Immunol Res (2015) 61:260–268
DOI 10.1007/s12026-014-8619-8

Successful adjunctive immunoglobulin treatment in patients affected by leukocyte adhesion deficiency type 1 (LAD-1)

Marco Yamazaki-Nakashimada · José L. Maravillas-Montero · Laura Berrón-Ruiz · Orestes López-Ortega · Noé Ramírez-Alejo · Ernesto Acevedo-Ochoa · Francisco Rivas-Larrauri · Beatriz Llamas-Guillén · Lizbeth Blancas-Galicia · Selma Scheffler-Mendoza · Alberto Olaya-Vargas · Leopoldo Santos-Argumedo

Published online: 21 December 2014

Abstract Two patients with a severe leukocyte adhesion deficiency type 1 (LAD-1) phenotype were analyzed by flow cytometry and functional assays to demonstrate the improper adhesive and phagocytic responses of their leukocytes. A single homozygous defect that involves a missense mutation (c.817G>A) that encodes for a G273R substitution in CD18 was identified in both patients. The adhesion and phagocytosis assays demonstrated the inability of patients' leukocytes to perform these functions. Expression of the LFA-1 (CD11a/CD18) on the co-transfected HEK 293 cells with the mutated form of CD18 was not detected. Finally, both patients have been treated with immunoglobulin as an adjunctive therapy with positive results. We propose that intravenous immunoglobulin treatment is safe and efficacious in LAD-1 patients before hematopoietic stem cell transplantation and helpful in controlling severe infections. Subcutaneous immunoglobulin appeared to help wound healing in refractory ulcers in these patients.

Keywords Primary immunodeficiency · LAD-1 · IVIG · SCIG · Adjunctive therapy · Wound healing

Introduction

Immunoglobulin replacement therapy is a life-saving treatment for individuals with primary immunodeficiency diseases (PIDs). Today, intravenous immunoglobulin (IVIG) and subcutaneous immunoglobulin (SCIG) are available for these patients. However, they are not used routinely in all PIDs [1]. The effectiveness of

Marco Yamazaki-Nakashimada and José L. Maravillas-Montero have contributed equally to this work.

M. Yamazaki-Nakashimada · F. Rivas-Larrauri · S. Scheffler-Mendoza
Departamento de Inmunología Clínica, Instituto Nacional de Pediatría, CP 04530 Mexico City, Mexico

J. L. Maravillas-Montero · L. Berrón-Ruiz · O. López-Ortega · N. Ramírez-Alejo · E. Acevedo-Ochoa · L. Santos-Argumedo (✉)
Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Avenida IPN 2508, CP 07360 Mexico City, D.F., Mexico
e-mail: lesantos@cinvestav.mx

J. L. Maravillas-Montero
Red de Apoyo a la Investigación, Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán"-Coordinación de la Investigación Científica, Universidad Nacional Autónoma de México, CP 14000 Mexico City, Mexico

L. Berrón-Ruiz · L. Blancas-Galicia · S. Scheffler-Mendoza
Unidad de Investigación en Inmunodeficiencias, Instituto Nacional de Pediatría, CP 04530 Mexico City, Mexico

B. Llamas-Guillén
Hospital del Niño y el Adolescente Morelense, CP 62765 Cuernavaca, Morelos, Mexico

A. Olaya-Vargas
Departamento Oncología, Unidad de Trasplantes de Células Madre Hematopoyéticas, Instituto Nacional de Pediatría, CP 04530 Mexico City, Mexico

immunoglobulin for reducing serious bacterial infections in X-linked agammaglobulinemia (XLA) and common-variable immunodeficiency (CVID) is well documented [2]. Immunoglobulins are also used for combined defects with significantly impaired antibody production [3].

Leukocyte adhesion deficiency type 1 (LAD-1) is a rare recessive inherited PID with an incidence of 1 in 1 million, involving a defect in neutrophil adhesion and characterized by skin ulcers, poor wound healing and recurrent bacterial infections [4]. This disease is caused by mutations in the CD18 gene (*ITGB2*) that encodes for the β_2 integrin subunit [5].

Each of the β_2 integrins is a heterodimer composed of one α chain (CD11a, CD11b, CD11c or CD11d) non-covalently linked to the common β_2 subunit CD18 [6, 7]. The association of both CD11 and CD18 subunits is required for normal surface-membrane expression and function of these receptors [8, 9].

Failure to produce a functional β_2 subunit results in the defective membrane expression of all β_2 integrin leukocyte integrins, thus generating subnormal adhesive properties and the clinical features of LAD-1 [5, 8, 10, 11].

In LAD-1, transfusions of granulocytes may be beneficial in severe infections [12]. At the moment, the only curative treatment for the severe phenotype is hematopoietic stem cell transplantation (HSCT) [5, 13]. Here, we present a molecular level-confirmed LAD-1 case, which has been treated with monthly IVIG diminishing the frequency of severe infections. SCIG was used successfully for the treatment of refractory ulcers.

Methods

Flow cytometry

Aliquots of whole blood were incubated with anti-CD18-FITC or anti-CD11b-FITC and γ1-FITC/γ2-PE/anti-CD45-PerCP isotype control (BD) 20 min at 4 °C in the dark. Erythrocytes were lysed with FACS lysing solution (BD), and remaining cells were washed with PBS containing 1 % BSA and fixed with PBS containing 1 % formalin. Samples were captured and then analyzed using FACSAria (BD Biosciences) and FlowJo 7.2.4 software (Tree Star Inc).

