BRIEF REPORT

Interleukin-12 and Interleukin-23 Blockade in Leukocyte Adhesion Deficiency Type 1

Niki M. Moutsopoulos, D.D.S., Ph.D., Christa S. Zerbe, M.D., Teresa Wild, M.S., Nicolas Dutzan, D.D.S., Laurie Brenchley, R.D.H., Giovanni DiPasquale, Ph.D., Gulbu Uzel, M.D., Karen C. Axelrod, R.N., Andrea Lisco, M.D., Lucia D. Notarangelo, M.D., George Hajishengallis, D.D.S., Ph.D., Luigi D. Notarangelo, M.D., and Steven M. Holland, M.D.

SUMMARY

A patient with leukocyte adhesion deficiency type 1 (LAD1) had severe periodontitis and an intractable, deep, nonhealing sacral wound. We had previously found a dominant interleukin-23–interleukin-17 signature at inflamed sites in humans with LAD1 and in mouse models of the disorder. Blockade of this pathway in mouse models has resulted in resolution of the immunopathologic condition. We treated our patient with ustekinumab, an antibody that binds the p40 subunit of interleukin-23 and interleukin-12 and thereby blocks the activity of these cytokines, inhibiting interleukin-23–dependent production of interleukin-17. After 1 year of therapy, our patient had resolution of his inflammatory lesions without serious infections or adverse reactions. Inhibition of interleukin-23 and interleukin-17 may have a role in the management of LAD1. (Funded by the National Institute of Allergy and Infectious Diseases and others.)

From the National Institute of Dental and Craniofacial Research (N.M.M., T.W., N.D., L.B., G.D.), the Laboratory of Clinical Infectious Diseases (C.S.Z., G.U., S.M.H.), and the Laboratory of Immunoregulation (A.L.), National Institute of Allergy and Infectious Diseases, and the Wound-Ostomy Care Nursing Service, Clinical Center (K.C.A.) — all at the National Institutes of Health, Bethesda, MD; the Pediatric Onco-Hematology and Bone Marrow Transplantation Unit, Children's Hospital, Spedali Civili of Brescia, Brescia, Italy (Lucia D. Notarangelo); the Department of Microbiology, School of Dental Medicine, University of Pennsylvania, Philadelphia (G.H.); and the Division of Immunology, Boston Children's Hospital and Harvard Medical School, Boston (Luigi D. Notarangelo). Address reprint requests to Dr. Holland at the National Institutes of Health, Bldg. 10/11N248 MSC 1960, Bethesda, MD 20892-1960, or at smh@nih.gov.

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LAD1 IS A PRIMARY IMMUNODEFICIENCY RESULTING FROM MUTATIONS IN *ITGB2*, which encodes the common CD18 subunit of the $\beta 2$ integrins; the $\beta 2$ integrins are required for the adhesion of neutrophils to endothelium and the transmigration of neutrophils into tissues. Patients with LAD1 typically have recurrent skin infections that can be chronic and refractory. Recurrent oral ulcers cause severe pain and difficulty eating, and severe periodontitis leads to complete loss of adult teeth in almost all patients.[1] The mucocutaneous lesions in LAD1 were widely assumed to be due to infections that were a result of the relative tissue neutropenia caused by the inability of LAD1 neutrophils to enter the tissue and control microbes.[2] However, recent work has shown that LAD1-associated oral disease is actually a microbe-induced hyperinflammatory response.[3,4]

LAD1 periodontal lesions have a strong interleukin-23–interleukin-17 signature, which drives local immunopathologic processes and bone resorption. Animal models of LAD1 have aberrant interleukin-23 and interleukin-17 responses in peripheral tissues[5,6] as well as spontaneous development of periodontitis that can be inhibited by blockade of the interleukin-23–interleukin-17 axis.[3] In humans, LAD1 periodontitis is typically progressive and refractory to conventional management and results in almost universal loss of the teeth and surrounding alveolar bone by late adolescence. Other than bone marrow transplantation, no directed approaches for the management of LAD1 are currently known. A video about LAD1 and its physiological basis is available at NEJM.org.

