

Letter to the Editor

Splicing defect of CD33 and inflammatory syndrome associated with occult bacterial infection

To the Editor:

The common denominators of human autoinflammatory diseases are recurring episodes of monophasic inflammation, with fever, a systemic inflammatory response, and a skin rash. There is no involvement of the adaptive immune system and, therefore, no memory. The additional organ involvement together with the periodicity of the febrile attacks defines the individual syndrome. Recent studies have identified causative mutations, for example, in the cryopyrin gene, leading to uncontrolled expression of IL-1. Many of the autoinflammatory diseases can be successfully treated with IL-1 blockade.

The sialic acid-binding immunoglobulin-like lectin (Siglec) family has been proposed to be involved in innate immune regulation but, so far, has not been linked to autoinflammatory diseases.

We report the case of a 26-year-old white woman, with unremarkable history except for hip surgery 9 years ago (Fig 1, A), suffering from recurrent attacks of fever ($>39^{\circ}\text{C}$),

with skin eruptions, lymphadenopathy, rheumatic pains, and reduced general condition. During fever, maculopapulous, nonpruritic skin lesions were found over the trunk and the proximal limbs (Fig 1, C). Skin histology documented edema and a neutrophilic infiltrate (Fig 1, B). On the basis of clinical presentation on the one hand and the exclusion of an infectious, autoimmune, allergic, or neoplastic disease on the other hand (see Table E1 in this article's Online Repository at www.jacionline.org), we suspected an autoinflammatory disease and tested the effect of the IL-1 receptor antagonist anakinra (Kineret, Amgen, Thousand Oaks, Calif). Within hours after the first injection, fever dropped, cardiovascular situation stabilized, skin eruptions paled, and within few days laboratory acute phase reaction normalized. Because of the absence of common genetic mutations causing autoinflammatory syndromes (see Table E1), we analyzed the patient's immune system. Flow cytometry analysis of peripheral blood revealed an almost complete lack of CD33 expression on patient granulocytes and monocytes compared with healthy controls (Fig 2, A). PCR analysis of total RNA from leukocytes showed the presence of CD33 transcripts, but with a significantly altered ratio between the long- and the short-splicing variant. Northern blotting confirmed that the patient produced predominantly the shorter splice