ITGB2 (CD18 gene) mutation analysis

Genomic DNA was obtained from EBV-B cell lines with DNAzol reagent (Life Technologies). Total RNA was extracted from peripheral blood mononuclear cells with TriZOL reagent (Life Technologies) and cDNA converted (SuperScript® III RT, Life Technologies). *ITGB2* gene was amplified with primers spanning complete coding sequence and from genomic DNA with primers spanning exon 7. The products were sequenced with ABI PRISM® Big Dye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) according to the manufacturer instructions.

Leukocyte adhesion assays and confocal microscopy

During 1 h at 37 °C, 96-well polystyrene plates (Nalge Nunc) or 8-well chamber slides (Lab-Tek) were coated with poly-L-lysine (0.01 %, Sigma) or fibronectin (2.5 µg/ml, Takara). The plates and slides were then washed twice with PBS 1× before adding 100 µl of heparinized whole blood per well. The cells were allowed to adhere for 1 h at 37 °C. After that, the plates and slides were carefully washed with 300 µl of PBS 1× and fixed with 4 % paraformaldehyde for 10 min.

For confocal microscopy, the cells were permeabilized with 0.01 % of Triton X-100 in PBS 1× and incubated with phalloidin–rhodamine and DAPI (molecular probes) for 15 min. The preparations were mounted in Vectashield (Vector Laboratories) and analyzed with an Olympus microscope using 40× or 60× objectives and Olympus FluoView software for processing images.

For quantitative adhesion measurements, we added crystal-violet (crystal-violet 7.5 g/l, NaCl 2.5 g/l, formaldehyde 1.57 % and methanol 50 %) to the 96-well plates for an additional 5 min. The cells were washed extensively three times with distilled water and solubilized with 10 % SDS, and the color retained was quantified at 540 nm (Multiskan Ascent, Thermo Scientific). After subtraction of non-specific colorimetric readings to obtain absolute binding, the absorbance for each well was registered, in at least four wells per condition, in two independent experiments.

Phagocytosis assays

A total of 200 µl of sheep red blood cells (SRBC) were opsonized with rabbit anti-SRBC IgG at a final dilution of 1:1,000 plus 100 µl of fresh human serum at a final dilution of 1:10 during 30 min at 37 °C and then washed with PBS 1× three times. A total of 500 µl of heparinized whole blood were plated on 35-mm glass-bottom Petri dishes (WillCo Wells) and incubated for 1 h at 37 °C. After this period, the non-adhered blood was carefully washed away with PBS 1× and then 3×10^6 opsonized SRBC were added incubating the plates for an additional hour. The plates were washed again to eliminate most of the SRBC and then observed at an Olympus microscope using differential interference contrast (DIC) and Olympus FluoView software for processing images.

Expression of CD11/CD18 in HEK 293 transfectants

A total of 1 μg of TRIzol (Life Technologies) extracted total RNA of PBMC, from both a healthy donor and the patient, which was reverse-transcribed using SuperScript II (Life Technologies). The resulting cDNA was used as template to amplify the complete coding regions of both LFA-1 components, using high-fidelity DeepVent DNA polymerase (New England Biolabs). The sequences of the oligonucleotides used to amplify CD18 were CTAGGTAC CGGACATGCTGGGCCTGCGCCC and CTAAGCGGCC GCCTCCTAACTCTCAGCAAACTTGG; for CD11a, the sequences used were CTGGTACCATGAAGGATTCCTG CATCACTGTG and CTAAGCGGCCGCTCAGTCCTTG CCACC. The resulting fragments were purified with the QIAquick Gel Extraction kit (QIAGEN) and cloned into the expression vector pTracer-CMV2 (Life Technologies), according to conventional procedures using the restriction enzymes KpnI and NotI (New England Biolabs). HEK 293 cells were grown in RPMI-1640 with 10 % FBS up to 60 % of confluence in six-well culture plates and then lipid-based-transfected with the different plasmids in Polyfect reagent (QIAGEN) according to the manufacturer's protocol. The cells were allowed to recover in RPMI with 10 % FBS after transfection for 72 h at 37 °C in 5 % CO2 incubator. Surface expression of CD11/CD18 antigens was analyzed by flow cytometry as described above.

Declaration of ethical approval

All studies presented here have been approved by the institutional research and ethics committee and have been performed in accordance with the 1964 Declaration of Helsinki and his later amendments. Written consent was obtained from parents and/or patients before blood was drawn.