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CASE PRESENTATION

A 19-year-old man with LAD1 (homozygous for *ITGB2* c.IVS15–2A→G; p.D750_K755) presented for evaluation of severe periodontal disease. He had delayed umbilical cord separation and recurrent urinary tract infections, otitis, and skin infections in early childhood. Testing that was performed when the patient had appendicitis at 4 years of age led to the diagnosis of LAD1, and he began prophylactic treatment with trimethoprim–sulfamethoxazole. At 5 years of age, he had enteric salmonellosis, and at 14 years of age he had mastoiditis. He has had frequent skin infections and several cases of pneumonia. His recurrent oral ulcers were treated with systemic glucocorticoids and acyclovir. Severe periodontitis began in his early teens, and he was advised to have his teeth extracted. During the 2 years before the current presentation, a sacral wound had progressed despite numerous courses of antibiotics and multiple deep, sharp débridements. Repeated use of systemic glucocorticoids to control the inflammatory reaction had caused adrenal insufficiency.

On admission, the patient was afebrile and apparently well but had severe periodontitis with generalized gingival inflammation and severe periodontal bone loss, particularly in the posterior areas; a large, deep, malodorous, inflamed sacral lesion was also noted (Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). Radiographs showed a left lingular pneumonia. His periodontitis was treated with deep dental cleanings at admission and again at 3 months. His sacral wound was treated with aggressive soaks, dressings, and topical glucocorticoids as well as broad-spectrum antibiotics. When he returned after 4 months of these standard-of-care treatments, he had resolution of his pneumonia but still had recurrent oral ulcers and persistent severe oral inflammation (approximately 90% of gingival areas bled on probing) (Fig. S1B in the Supplementary Appendix); the sacral wound was unchanged. The patient's CD18 expression was 34% of the control value but could not be further augmented by cell activation, a finding consistent with a moderate form of LAD1.

PATHOLOGIC PRODUCTION OF INTERLEUKIN-23 AND INTERLEUKIN-17

Biopsy of the patient's gingival tissue revealed dense lymphocytic infiltrates with intense interleukin-17 staining (Fig. 1A). *IL17A* and *IL23A* (encoding the p19 subunit of interleukin-23) messenger RNA (mRNA) levels in lesional tissues were also substantially increased and were similar to the levels seen in other patients with LAD1 (Fig. 1C and 1D). Flow-cytometric analysis of cells extracted from the gingival lesions confirmed the presence of elevated levels of interleukin-17–producing cells within the lesions; the CD4+ T-cell compartment was the major source of interleukin-17 production (Fig. 1E and 1F). We were reluctant to perform a biopsy of the patient's sacral wound because of previous inflammatory reactions that had occurred after manipulation. Therefore, we examined similar sacral tissue resected from another patient with LAD1 and found abundant interleukin-17–producing T cells in the inflamed tissue. This finding of excessive interleukin-17–producing T cells in inflamed periodontal and cutaneous LAD1 lesions suggested that the immunopathologic processes at the two sites might be similar (Fig. 1B) and suggested that inhibition of the interleukin-23–interleukin-17 axis might be helpful.

BLOCKADE OF THE P40 SUBUNIT OF INTERLEUKIN-23 AND INTERLEUKIN-12

In view of the patient's progressive periodontal disease and persistent wound, and being aware of the natural history of these processes in LAD1, we elected to target the excessive interleukin-17 levels that were associated with his immunopathologic condition. Human interleukin-17–mediated diseases have been approached in several ways, including treatment with ustekinumab, the antibody that binds the p40 subunit common to interleukin-12 and interleukin-23. Ustekinumab inhibits interleukin-12 and interleukin-23 signaling and the downstream interleukin-17 response and is effective in the treatment of plaque psoriasis, with few associated infections found in several large trials.[7-9] We used ustekinumab off-label with the dosing regimen