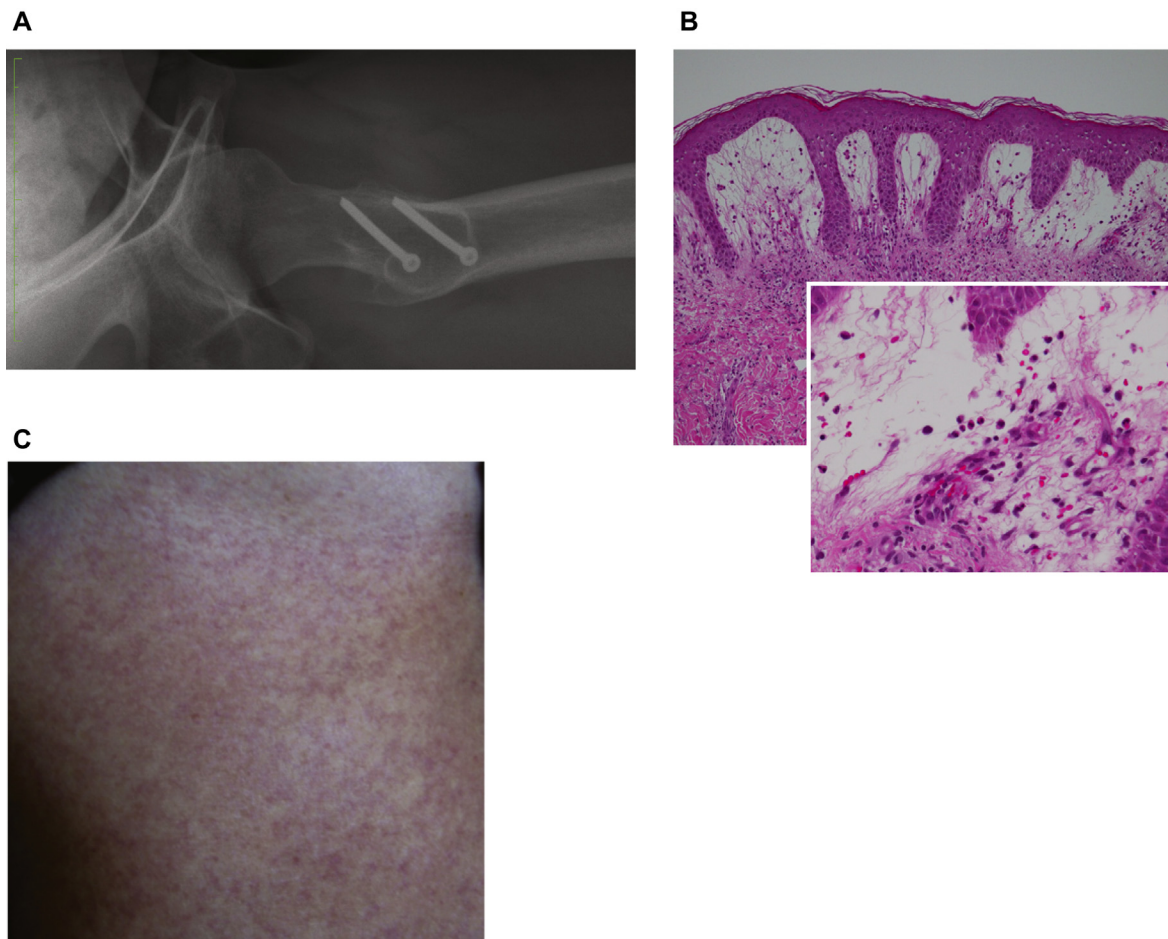


FIG 1. A, Axial x-ray of the left hip joint, showing 2 screws in the trochanter major that were confirmed to be infected with *S aureus*. B, Histologic specimen of skin eruption during flare, showing subepidermal edema and a neutrophilic infiltrate. C, Macroscopic picture of maculopapulous skin eruptions during flare.

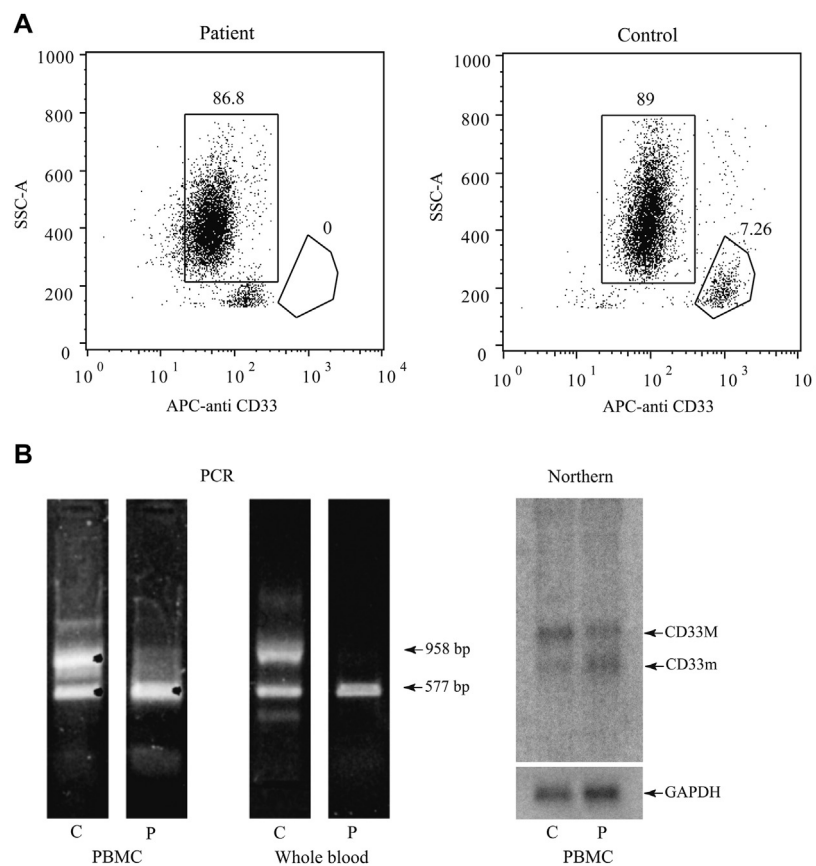


FIG 2. A, Flow-cytometry plots showing CD33 expression on granulocytes (big population) and monocytes (small population) in patient (left) and an age- and sex-matched healthy control (right). B, Left: Analysis of CD33 mRNA from leukocytes and whole blood of the patient (P) and a healthy control (C). Bands of 958 bp (CD33M) and 577 bp (CD33m). Right: Northern blot of total RNA from leukocytes. APC, Allophycocyanin; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; SSC-A, side scatter-A.

variant CD33m (Fig 2, B). Sequencing of the gene showed no mutation in the splicing regions, indicating that the splicing defect is due to a dysregulated splicing machinery.