Results

Case report

Patient 1

A 3-month-old male patient with a family background of first degree consanguinity and a deceased sister secondary to sepsis at 6 month of age was referred to our hospital with omphalitis and neutrophilia. He presented delayed umbilical cord separation at 30 days of life. At the time of hospitalization, his blood cell count revealed leukocytosis (58.4×10^3 μl) and neutrophilia (28×10^3 μl). The dihydrorhodamine 123 assay was normal (neutrophil oxidative index = 22). He also manifested gastroenteritis, otitis, complicated balanoposthitis, ecthyma gangrenosum, gluteal cellulitis and oral candidiasis. Monthly intravenous gamma globulin therapy was started and has been continued until today (500 mg/kg/month). At 2 years old, umbilical cord blood stem cell transplantation was performed without engraftment. At 2 years 8 months of age, he presented perianal ulcers without response to antibiotics leading to surgical debridement (Fig. 1a). We initiated administration of granulocyte colony-stimulating factor (G-CSF) as previously recommended with little improvement (4). The lesions persisted for 2 months without healing. Subcutaneous gamma globulin at the perianal site was initiated (135 mg/kg/week for 4 weeks) with important improvement (Fig. 1b). A second haploidentical stem cell transplant was performed but without engraftment. At present, he is waiting for third stem cell transplantation.

Patient 2

A 10-month-old male with family background of second-degree consanguinity was admitted to our hospital. Three days after birth, he was admitted to another hospital due to severe omphalitis with culture positive for a multiresistant *Escherichia coli*, treatment with meropenem started with poor response. The extension of the lesion rapidly increased with necrosis (Fig. 1c). Debridement of necrotic tissue was performed, but the lesion did not heal and

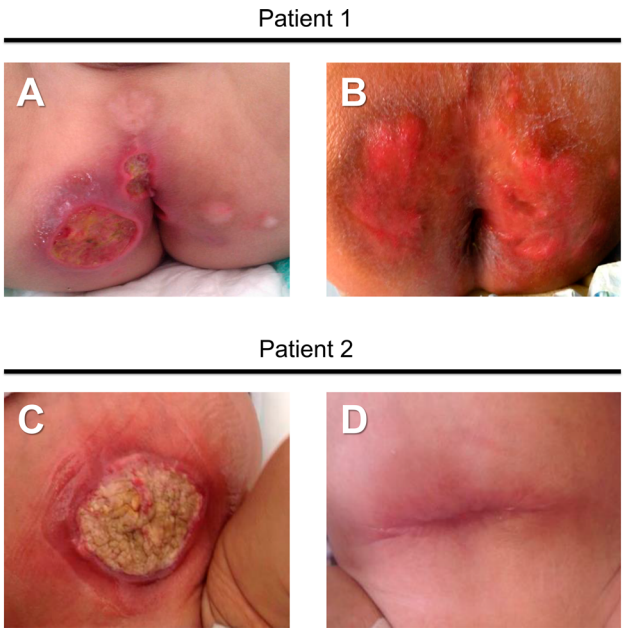

Fig. 1 Successful lesion treatment with SCIG. **a** Refractory non-healing wound in the perianal area of patient 1. **b** Nine months after four weekly doses of subcutaneous immunoglobulin, the lesion completely healed. **c** Refractory non-healing wound in periumbilical area of patient 2. **d** After 3 months of treatment of subcutaneous immunoglobulin, the lesion completely healed

progress. Blood tests revealed leukocytosis (29×10^3 µl) and neutrophilia (18×103 µl). We started administration of granulocyte colony-stimulating factor (G-CSF) in the peri-umbilical wound and subcutaneous gamma globulin with improvement and remission of the local defect (Fig. 1d). The patient required further admissions due to cytomegalovirus infection (with PCR positive for CMV), treated with ganciclovir. Others events of pneumonia, urinary infection, gastroenteritis and sepsis were treated with early antibiotic therapy with good clinical course. Our patient also coursed with autoimmune manifestation characterized by hemolytic anemia treated with prednisone (0.3 mg/kg/day). Due to severe and recurrent infections, the patient is waiting for hematopoietic stem cell transplantation, and currently, he is treated with prophylactic antibiotic and monthly intravenous gamma globulin therapy.

The clinical laboratory flow cytometry data indicated that nearly no surface expression CD18 (Fig. 2a) or CD11b (Fig. 2b) on the patients' leukocytes could be found, in contrast to a normal control. In any case, there is a clear reduction in the levels of both integrin subunits, scoring below 1 % for each cell population depicted here, when compared with the control sample that shows nearly 100 % of expression on each population.

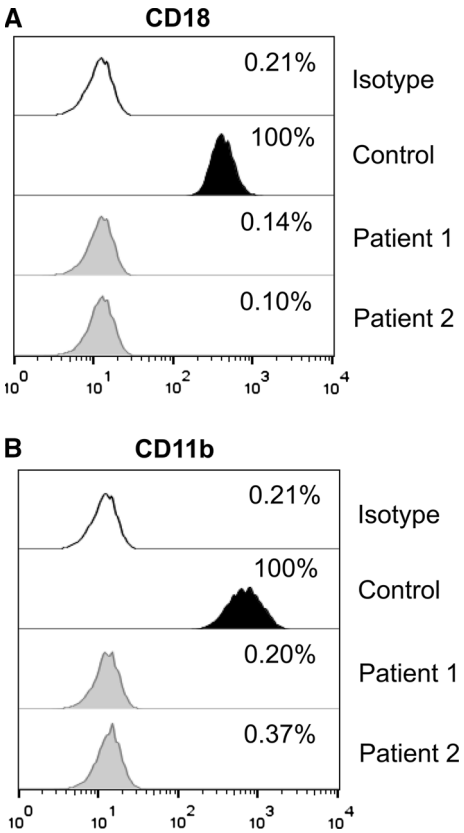

Fig. 2 Expression of CD18 and CD11b on leukocyte populations. Total blood samples collected from the patients were stained with monoclonal fluorescent-labeled antibodies against CD18 and CD11b. For selecting the displayed populations, the cells were gated using FSC and SSC parameters selecting the corresponding granulocyte population. Percentages of cells expressing each molecule are displayed next to each histogram