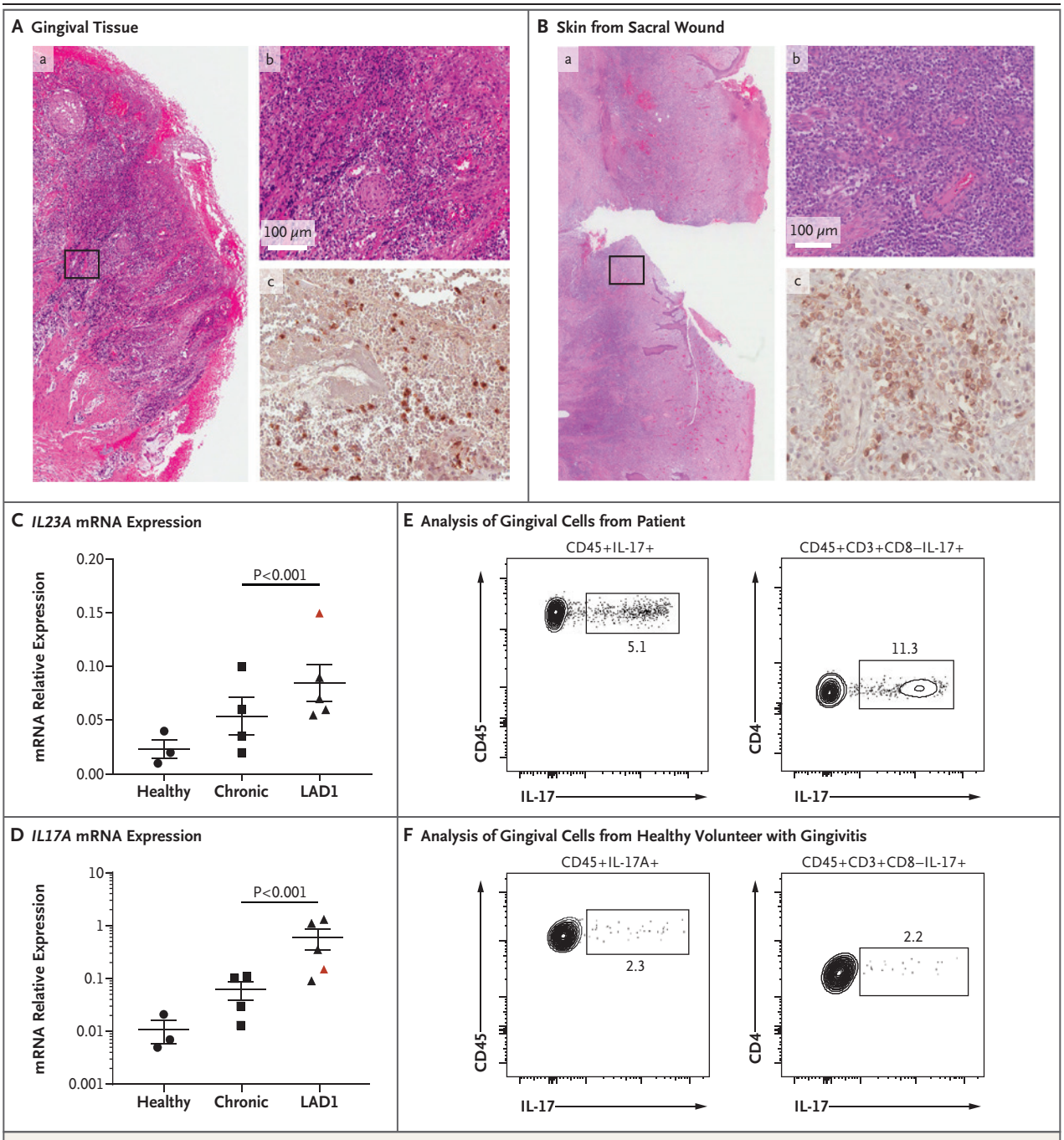

Figure 1. Interleukin-17–Dominated Inflammation in Leukocyte Adhesion Deficiency Type 1 (LAD1).