One and a half years after the first occurrence of symptoms, anakinra failed to induce remission and additional glucocorticoids could not control the symptoms. The patient was hospitalized, and blood cultures and hip joint fluid were tested positive for *Staphylococcus aureus*. Antibiotic treatment and surgical removal of the 2 infected screws (Fig 1, A) led to a full recovery. Subsequently, the patient remained healthy without further febrile attacks.

CD33 (Siglec-3) is the third member of the Siglec family of transmembrane receptors that bind carbohydrates, in particular sialic acid, from the surface of cells, viruses, and bacteria.¹ The extracellular domain consists of 2 immunoglobulin-like domains, one N-terminal Ig-V domain that interacts with sialic acid, and one Ig-C2 domain. Two isoforms of CD33 have been described that result from alternative splicing of exon 2 encoding the Ig-V domain.² CD33M contains both immunoglobulin domains and is usually the predominant isoform in healthy individuals, whereas CD33m lacks the extracellular binding N-terminal Ig-V domain. Most commercial mAbs exclusively recognize the CD33M isoform.³ Consequently, flowcytometric analysis of PBMCs from the patient's blood suggested a lack of CD33. However, CD33 transcripts could be detected in total RNA from

leukocytes by PCR with a primer set specific for the shorter isoform (Fig 2, B, left). Northern blotting of total RNA confirmed the altered ratio of CD33m/CD33M compared with an age- and sex-matched healthy control (Fig 2, B, right). Sequencing of genomic DNA from leukocytes revealed no mutations. In particular, all donor and acceptor sites for splicing were found to be normal (see this article's Methods section in the Online Repository at www.jacionline.org for details). It is therefore likely that the patient harbors a polymorphism or mutation in another crucial component of the splicing machinery.

The role of CD33 is not yet known in detail, but it is believed that the Siglecs participate in the modulation of leukocyte function by repressing cell activation and proliferation.^{4,5} In fact, reduction of CD33 at the surface of monocytes by antibody treatment or by RNA-mediated interference resulted in the secretion of elevated levels of the proinflammatory cytokines IL-1 β , TNF- α , and IL-8.⁶ Because the predominant CD33 isoform of the patient lacks the Ig-V domain that is known to interact with sialic acid, we hypothesized that the patient cannot counteract cellular activation efficiently enough. Although the disease course excludes the CD33 deficiency as a cause of a (novel) autoinflammatory syndrome, it is conceivable that the reduced levels of CD33M on the patient's leukocytes led to inflammatory overreactions to a latent, occult bacterial infection.

The dual role of *S aureus* infection in humans, on the one hand to act as a commensal bacterium in healthy carriers and on the other hand to be a deleterious invasive pathogen, is not yet completely understood. Recently, an immune regulatory role for TLR2 has emerged and been linked to the protective effects of *S aureus* during host immune responses to microbial toxins.^{7,8} Similar functions might be attributed to CD33 signaling. The CD33 splicing anomaly in our patient persisted after antibiotic treatment, which suggests that altered alternative splicing of CD33 was not induced by *S aureus*. Because all symptoms disappeared on surgical removal of the screws, we hypothesize that an *S aureus* biofilm in the hip repeatedly induced bacteremia, mimicking an autoinflammatory syndrome until the development of overt sepsis and septic arthritis in the context of more profound pharmacological immunosuppression.

In summary, this is the first description of an almost complete loss of the long splicing variant of CD33, containing the ligand-binding region, on peripheral leukocytes of a young female patient. Despite a negative cellular feedback role of this molecule, the autoinflammatory phenotype of the disease was not caused by this deficiency but rather by an occult bacterial infection. It is, however, conceivable that the defect resulted in an overshooting inflammatory response to bacterial stimuli.

Maria L. Balmer, MD, PhD^{a,b}

Beat Trüeb, PhD^c

Lei Zhuang, PhD^c

Emma Slack, PhD^a

Helmut Beltraminelli, MD^d

Peter Matthias Villiger, MD^c

From ^athe Department of Clinical Research, University of Bern, Bern, Switzerland;

^bMedizinische Poliklinik, University Hospital and University of Basel, Basel, Switzerland; and the Departments of ^cRheumatology, Clinical Immunology and

Allergy and ^dDermatology, University Hospital and University of Bern, Bern, Switzerland. E-mail: Peter.Villiger@Insel.ch.

This study has been supported by the research grant of the Department of Rheumatology and Clinical Immunology/Allergy, University Hospital and University of Bern, Switzerland.

