between 2013 and 2021 are
described. Remarkably, four of the nine cases (44.4%) were diagnosed with or evolved to AML. Although causality cannot be established between the FcγRIIIB deficiency and the progression to AML, the proportion of patients with this severe disease within the FcγRIIIB-deficiency cohort was truly alarming. In fact, FcγRIIIB deficiency was detected eight-times more frequently among patients with blood disorders than among those with immune-system alterations. Thus, whereas the frequency of FcγRIIIB deficiency among immunological patients remained within the expected ranges in Spain (0.05%), the frequency was much higher in patients with blood disorders (0.42%), and much more pronounced among patients with ITP (4.34%), therapy related myeloid neoplasms (1-61%), MDS with excess blasts (1-28%) or AML (0-75%).

There is not an easy explanation for the association of FcγRIIIB deficiency with the accelerated progression to AML observed in patients with clonal myeloid disorders in our present series. Nonetheless, it is well known that CNVs represent a significant source of genetic diversity that may cause cancer predisposition inherited in a Mendelian fashion. Although our present results suggest that FcγRIIIB deficiency in patients with clonal myeloid disorders could be an adverse prognostic factor, its low frequency makes it necessary to carry out multivariate analyses in large multicentre series to determine its independent prognostic value in relation to other well-characterised predictive variables. Besides, outside the context of clonal myeloid disorders (ICUS, ITP, PNH, etc.), our present data indicate that this deficiency may have a more controversial meaning.

In line with most cases reported in the literature,6,7 we cannot rule out that some of our present cases may just be random. Nonetheless, two cases were screened due to thrombocytopenia, one of them associated to antiphospholipid syndrome. A link among ITP, SLE12 and low copy number of FCGR3B gene1,2 has been well established. The patient with chronic thrombocytopenia debuted with the first pregnancy and therefore FcγRIIIB-related isomunisation should not be ruled out. Another two of the nine cases in our present series were screened because of anaemia and finally diagnosed with ICUS. In line with these two cases, two apparently sporadic cases with anaemia related to PNH8 and severe idiopathic aplastic anaemia9 have been reported. Regarding our case with the Bud–Chiari syndrome, it should be taken into account that polycythaemia vera and essential thrombocytaphaemia are the most frequent aetiologies.13 However, the patients with immune-system disorders, bronchiectasis and normally functioning neutrophil could be among the expected casual findings.

The low frequency of the FCGR3Bnull genotype in the general population makes it difficult to achieve sufficient power for calculation. This may be the reason why there are contradictory reports describing that low copy numbers of FCGR3B (one) are associated with immunopathology2,3,14 while FCGR3Bnull genotype is not, although one report suggested an association with SLE.15 In this line, many immunophenotype analysts are detecting and describing this alteration but, in general, clinical haematologists are unaware of the implications of this genetic alteration and the existing literature does not impel them to explore and follow-up these patients more closely, so this association may have gone mostly unnoticed up to now.

Therefore, although these results should be validated in larger independent series, they suggest that we should reconsider whether FCGR3Bnull cases that appear in haematology clinics worldwide are casual. Results of our present series indicate that these patients could have cumulative incidence of AML as high as those with MDS with excess blasts or therapy related myeloid neoplasms, and therefore, they should be clearly informed by immunophenotype analysts and haematologists should monitor these patients more closely.

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Author contributions

Alfredo Minguela analysed the data, wrote the manuscript and approved the final version for submission. Eduardo J. Salido supervised the study and contributed with the manuscript writing. Montes-Ares Olga, María F. Soto-Ramírez, Juan D. Leal, María C. García-Varay, Adela Periago, Mercedes Berenguer and Miguel Blanquer collaborated in the review of the patients’ medical records. José A. Campillo collaborated in the immune-phenotype analysis and supervised the research.

Conflict of interest

The authors declare no conflicts of interest.

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