Panels A and B show histologic sections from gingival tissue (A) and sacral-wound skin (B), stained with hematoxylin and eosin at low magnification (subpanel a) and higher magnification (subpanel b), as well as with interleukin (IL)–17 (subpanel c) (brown staining is positive). Panels C and D show expression of *IL23A* (encoding the p19 subunit of IL-23) messenger RNA (mRNA) (C) and *IL17A* mRNA (D) in tissues from patients with no oral inflammation (healthy), chronic periodontitis (chronic), and LAD1 periodontitis (LAD1; the current patient is indicated in red). Panels E and F show the results of a flow cytometric analysis of cells isolated from the gingival tissues of our patient (E) and a healthy volunteer with gingivitis (F). IL-17 production in total hematopoietic (CD45+) cells and in CD45+CD3+CD8– CD56– cells (presumed to be CD4+ T cells) is shown.

approved for psoriasis: 45 mg administered subcutaneously at baseline, at week 4, and every 12 weeks thereafter. Throughout the treatment period, there were no adverse reactions associated with the injections, and the patient continued to receive standard-of-care dental cleanings and antibiotic prophylaxis with trimethoprim–sulfamethoxazole.

The oral pathologic features were dramatically reduced within weeks after the first injection was administered. Three weeks after treatment initiation, the patient's oral tissues were clinically healthier. Indeed, bleeding on probing, which is an indicator of gingival inflammation, was reduced to only 40% of sites, as compared with 90% of sites before treatment (Fig. 2B). Levels of tissue markers of inflammation, such as interleukin-23 and interleukin-17, went from being very high relative to levels found in volunteers with healthy gingiva and patients with chronic periodontitis (Fig. 1) to almost undetectable by 3 weeks after treatment (Fig. 2C and 2D). Clinically detected inflammation continued to decline relative to pretreatment levels throughout the period of treatment (Fig. 2B). Similarly, the expression of tissue interleukin-17, a direct downstream target of anti–interleukin-23 therapy, remained low throughout the entire period of treatment (Fig. 2D). Transcription of interleukin-17–regulated genes (*CSF3* and *CXCL1*) was also partially inhibited. *IL23A* mRNA expression is not directly targeted by ustekinumab and was not inhibited in the long term. We attributed a temporary initial reduction in *IL23A* mRNA expression to the overall reduction of inflammation immediately after treatment (Fig. 2C). During the entire treatment period, the patient reported a dramatic subjective reduction in oral inflammation. The reductions were most marked during the first 4 to 6 weeks after each injection of ustekinumab; however, the patient became more aware of oral inflammation during the final month before the next dose was scheduled to be given. *IL17A* mRNA expression in his gingival tissues that was measured immediately before redosing at months 10 and 14 showed some rebound but not to pretreatment levels, a finding consistent with the patient's subjective reports (Fig. 2D). Despite these rebounds, he had a marked reduction in oral and periodontal inflammation throughout the period of treatment (Fig. 2A and 2B) without progression of bone resorption or tooth loss.

Similar to the patient's oral pathologic condition, his sacral wound improved dramatically. After the third injection, wound improvement was readily evident, with a marked reduction in size. By 10 to 14 months, the wound was completely healed with minimal residual scarring (Fig. 2E). During this period, the patient stopped taking glucocorticoids, and endogenous glucocorticoid production gradually returned. Throughout the course of treatment, no new skin lesions and markedly fewer oral ulcers developed.

DISCUSSION

Periodontitis, oral ulcers, and chronic necrotic skin lesions are common in LAD1, but their treatment has remained largely empirical. Despite receiving periodontal care, patients with LAD1 lose teeth and alveolar bone. Chronic necrotic skin lesions, often incorrectly described as pyoderma gangrenosum (biopsy samples characteristically show no neutrophils), are common in LAD1 and are typically treated with antibiotics, surgery, or glucocorticoids, yet they still result in characteristic scarring. Case reports of successful treatment of these lesions with high-dose immune globulin,[10] tumor necrosis factor (TNF) blockade,[11] or glucocorticoids attest to the fact that immune modulators are often used as treatment, despite the lack of a mechanistic foundation to support their use. Interestingly, TNF blockade also inhibits the differentiation of type 17 helper T cells and the production of interleukin-17, potentially affecting the interleukin-17 axis in LAD1, as well.[12,13]

