Neutralizing Anti–DNase 1 and –DNase 1L3 Antibodies Impair Neutrophil Extracellular Traps Degradation in Hidradenitis Suppurativa

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Hidradenitis suppurativa (HS) is a debilitating inflammatory skin disorder characterized by abscess-like nodules and boils resulting in fistulas and tissue scarring. We previously reported evidence of an autoimmune signature in HS, characterized by enhanced neutrophil extracellular trap (NET) infiltration in HS skin lesions and dysregulation of the adaptive immune system characterized by the presence of autoantibodies. Timely removal of NETs is critical for tissue homeostasis to prevent a dysregulated generation of modified autoantigens and tissue damage. DNases 1 and 1L3 play important roles in proper NET removal. We tested the hypothesis that NETs in patients with HS are not effectively cleared owing to the presence of antibodies against DNase 1 and DNase 1L3. We report that HS serum poorly degraded NETs. Addition of exogenous DNase 1 restored NET degradation capabilities in a subset of HS samples. DNase 1 activity was significantly decreased in HS sera. Anti–DNase 1 and –DNase 1L3 antibodies were detected in serum samples and skin lesions from patients with HS. Purified IgGs from HS decreased DNase 1 activity and NET degradation. Taken together, this identification of neutralizing antibodies against nucleases in HS expands the understanding of the pathogenesis of this disease to support an autoimmune mechanism in its underlying pathogenesis.

INTRODUCTION

Hidradenitis suppurativa (HS) is a chronic skin condition characterized by swollen, painful lesions located in the axillae, groin, and other intertriginous areas (Saunte and Jemec, 2017). HS is estimated to affect around 1% of the general population, having a higher prevalence among women of African American descent (Garg et al., 2017; Vlassova et al., 2015). The etiology of HS remains unknown, but smoking, obesity, and hormonal factors have been associated with the condition (Saunte and Jemec, 2017). Neutrophils are highly abundant in HS skin lesions, and HS neutrophils display enhanced capacity to form neutrophil extracellular traps (NETs) that are detected in skin lesions (Byrd et al., 2019). Timely and efficient removal of NETs is crucial for tissue homeostasis and to prevent dysregulated autoantigen generation. Indeed, impaired NET degradation has been documented in several autoimmune and autoinflammatory conditions (Hakkim et al., 2010; Mistry et al., 2018) in association with clinical manifestations; for example, lack of NET degradation is associated with nephritis in patients with lupus (Hakkim et al., 2010). Improper NET clearance can be attributed to a variety of factors, including inhibition of DNase 1 activity by natural DNase 1 inhibitors, protection from NET degradation by antibodies that target these structures, or impairment of nuclease activity by antibodies against nucleases, including the DNase 1 and DNase 1L3 endonucleases.

NETs can externalize autoantigens and alarmins that can activate the adaptive immune system, promoting pathogenic immune responses (Carmona-Rivera et al., 2017). Dysregulation of the immune system has been reported in HS. Of particular interest, B cells and plasma cells are present in HS skin lesions, and these cells display an activated immunophenotype in circulation (Byrd et al., 2019; Frew et al., 2020; Gudjonsson et al., 2020). IgG levels are elevated in sera from patients with HS, and antibodies against extracellular matrix and nuclear and citrullinated antigens are also prevalent in this disease (Carmona-Rivera et al., 2022). We hypothesized that antibodies against DNases are present in patients with HS and may contribute to the impaired NET degradation exhibited in this condition. In this study, we found that over 90% of patients with HS do not degrade NETs. The addition of exogenous DNase 1 partially restored degradation capacity in HS sera but also revealed a subset of patients with...
HS who did not respond to the treatment. Antibodies against DNase 1 and DNase 1L3 were present in both serum and skin lesions from patients with HS and contributed to reduced DNase1 activity and impaired capacity for NET degradation. Furthermore, the presence of these antibodies correlated with chronic states of HS.

RESULTS

Impaired NET degradation in patients with HS

We analyzed the NET degradation capabilities of 26 serum samples collected from 8 healthy control and 18 patients with HS. The demographics of the HS cohort are displayed in Table 1. The proportion of patients with HS (male to female) and the three disease stages (Hurley stages I, II, III) were represented across the cohort of patients. NETs were treated with either patient or control serum, and Sytox green was used to measure the remaining DNA, as a readout of NET clearance. Undigested NETs displayed significantly higher levels of fluorescence than NETs incubated with control serum (Figure 1a). NETs incubated with HS serum exhibit significantly higher fluorescence intensity than those incubated with control sera, with levels similar to those found in undigested NETs (Figure 1a), indicating a lack of degradation capabilities. DNA fluorescence imaging analysis showed that control serum degraded NETs, whereas sera from patients with HS did not (Figure 1b). Indeed, all control serum samples tested degraded NETs, whereas only 6% of HS samples could dismantle NETs (degraders). The remaining 94% of HS samples show impairments in NET degradation (non-degraders) (Figure 1c). These results suggest that serum from patients with HS displays poor capability for proper NET degradation in vitro.