Sequence variations detected in *ITGB2*

We sequenced the coding exonic regions of CD18 gene (*ITG2B*) in cDNA prepared from the patients' peripheral blood mononuclear cells (PBMCs), finding a c.817G>A nucleotide substitution in exon 7 of *ITG2B* in both patients (Fig. 3a). This is a previously reported missense mutation [14] that leads to the replacement of the normally encoded glycine by an arginine (G273R, Fig. 3b). This mutation was confirmed by sequencing the exon 7 of *ITGB2* in patients' gDNA. Interestingly and again in the two patients, another single nucleotide substitution, c.819G>A, was detected (Fig. 3a, b). This substitution resides in the same codon as the previously mentioned mutation, but does not result in any amino acid alteration, thus representing a previously characterized polymorphism [15]. Both patients were shown to be homozygous for the mutation c.817G>A by sequencing of their genomic DNA.

Patients' leukocytes present adhesion and phagocytosis defects

In order to demonstrate that the detected sequence variations in *ITGB2* gene actually impact in the β_2 integrin functions, we performed some functional assays using blood samples of the patients.

First, we tested the ability of patients' leukocytes to remain attached to a surface cover either with a general adhesive substrate, such as poly-L-lysine [16], or one of the many described ligands of β_2 integrins, such as fibronectin [17]. As expected for any LAD-1 patient, blood leukocytes, predominantly PMN cells, presented a clear defect in adhering to both substrates when compared with leukocytes obtained from a healthy donor (Fig. 4a). The control cells attached to fibronectin and developed some adhesive membrane filopodia-like processes that were not observed in the few leukocytes from the patients that remained attached to the slides (Fig. 4b). To quantify the adhesive response of these cells, we used a colorimetric assay with a crystal-violet staining of the adhered leukocytes to the same substrates. As showed in Fig. 4c, for every substrate and even with the plain plastic plates, the patients' blood cells were significantly less adhesive than the control samples.

According to the previous results, we were also able to observe a pronounced reduction in the ability of phagocytosis in the patients' leukocytes. When these cells were

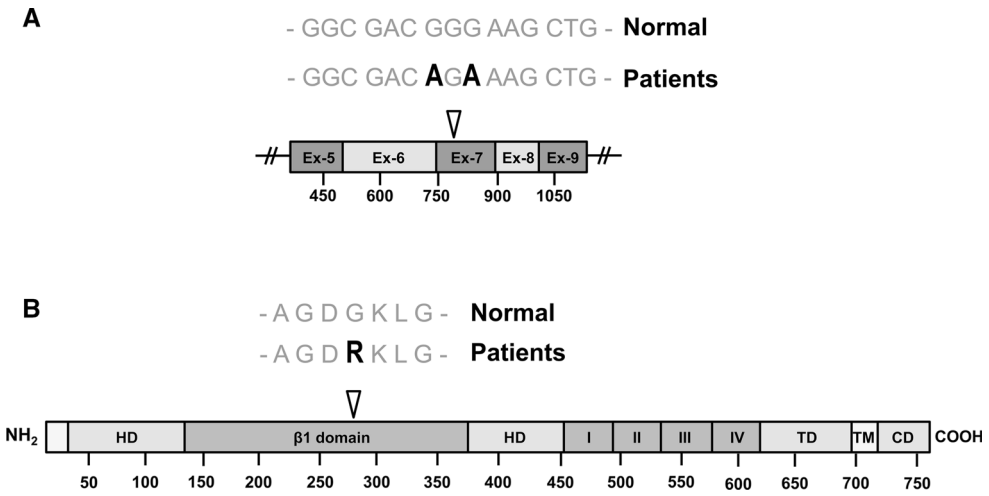

Fig. 3 Sequence variations revealed in the *ITGB2* gene. **a** Sequencing of cDNA samples extracted from patients' PBMCs revealed two single nucleotide changes in *ITGB2*: c.817G>A (missense mutation) and c.819G>A (polymorphism), both located at exon 7. **b** Schematic representation of the CD18 protein showing that the missense mutation found in both patients generates a glycine for arginine substitution in the β1 domain of the protein. *HD* hybrid domain, *I–II–III–IV* cysteine-rich repeats, *TD* tail domain, *TM* transmembrane domain, *CD* cytoplasmic domain

fed with opsonized SRBC and compared with the control cells, we clearly observed that the internalization of the SRBC was abolished in the patients' leukocytes (Fig. 4d).

The G273R substitution in CD18 abolishes the expression of surface of LFA-1

As was previously stated, the mutations in CD18 prevent the cell surface expression of β_2 integrins. We corroborated this observation by cloning the full coding sequence of the mutated *ITGB2* patients' genes into an expression vector and subsequently co-expressed it with the α subunit CD11a, in HEK 293 cells. We were unable to detect membrane expression of either LFA-1 subunits (nor CD18 or CD11a). When we used a wild-type *ITGB2* sequence as a control, we could get some transfectants co-expressing both integrin subunits as expected (Fig. 5).