We pursued the blockade of the p40 subunit common to interleukin-12 and interleukin-23 on the basis of strong biologic evidence from patients with LAD1 and animal models of the disorder. Indeed, we have recently demonstrated that the interleukin-23 pathway is profoundly dysregulated in the absence of tissue neutrophils. Specifically, at barrier sites such as the oral mucosa, and probably in the skin and gastrointestinal tract, an otherwise physiologic interleukin-23 response induced by the local microbiome becomes dysregulated and excessive in the absence of tissue neutrophils.[6,14] The phagocytosis of apoptotic neutrophils (efferocytosis) by tissue macrophages acts as a signal to down-regulate the production of interleukin-23 and therefore the downstream cytokines interleukin-17 and granulocyte colony-stimulating factor (G-CSF).[6,15] The

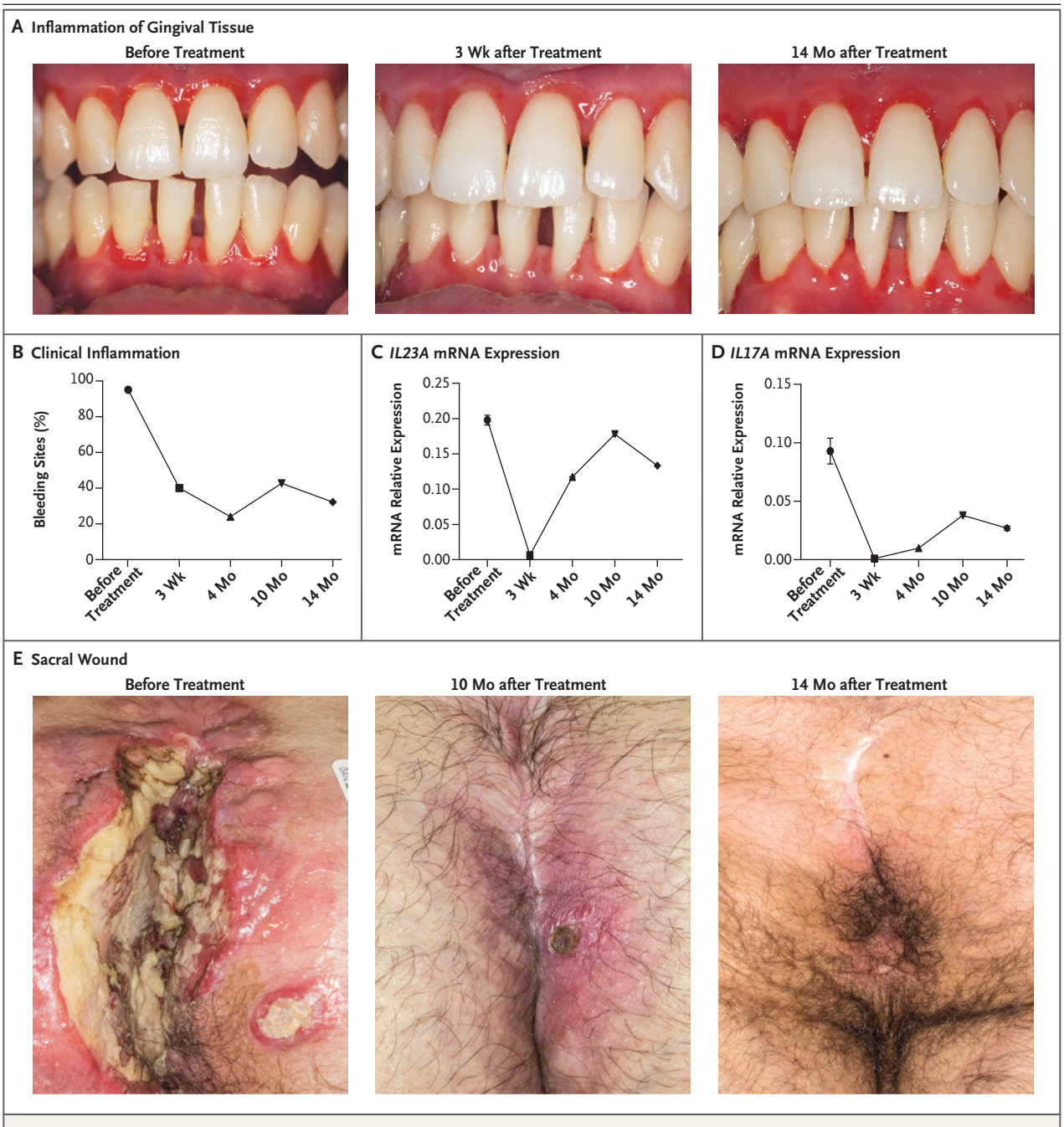

Figure 2. Response to Ustekinumab Treatment.

Panel A shows clinical photographs of the gingival tissues of our patient before treatment and at 3 weeks and 14 months after treatment. Panel B shows clinical detection of inflammation in the oral cavity (percentage of sites with bleeding on probing) according to time relative to treatment. Panels C and D show relative levels of expression of *IL23A* mRNA (C) and *IL17A* mRNA (D) before and during treatment. Panel E shows clinical photographs of the sacral wound before treatment and at 10 and 14 months after treatment. No sharp débridements were performed during this period.

disruption of this circuit as a result of the paucity of tissue neutrophils in LAD1 leads to exaggerated interleukin-23 and interleukin-17 responses, which appear to account for local LAD1 immunopathologic processes. This hypothesis is supported by our findings that anti–interleukin-23 and anti–interleukin-17 prevent periodontal inflammation and bone loss in lymphocyte function–

associated antigen 1 (LFA1)–deficient mice, which mimic the human LAD1 periodontal phenotype.[3] The success of ustekinumab in the treatment of our patient's periodontitis and chronic sacral wound further supports this model. An appropriate (microbial) stimulus is required to stimulate this disinhibited interleukin-23–interleukin-17 axis, which explains why the pathologic inflammatory response in patients with LAD1 develops at barrier sites (gingiva, skin, and colon) that are subjected to continuous microbial stimulation.