

SHORT COMMUNICATION

Recurrent Pyoderma Gangrenosum and Cystic Acne Associated with Leucocyte Adhesion Deficiency due to Novel Mutations in *ITGB2*: Successful Treatment with Infliximab and AdalimumabAnders Vahlquist^{1#}, Lena Douhan Håkansson^{2*}, Lars Rönnblom¹, Malgorzata Karawajczyk¹, Anders Fasth³, Marielle E. van Gijn⁴, Dirk Roos⁵ and Per Venge¹¹Department of Medical Sciences, Uppsala University; ²Department of Clinical Chemistry and Pharmacology, University Hospital, SE-751 85 Uppsala; ³Department of Pediatrics, University of Gothenburg, Gothenburg, Sweden; ⁴Department of Medical Genetics, Genome Diagnostics, University Medical Centre, Utrecht, The Netherlands and ⁵Sanquin Research, and Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands. *E-mail: lena.douhan.hakansson@akademiska.se

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Pyoderma gangrenosum (PG) is not unfrequent in adult patients, especially in association with inflammatory gut or haematologic diseases (1). An onset of PG in early childhood is extremely rare (2) and should always lead to a suspicion of an underlying genetic abnormality related to the group of autoinflammatory diseases. One such disease is the autosomal dominant syndrome called PAPA (Pyogenic sterile Arthritis with Pyoderma gangrenosum and Acne; MIM 604416), caused by *CD2BP1* (*PSTPIPI*) mutations (3).

PG has also been noted among patients with leucocyte adhesion deficiency (LAD) syndrome (4–6), an autosomal recessive disease that occurs in 3 different forms. LAD-I MIM 116920 is caused by mutations in *ITGB2* coding for the β_2 -integrin CD18 (7).

Here we report our long-term observations of a 26-year-old man who had his first PG at the age of 3 and later developed relapsing PG and nodulo-cystic acne.

MATERIAL AND METHODS (See Appendix S1¹)

CASE REPORT

The patient was born healthy after an uneventful pregnancy (no consanguinity). His mother suffers since many years from recurrent synovitis of unknown aetiology, whereas his father and elder sister are reportedly healthy. At 16 months of age, the patient contracted pertussis followed by disseminated impetigo with fever, otitis media, balanitis and extensive molluscum contagiosum infections. At 3 years of age he developed an abscess at his back that was treated with penicillin. Soon thereafter, multiple ulcerating abscesses appeared on his chest, which did not respond to antibiotics. Low-grade fever, leukocytosis ($33 \times 10^9/l$; normal range $3-9 \times 10^9/l$) and an elevated C-reactive protein (CRP) (116 $\mu g/l$; N: <4) were noted. Systemic betamethasone therapy reduced CRP to 11 within a day and initiated healing of the skin ulcers. The haematological variables normalised and have been unremarkable ever since. A diagnosis of juvenile PG was made and treatment with prednisolone, sulfasalazine, zinc sulphate and isoxacillin was initiated and maintained for one year. At this time, the boy's granulocyte function was examined by one of us (AF), showing a reduced chemotaxis and a reduced production of formazan in the nitroblue tetrazolium test (NBT) of 50% compared to normal blood donors.

Between 4 and 11 years of age the patient was free of symptoms, except for extensive scarring and pigment abnormalities in healed areas of PG (Fig. S1¹). In 1998, he suddenly developed new skin ulcerations with pain, pus and other clinical features of PG (no biopsy taken). This time he was treated with oral cyclosporine 2 mg/kg/day, methotrexate (MTX) 5 mg/week and prednisolone up to 60 mg/day, which improved the ulcers, but eventually caused intolerable side-effects such as nausea, steroid-induced myositis and retarded skeletal growth. In 2002, at the age of 15, he was referred to the Department of Pediatrics, Uppsala University Hospital, where etanercept (25 mg twice per week) was initiated, replacing other drugs. Instead of improving, new skin lesions developed in the face and the patient was referred to one of us (AV) for advice. The facial lesions were diagnosed as probably steroid-induced nodulo-cystic acne, which together with the PG-like lesions on other parts of the body suggested a diagnosis of PAPA, especially since the mother had recurrent synovitis. However, no abnormalities were later detected in the *PSTPIPI* gene (MEVg). Investigations of granulocyte function revealed a reduced expression of β_2 -integrins, normal expression of Fc γ -receptors and selectin receptors, a normal respiratory burst and a normal phagocytosis (Table I, Table S1¹, Fig. S2¹). These findings were consistent with moderate and incomplete LAD-I. In contrast, granulocytes from the patient's parents and sister demonstrated a normal expression of β_2 -integrins (see Table I). Eventually, LAD-I was genetically confirmed in the patient in 2008 by one of us (DR) who found 2 heterozygous mutations in the *ITGB2* gene, one splice mutation from the mother (intron 14+1delG) and one point mutation from the father (c.130A>C; p.Thr44Pro). His healthy sister did not carry any of these mutations.