Disclosure of potential conflict of interest: M. L. Balmer has been supported by an SNF MD-PhD grant. E. Slack is employed by ETH, Zurich, Switzerland, and has been supported by an SNF Ambizione Fellowship and a grant from ETH. P. M. Villiger is a Board member for MSD, Pfizer, Abbott, Novartis, and Roche and is a member of an EEU Expert Board. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Cao H, Crocker PR. Evolution of CD33-related siglecs: regulating host immune functions and escaping pathogen exploitation? *Immunology* 2011;132:18-26.
2. Hernández-Caselles T, Martínez-Esparza M, Pérez-Oliva AB, Quintanilla-Cecconi AM, García-Alonso A, Alvarez-López DMR, et al. A study of CD33 (SIGLEC-3) antigen expression and function on activated human T and NK cells: two isoforms of CD33 are generated by alternative splicing. *J Leuk Biol* 2006;79:46-58.
3. Pérez-Oliva AB, Martínez-Esparza M, Vicente-Fernández JJ, Corral-San Miguel R, García-Peñarrubia P, Hernández-Caselles T. Epitope mapping, expression and post-translational modifications of two isoforms of CD33 (CD33M and CD33m) on lymphoid and myeloid human cells. *Glycobiology* 2011;21:757-70.
4. Crocker PR, Redelinghuys P. Siglecs as positive and negative regulators of the immune system. *Biochem Soc Trans* 2008;36:1467-71.
5. von Gunten S, Bochner BS. Basic and clinical immunology of Siglecs. *Ann New York Acad Sci* 2008;1143:61-82.
6. Lajaunias F, Dayer J-M, Chizzolini C. Constitutive repressor activity of CD33 on human monocytes requires sialic acid recognition and phosphoinositide 3-kinase-mediated intracellular signaling. *Eur J Immunol [In Vitro]* 2005;35:243-51.
7. Chau TA, McCully ML, Brintnell W, An G, Kasper KJ, Vinés ED, et al. Toll-like receptor 2 ligands on the staphylococcal cell wall downregulate superantigen-induced T cell activation and prevent toxic shock syndrome. *Nat Med* 2009;15:641-8.
8. Lai Y, Di Nardo A, Nakatsuji T, Leichtle A, Yang Y, Cogen AL, et al. Commensal bacteria regulate Toll-like receptor 3-dependent inflammation after skin injury. *Nat Med* 2009;15:1377-82.

<http://dx.doi.org/10.1016/j.jaci.2013.03.027>

METHODS

Histology

Skin biopsies were paraffin-embedded and stained with hematoxylin and eosin according to standard protocols. Results are shown in Fig 1, B.

Cellular studies

PBMCs of the patient stimulated *in vitro* with LPS revealed increased IL-1 β production in comparison to PBMCs of healthy controls. Moreover, the threshold for shedding of CD62L (an early cell activation marker) in response to LPS stimulation was strongly reduced as compared with healthy control granulocytes and monocytes (data not shown).

FACS analysis

Whole blood (citrate, 25 μ L) was stained with 25 μ L PBS/BSA2% containing APC-anti-CD33 antibody (BioLegend, San Diego, Calif) for 15 minutes. Red blood cells are then lysed in 200 μ L of FACS Lysis solution (BD Biosciences, San Diego, Calif) and samples acquired on a FACS Array (BD Biosciences). Blood monocytes and granulocytes are distinguished on the basis of side-scatter and CD33 expression by using FlowJo Software (Tree Star, Ashland, Ore).

Cloning and sequencing of mRNA

Total RNA was extracted from blood lymphocytes with the QIAamp RNA Blood Mini Kit from Qiagen (Hilden, Germany) and from PBMCs with the GeneElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich, St Louis, Mo). The RNA was transcribed into first-strand cDNA by reverse transcriptase from Moloney Murine Leukemia Virus (1.5 u/ μ L) as suggested by the supplier of the enzyme (Invitrogen, Carlsbad, Calif). Random hexamers served as primers. Aliquots of the cDNA were amplified by PCR using Fast Start Taq DNA Polymerase (Roche Diagnostics, Mannheim, Germany) and the following primer pairs: nucleotides 15-972 (NM_001772) AAGCCCTG GAAGCTGCTTCCTC/TGGTGTTCGGGGAGGCTGAC (product length

958 bp and 577 bp, respectively; 39 cycles of 1 minute 95°C, 1 minute 66°C and 1 minute 72°C), nucleotides 929-1153 AATGACACCCACCCTACCAC/GATGCTCTTGTGGGTTTCCTC (product length 225 bp; 35 cycles of 1 minute 95°C, 1 minute 58°C, and 1 minute 72°C). The PCR products were resolved on a 1% agarose gel. Bands of interest were excised, purified with the GeneElute Gel Extraction Kit (Sigma-Aldrich), and sequenced by the dideoxy chain termination method on an ABI 3730 cycle sequencing platform.