Discussion

Clinically, LAD-1 cases are subdivided into severe and moderate. In the severe form of the disease (less than 1 % expression of CD18), the prognosis is very poor without hematopoietic stem cell transplantation [18]. The clinical presentation of the patients analyzed here fits exactly with the previous descriptions, presented all the clinical features mentioned in the literature, and also can be categorized as severe forms of LAD-1 due to the less than 1 % of CD18 expression found in each of their blood leukocyte populations.

As previously reported by different groups, the inability to express CD18 in the surface of leukocytes together with the different integrin α subunits (CD11a, CD11b, CD11c and CD11d) is typically caused by mutations in the *ITGB2* gene [9]. Currently, 86 different allelic mutations related to LAD have been reported for this gene including deletions, insertions, splice site, nonsense and missense mutations; interestingly, most of these variations correspond to missense mutations and are located at the region corresponding to the β1 domain of CD18 [9].

In cDNA samples of the patients described in this study, we were able to detect two sequence changes that are both located in the mentioned β1 domain of CD18: c.817G>A and c.819G>A. When genomic DNA was analyzed, we confirmed the patients' homozygosity for both changes located in exon 7 of *ITGB2*. We suspected that the first change, which leads to a G273R substitution, could be responsible for the LAD phenotype in the patients. Indeed, we found that this mutation was previously reported by Hogg et al. [14]. The second change in the 819 position, which would not change the glycine encoded normally at this position, was also reported as a polymorphism by different authors [9]. It is important to highlight that in our patients, both point sequence changes are present in the same 273 codon of the *ITGB2* gene, although the c.817G>A is the mutation responsible for the amino acid substitution in the CD18 protein. Based on our study and the studies of the patients mentioned above, it likely represents the cause of the disease in our cases [9, 14].

The functional analyses performed with blood cells of the patients supported the diagnosis of LAD-1 because the