In 2003, based on tentative diagnoses of acne and PG related to LAD-I, long-term treatment with high dose tetracycline (2 g/day) and azathioprine 50 mg \times 2 (for tapering of high-dose corticosteroid therapy) was prescribed, which resulted in an improvement especially of facial lesions. In 2004, a sudden bout of acne-like abscesses on the trunk and neck motivated a change of acne therapy to isotretinoin 50 mg/day; however, this worsened the PG-like lesions on the trunk and extremities. The patient was re-started on high-dose tetracycline, combined with prednisolone 60 mg/day and a new TNF-blocker, adalimumab 40 mg every second week. After 2 months, prednisolone could be tapered without reactivation of PG. On this systemic therapy (and topical tacrolimus 0.1% on healed PGs) the patient was free of relapses for 2 years. In May 2007 new PGs appeared, which motivated a switch from TNF- to interleukin-1 (IL-1)-blockade, using the IL-1 receptor antagonist anakinra (Kineret). PAPA syndrome involves abnormal pyrin activation and increased IL-1 β levels (3) that ideally should respond to anti-IL-1 treatment. Since 2 months of this therapy did not improve the ulcers (Fig. 1a), infliximab (2.5 mg/kg) was started which resulted in a rapid improvement within 1 week and a healing of

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Table I. Granulocyte cell surface expression of β_2 -integrins, CD65 and P-selectin ligand (CD162) in the patient and his relatives

Cell surface antigen (antibody clone)	Patient % (mean)	Sister % (mean)	Mother % (mean)	Father % (mean)	Ref.* % (mean)
CD11a (MHM24)	7.9 (0.65)	99 (6.4)	99 (7.1)	100 (6.6)	>95 (>5.5)
CD11b (Bear 1)	2.1 (0.1)	99 (4.5)	99 (6.0)	99 (8.7)	>95 (>3.0)
CD11b(2LPM19c)	17 (0.17)	100 (3.4)	99 (4.8)	100 (7.0)	>95 (>2.0)
CD18 (MHM23)	9 (0.43)	99 (5.2)	98 (3.4)	100 (4.9)	>95 (>3.0)
CD65	99 (63)	99 (52)	100 (54)	99 (36)	>95 (>20)
CD162	100 (16)	99 (11)	99 (9.9)	100 (17)	>95 (>9)

Analysis performed in December 2004. For methods see Appendix S1¹.

*Reference interval established at the laboratory.

all ulcers within 2 months (Fig. 1b). Infliximab infusions every 8 weeks (plus azathioprine to reduce the risk of immunisation) were maintained for almost 5 years with excellent results. Occasional bacterial skin abscesses were incised and treated with flucloxacillin 1 g \times 3/day for 1–2 weeks. In Nov 2011, new ulcers started to appear on the patient's legs despite a doubled infliximab dose (5 mg/kg at 6 weeks interval) and addition of prednisolone up to 70 mg/day. The patient was then switched back to adalimumab and MTX injections 10 mg/week before testing intravenous immunoglobulins (IVIg) twice during one month, alas with pronounced worsening after 6 weeks (Dr. N. Kuzmina, personal communication). The patient is now back on infliximab, MTX and high-dose prednisolone with noticeable improvement. During the whole treatment period since 2007, the patient has remained in good general health with no systemic infections or prominent adverse effects of the treatment.

DISCUSSION

The patient was found to be compound heterozygous for 2 *ITGB2* mutations, a known cause of LAD-I (7). The mutations are predicted to replace threonine-44 in CD18 by proline (father's mutation) and to cause skipping of exon 14 from CD18 mRNA, leading to a frameshift and premature termination of CD18 protein synthesis (mother's mutation). This last mutation will almost certainly lead to absence of CD18 protein expression, but the Thr44Pro substitution may preserve some residual CD18 protein expression and function. Clearly the patient has not the complete clinical and laboratory picture of LAD-I: (i) he did not have problems with umbilical detachment, delayed wound healing and paradontitis, (ii) he has no basal leukocytosis (except during corticosteroid therapy)

and the granulocyte function tests showed normal migration and phagocytosis, and (iii) his respiratory burst test is in the lower range of normal values. However, the expression of β_2 -integrins on granulocytes is abnormal in consistency with a leaky, hypomorphic LAD-I disease. Although the patient presented with disseminated bacterial and viral infections in early childhood consistent with immunosuppression due to LAD-I, he subsequently had only rare episodes of skin abscesses that were difficult to distinguish from deep PGs or cystic acne.

PG is generally considered to be a disorder of the neutrophil granulocytes, with granulocytes and macrophages infiltrating the skin, leading to tissue necrosis despite absent pathogens (1). In contrast, PG-like necrotic ulcers described in association with LAD-I usually show a relative lack of infiltrating neutrophils (4, 5), a finding which is readily explained by reduced chemotaxis and inability to attach to lesional endothelium of the defect granulocytes. Intuitively, one may think that such a defect should result in tissue inertia rather than injurious inflammation as observed in our and previous patients with LAD and PG. Speculatively, a hypomorphic mutation in *ITGB2* with some residual expression of CD18, as found in our patient, might account for the paradox. It is also possible that some patients with LAD-I are able to develop compensatory inflammatory mechanisms – perhaps involving TNF- α – which may bypass certain pathogenic aspects of LAD-I. In theory, unopposed activation of such a compensatory mechanism could lead to pustular and necrotic skin reactions also in the absence of functional neutrophils. Incidentally, such a hypothesised hyper-inflammatory response in LAD-I patients with PG may provide a clue as to why these individuals appear less susceptible to severe bacterial infections (4, 5) as compared to LAD-I patients with no obvious signs of skin hyper-reactivity (for review see ref. 12).

The treatment of LAD-I is complex and often disappointing. Isolated reports of successful haematopoietic stem cell transplantation have appeared (13), but this is only indicated when all other therapies have failed. Our treatment experience points to infliximab, adalimumab as the most valuable remedies for this disease. Inflixi-



Fig. 1. Clinical picture at age 20 years: A long-standing pyoderma gangrenosum on the inside of the thigh (a) after 2 months of anakinra treatment, and (b) after 2 months of infliximab infusions 200 mg on weeks 0, 2 and 6.

mab has been previously described for idiosyncratic PG (14). Any effect of high-dose IVIG therapy (15) was unnoticeable in our patient after 6 weeks.

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