Isolation and sequencing of genomic DNA

Genomic DNA was isolated from whole blood with the Wizard Genomic DNA Purification Kit from Promega (Madison, Wis). Individual exons were amplified from the genomic DNA with Fast Start Taq DNA Polymerase (Roche Diagnostics, Mannheim, Germany) by using the following primer pairs: exons 1-2 AAGCCCTGGAAGCTGCTTCCTC/TTCCCGGAACCAG-TAACCATGAAC (annealing temperature 60°C), exon 2 TGTGGGCAGGT GAGTGGCTGTG/TGCCCTGCAGTACCCATGAACCTC (annealing temperature 60°C), and exons 2-3 TACAAATCTCCCCAGCTCTCTGTG/AGAGCAGGTCAGGTTTTTGGAGTG (annealing temperature 59°C). The products were resolved on an agarose gel and processed for sequencing as described above.

Northern blot

RNA from lymphocytes (5 μ g/lane) was separated on a 1% agarose gel in the presence of formaldehyde and subsequently transferred to a GeneScreen Nylon membrane (PerkinElmer, Waltham, Mass) by vacuum blotting. A radioactively labeled probe was prepared from the CD33 cDNA (nucleotides 15-972) by the random primed oligolabeling method using [α -³²P] dCTP. The nylon membrane was hybridized with this probe overnight in a buffer containing 50% formamide. Subsequently, the membrane was washed under standard stringency (1 $\times$ SSC) and exposed to x-ray film (Kodak BioMax MS, Rochester, NY).

All experiments have been performed in agreement with the patient and the patient provided written informed consent.

TABLE E1. Laboratory results and additional examinations

Laboratory values (normal range)	Patient value
Leukocytes (3.5-10.5 G/L)	14.8 G/L
Neutrophils (1.6-7.4 G/L)	13.9 G/L
Eosinophils (0.02-0.4 G/L)	0.02 G/L
Monocytes (0.2-0.93 G/L)	0.37 G/L
Lymphocytes (1.1-3.5 G/L)	0.52 G/L
CRP (<5 mg/dL)	24 mg/dL
BSR	23 mm/h
LDH (<480 U/L)	565 U/L
ALT (6-37 U/L)	65 U/L
AST (10-37 U/L)	44 U/L
Creatine kinase (<170 U/L)	88 U/L
IgM (<2.3 g/L)	3.89 g/L
Serum albumin (30-52 g/L)	40 g/L
HIV, enterovirus, adenovirus, <i>Rickettsia conorii</i> , <i>Coxiella burnetii</i> , parvovirus B19, HHV-6&7 (IgM), HSV (IgM), cytomegalovirus (IgM), EBV (IgM), blood cultures	Negative
ANA (<1:80)	1:80
dsDNS, nDNS, SS-A, SS-B, Scl-70, Jo-1, mitochondria, CCP, RF, c-ANCA, p-ANCA, histones	Negative
Additional examinations	
Echocardiography	Normal
Chest x-ray	Normal
Bone marrow biopsy	Normal
Computed tomography scan	Slight hepatomegaly and small amount of ascites
Genetic mutations for FMF, HIDS, CAPS, and TRAPS	Absent
Allergic testing (painkillers)	Negative

ALT, Alanine aminotransferase; ANA, antinuclear antibodies; AST, aspartate aminotransferase; BSR, blood sedimentation rate; CAPS, cryopyrin-associated periodic syndrome; CCP, anti-cyclic citrullinated peptide antibodies; CRP, C-reactive protein; dsDNS, double-stranded deoxyribonucleic acid; EBV, Epstein Barr virus; FMF, familial mediterranean fever; HHV-6&7, human herpesvirus 6&7; HIDS, hyper-IgD syndrome; HIV, human immunodeficiency virus; HSV, herpes simplex virus; Jo-1, anti-histidyl-tRNA synthetase antibodies; LDH, lactate dehydrogenase; nDNS, nuclear deoxyribonucleic acid; Scl-70, anti-topoisomerase I antibodies; SS-A/B, anti-Sjogren syndrome A/B antibodies; TRAPS, TNF receptor associated periodic syndrome; RF, rheumatoid factor; c-ANCA, cytoplasmic anti-neutrophil cytoplasmic antibodies; p-ANCA, perinuclear anti-neutrophil cytoplasmic antibodies.