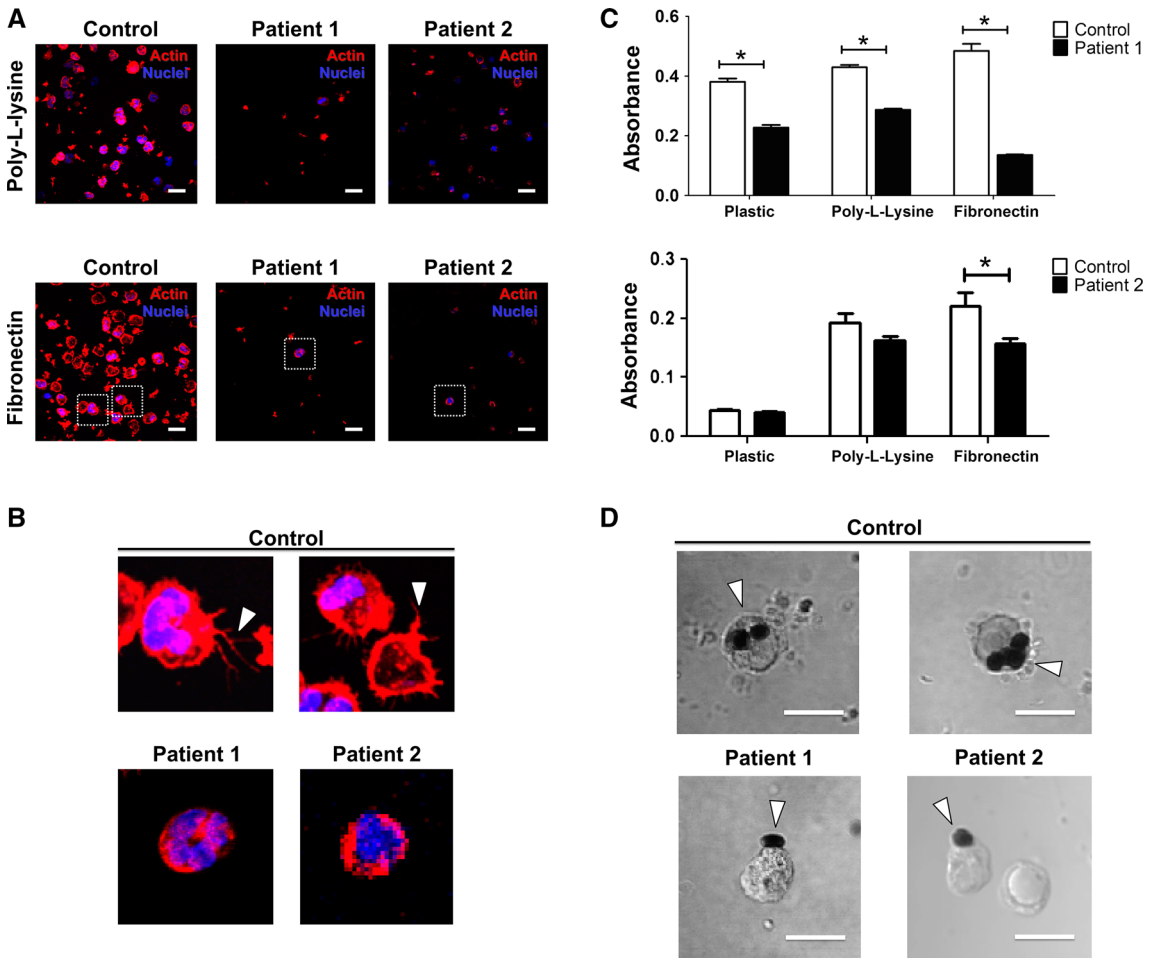

Fig. 4 Adhesive and phagocytic defects in patients' leukocytes. **a** Equal volumes of total blood from the patients and a healthy donor were poured over microchambers covered with poly-L-lysine or fibronectin to allow attachment of the leukocytes and platelets. After an incubation period, the red blood cells were removed by washing and the remaining cells were stained to detect F-actin with phalloidin-rhodamine and the nuclei with DAPI. **b** *Inserts of the squares* marked in (**a**) for a closer look of the spread leukocytes (PMN by nuclei appearance) show the formation of membrane extensions enriched in F-actin present only in control cells, but not visible at the few patients' leukocytes that remained attached after wash. **c** Quantitative adhesion measurements of the patient 1, patient 2 and control blood cells by a colorimetric assay. The *bars* indicate the mean absorbance obtained for each of the substrates used. $n = 2$. **d** Impaired phagocytosis of SRBC in the PBMCs of the patients, a comparison with normal PBMCs obtained from a healthy donor is shown. The *arrows* indicate the erythrocytes. *Scale bars* 10 μm

blood leukocytes, mostly PMN cells as seen in the microscopic images, did not remain attached to the distinct substrates used in these adhesion experiments. Accordingly, the phagocytic capacity of these cells was also found abolished as determined by the inability of patients' leukocytes to ingest opsonized SRBC when compared with normal control cells. The damage in LAD-1 PMN phagocytosis has been previously described and is also evident in our patients, correlating with the clinical presentation. Of note, the efficiency of the respiratory burst in these phagocytes, as measured by the oxidation of dihydrorhodamine (DHR) 123, scored into normal values but still slightly low compared to healthy controls (data not shown). This observation is not surprising due to previous reports indicating the relationship between alterations in respiratory burst responses of granulocytes and the deficiency of β_2 integrins in LAD patients [19].

To demonstrate that the mutated *ITGB2* gene is the causative agent of LAD-1 in our patients, we cloned the coding sequence of it for expressing in HEK 293 cells together with normal CD11a. When compared these cells with those expressing a wild-type LFA-1 form (co-transfected with a normal *ITGB2*), we detected both CD18 and CD11a only when the wild-type *ITGB2* was transfected. When G273R-mutated *ITGB2* from either patient was used, we did not detect any surface expression of CD18 or CD11a in these cells. Thus, in agreement with Hogg et al. [14], this particular mutation precludes the surface expression of the β_2 integrin dimers. All these studies support the diagnosis of a severe form of LAD.