[4] Consistent with this hypothesis, antibiotic treatment alone has not been very successful in addressing the immunopathologic complications in LAD1. Despite the fact that such treatment can suppress the microbial burden, it does not sterilize mucosal sites and thereby permits persistent stimulation by residual bacteria, which is apparently adequate to activate a dysregulated interleukin-23–interleukin-17 response.

Treatment of our patient with ustekinumab for more than 1 year was associated with resolution of refractory oral inflammation and healing of a severe sacral wound. The dosing of ustekinumab that is normally used for psoriasis was effective in early complete blocking of interleukin-17 and related cytokines and chemokines in the gingiva for at least 3 weeks. Whether a shorter treatment interval would have more clinical benefit is unclear. However, as we try to understand the oral and cutaneous lesions in LAD1, it is important to recall that skin-wound closure and complete epithelialization minimizes microbial stimulation; in contrast, the periodontal pocket is an open environment that is continuously subjected to a high level of microbial stimulation. Therefore, although a limited course of this targeted therapy might help in the treatment of a closable wound, LAD1 periodontitis is an ongoing process that is likely to require more long-term therapy. The treatment of additional patients with LAD1 is needed to help clarify the role of ustekinumab in the management of immunopathologic processes in LAD1 and the long-term safety of the drug in light of the underlying immunodeficiency in these patients.

This single case of ustekinumab treatment in LAD1 highlights the critical role of neutrophils not only in defense against infection, as has historically been recognized, but also in the regulation of inflammation itself.[2] Whether the immunopathologic consequences of tissue neutropenia in LAD1 are replicated in other forms of neutropenia remains to be seen.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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