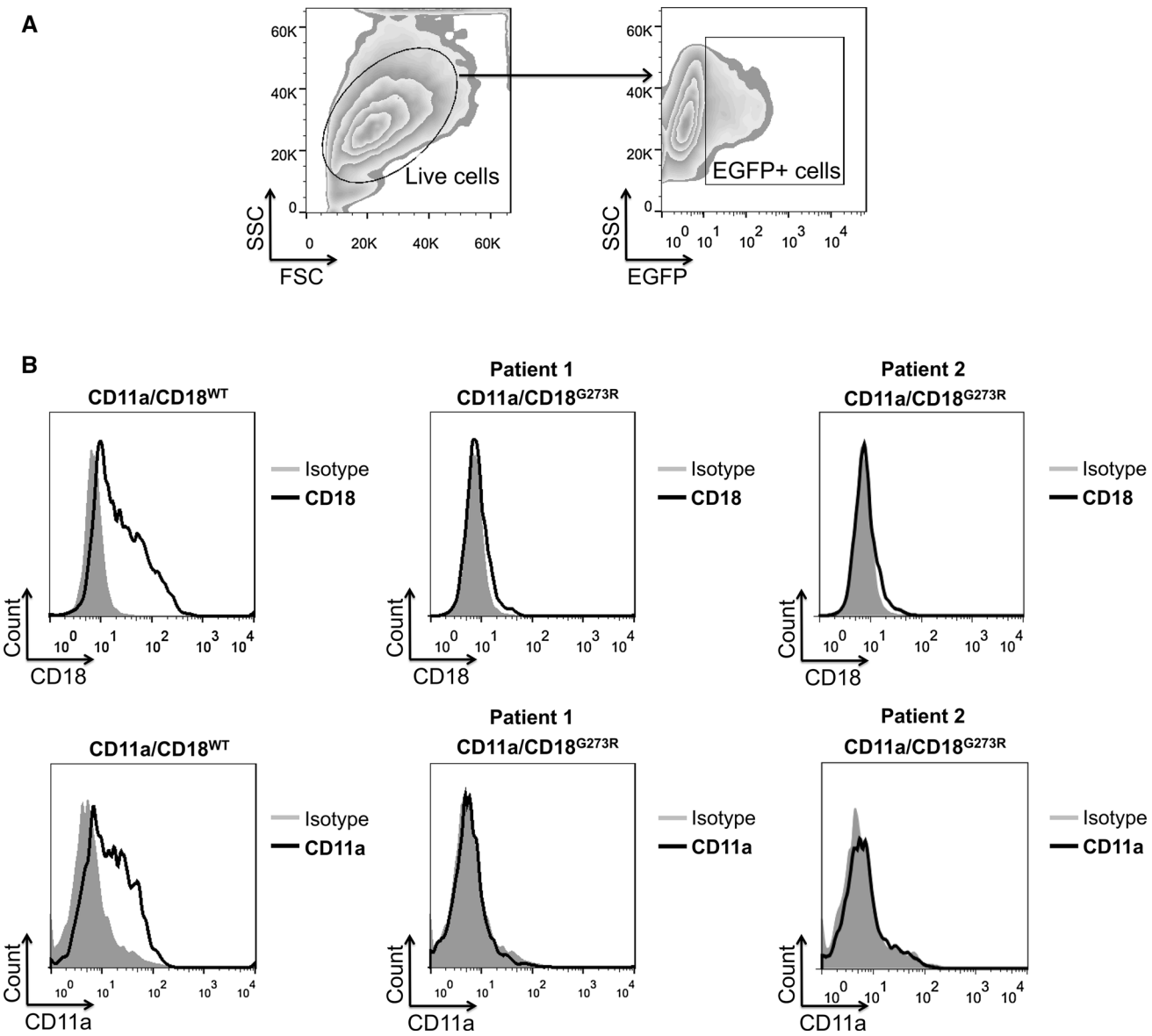

Fig. 5 The co-expression of mutated *ITGB2* and normal CD11a in HEK 293 cells fails to generate membrane LFA-1. **a** Plasmids encoding the mutated patients' CD18, a wild-type (WT) CD18 and WT CD11a, were transfected into HEK 293 cells. As the vectors encode EGFP as a reporter, we could gate the viable EGFP+ cells as transiently transfected. **b** Histograms showing the expression levels of the different constructions after co-transfection: the normal control form of CD18 (CD18WT), the mutated form of the patients (CD18G273R) and CD11a. *MFI* mean fluoresce intensity

Although the use of immunoglobulin has not stopped the presentation of infections, it has helped in terms of the severity of the infections, eventually preventing a fatal disease as, for example, in the second patient that currently is 4 years old. IVIG has been previously been used in LAD. Nord et al. [20] reported a 31-year-old male with LAD-1 who presented with recurrent ulcerations on the extremities diagnosed as pyoderma gangrenosum-like lesions that were refractory to steroids, infliximab and mycophenolate mofetil. IVIG was used successfully leading to the healing of his lesions. Because of the severe recurrent infections that the patient presented despite antibiotic prophylaxis, we started IVIG treatment which helped diminish the number and severity of infections. Although in LAD-1 patients the defect lies in the leukocyte migration, intravenous immunoglobulin could prevent dissemination of microorganisms via bloodstream as well as act locally in the site of infection. Our patients also presented non-healing ulcers, and local SCIG was used leading to the healing of the ulcer. Local subcutaneous immunoglobulin has previously been reported in CVID suggesting a local mechanism of protection. Lin et al. [21] report an 18-year-old male affected

with CVID, who presented warts resistant to conventional therapy. Weekly infusions of subcutaneous immunoglobulin were associated with the resolution of the cutaneous warts within 2 months. On the other hand, subcutaneous immunoglobulin could exert a local immune-modulatory mechanism helping the healing of the wounds. The function of granulocytes in wound healing has been extensively studied, but there are few reports describing the effects of B cells in wound healing. In aseptic wound healing, very few B cells migrate to the wound site. Nishio et al. [22] recently hinted the importance of immunoglobulin in wound healing. Antibodies bound to damaged tissues might induce phagocytosis by Fcγ receptors present on neutrophils and macrophages. In mice, the topical administration of a neutralizing antibody recognizing aminopeptidase-N, an enzyme implicated in the regulation of epidermal–dermal interaction and expression of adhesion molecules, led to acceleration of wound closure [23]. Antibodies present in pooled human IVIG blocked Fas-mediated keratinocyte death in vitro. Patients suffering from toxic epidermal necrolysis (TEN) were treated with IVIG; disease progression was rapidly reversed, and the outcome was favorable [24]. Thus, inhibiting Fas-mediated keratinocyte apoptosis may contribute in the efficacy of the immunoglobulin treatment for wounds.

Conclusions

We present two patients with severe LAD-1 who has been treated with immunoglobulin as an adjunctive therapy. We propose that IVIG is safe and efficacious in LAD-1 patients before HSCT and helpful in controlling severe infections. SCIG also appeared to help wound healing in refractory ulcers in these patients.

Acknowledgments We thank to Dr. Héctor Romero-Ramírez and M.Sc. Víctor H. Rosales-García for their help at different stages of this work. Supported by Instituto de Ciencia y Tecnología del Distrito Federal (ICyTDF) (Grant PICSA12-157) and Consejo Nacional de Ciencia y Tecnología (CONACyT) (Grant 153733 awarded to L.S.A.). J.L.M.M. and N.R.A. were supported by doctoral scholarships 203768 and 456878 from CONACyT. L.B.R was the recipient of a doctoral (176353) and postdoctoral (CVU 44813) scholarship from CONACyT.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Gelfand EW, Ochs HD, Shearer WT. Controversies in IgG replacement therapy in patients with antibody deficiency diseases. J Allergy Clin Immunol. 2013;131(4):1001–5.
2. Maarschalk-Ellerbroek LJ, Hoepelman IM, Ellerbroek PM. Immunoglobulin treatment in primary antibody deficiency. Int J Antimicrob Agents. 2011;37(5):396–404.
3. Garcia JM, Espanol T, Gurbindo MD, Casas CC. Update on the treatment of primary immunodeficiencies. Allergol Immunopathol (Madr). 2007;35(5):184–92.
4. Li L, Jin YY, Cao RM, Chen TX. A novel point mutation in CD18 causing leukocyte adhesion deficiency in a Chinese patient. Chin Med J (Engl). 2010;123(10):1278–82.
5. Etzioni A. Defects in the leukocyte adhesion cascade. Clin Rev Allergy Immunol. 2010;38(1):54–60.
6. Campbell ID, Humphries MJ. Integrin structure, activation, and interactions. Cold Spring Harb Perspect Biol. 2011;3(3):a004994.
7. Luo BH, Carman CV, Springer TA. Structural basis of integrin regulation and signaling. Annu Rev Immunol. 2007;25:619–47.
8. Tan SM. The leucocyte beta2 (CD18) integrins: the structure, functional regulation and signalling properties. Biosci Rep. 2012;32(3):241–69.
9. van de Vijver E, Maddalena A, Sanal O, Holland SM, Uzel G, Madkaikar M, et al. Hematologically important mutations: leukocyte adhesion deficiency (first update). Blood Cells Mol Dis. 2012;48(1):53–61.
10. Hogg N, Patzak I, Willenbrock F. The insider's guide to leukocyte integrin signalling and function. Nat Rev Immunol. 2011;11(6):416–26.
11. Etzioni A. Genetic etiologies of leukocyte adhesion defects. Curr Opin Immunol. 2009;21(5):481–6.
12. Mellouli F, Ksouri H, Barbouche R, Maamer M, Hamed LB, Hmida S, et al. Successful treatment of Fusarium solani ecthyma gangrenosum in a patient affected by leukocyte adhesion deficiency type 1 with granulocytes transfusions. BMC Dermatol. 2010;10:10.
13. Etzioni A. Leukocyte adhesion deficiencies: molecular basis, clinical findings, and therapeutic options. Adv Exp Med Biol. 2007;601:51–60.
14. Hogg N, Stewart MP, Scarth SL, Newton R, Shaw JM, Law SK, et al. A novel leukocyte adhesion deficiency caused by expressed but nonfunctional beta2 integrins Mac-1 and LFA-1. J Clin Invest. 1999;103(1):97–106.
15. Roos D, Law SK. Hematologically important mutations: leukocyte adhesion deficiency. Blood Cells Mol Dis. 2001;27(6):1000–4.
16. Maravillas-Montero JL, Gillespie PG, Patino-Lopez G, Shaw S, Santos-Argumedo L. Myosin 1c Participates in B Cell cytoskeleton rearrangements, is recruited to the immunologic synapse, and contributes to antigen presentation. J Immunol. 2011;187(6):3053-63.
17. Theodoridis AA, Eich C, Figdor CG, Steinkasserer A. Infection of dendritic cells with herpes simplex virus type 1 induces rapid degradation of CYTIP, thereby modulating adhesion and migration. Blood. 2011;118(1):107–15.
18. Qasim W, Cavazzana-Calvo M, Davies EG, Davis J, Duval M, Eames G, et al. Allogeneic hematopoietic stem-cell transplantation for leukocyte adhesion deficiency. Pediatrics. 2009;123(3):836–40.
19. Zhou MJ, Brown EJ. CR3 (Mac-1, alpha M beta 2, CD11b/CD18) and Fc gamma RIII cooperate in generation of a neutrophil respiratory burst: requirement for Fc gamma RIII and tyrosine phosphorylation. J Cell Biol. 1994;125(6):1407–16.
20. Nord KM, Pappert AS, Grossman ME. Pyoderma gangrenosum-like lesions in leukocyte adhesion deficiency I treated with intravenous immunoglobulin. Pediatr Dermatol. 2011;28(2):156–61.
21. Lin JH, Wang KY, Kraft S, Roberts RL. Resolution of warts in association with subcutaneous immunoglobulin in immune deficiency. Pediatr Dermatol. 2009;26(2):155–8.

22. Nishio N, Ito S, Suzuki H, Isobe K. Antibodies to wounded tissue enhance cutaneous wound healing. Immunology. 2009;128(3): 369–80.
23. Lai A, Hosseini-Tabatabaei A, Hartwell R, Rahmani-Neishaboor E, Kilani RT, Ghahary A. Topical application of aminopeptidase N-neutralizing antibody accelerates wound closure. Mol Cell Biochem. 2013;372(1–2):95–100.
24. Viard I, Wehrli P, Bullani R, Schneider P, Holler N, Salomon D, et al. Inhibition of toxic epidermal necrolysis by blockade of CD95 with human intravenous immunoglobulin. Science. 1998; 282(5388):490–3.