Partial restoration of NET degradation by addition of nuclease

To elucidate the factors underlying the poor NET degradation capacity displayed in HS serum samples, we tested whether exogenous DNase 1 or micrococcal nuclease (MNase) could restore NET degradation capability in this disease in vitro. Addition of exogenous DNase 1 to HS serum samples significantly increased the degradation capacity when compared with HS serum alone (Figure 2a and b). Overall, 39% of the HS serum samples tested did not exhibit a restored capability for NET degradation after DNase 1 addition (Figure 2d), suggesting that some other factors may interfere with proper NET degradation, such as the presence of a DNase 1 inhibitor or physical protection of the NETs from degradation by DNase 1. Furthermore, the addition of MNase to HS serum significantly increased its ability to degrade NETs (Figure 2a and c). However, after the addition of MNase, 44% of the HS serum samples tested did not display restored NET degradation capabilities (Figure 2d), further supporting the presence of factor(s) in HS serum samples that protect NETs from nuclease activity.

Anti-NET autoantibodies have been previously associated with impairments in NET degradation in conditions such as systemic lupus erythematosus (SLE) (Hakkim et al., 2010). Therefore, we tested for their presence in HS serum. We did not find significant levels of anti-NET antibodies, but they correlated negatively with impairments in NET degradation ($r = -0.4536, P = 0.0458$) and with the presence of recombinant DNase 1 ($r = -0.4929, P = 0.0322$) (Figure 2e and f). In contrast, antibodies against NETs did not associate ($r = 0.3821, P = 0.0803$) with impairments of NET degradation after the addition of recombinant MNase (Figure 2g), suggesting a minor role of anti-NET antibodies in impairing DNA degradation in patients with HS. To validate that anti-NET antibodies are present in HS and may contribute, at least in part, to decreased NET degradation, we performed immunofluorescence analysis in HS skin lesions. Confocal analysis showed partial colocalization of extracellular citrullinated histone H4, a marker for NETs, and IgG (Figure 2h). This suggests that anti-NETs antibodies may be present in HS skin lesions and may partially protect NETs from being effectively degraded.

DNase 1 activity is reduced, and anti-DNase antibodies are present in HS serum

To gain further insights into the mechanisms that impair NET degradation in HS serum, we assessed the activity of DNase 1 in HS samples using a commercially available assay. We also included neutrophilic dermatosis, pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA), given that serum from these patients has also been reported to display impaired NET degradation capabilities.

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Table 1. Demographics of the Patients with HS Whose Serum and Tissue Were Tested in this Study

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>37.5 (17.5)</td>
</tr>
<tr>
<td>Female sex, n (%)</td>
<td>12 (67%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>14 (78%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>3 (17%)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>History</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>Current tobacco use, n (%)</td>
<td>3 (17%)</td>
</tr>
<tr>
<td>Previous smoker, n (%)</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Never smoked, n (%)</td>
<td>13 (72%)</td>
</tr>
<tr>
<td>BMI</td>
<td>35.02 (11.47)</td>
</tr>
<tr>
<td>HS tissue</td>
<td>HS Patients (n = 25)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; IQR, interquartile range. Values are expressed as the median (IQR) unless otherwise indicated.

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degradation (Mistry et al., 2018). Indeed, we found that sera from both patients with HS and those with PAPA display a significant reduction of DNase 1 activity when compared to control serum (Figure 3a). G-actin is a natural inhibitor of DNase 1 that has also been reported to impair NET degradation (Hakkim et al., 2010). Of note, the levels of G-actin in HS serum samples were significantly decreased compared with those in the controls (Figure 3b), suggesting that G-actin is not a contributing factor to the reduced DNase 1 activity seen in HS serum.

Because we previously reported the presence of autoantibodies in patients with HS (Carmona-Rivera et al., 2022), we hypothesized that patients with HS may develop antibodies against nucleases, similar to what has been described in other diseases such as SLE (Hartl et al., 2021). The most abundant and efficient nucleases present in circulation are DNase 1 and DNase 1L3 (Napirei et al., 2005). Therefore, we decided to test for the presence of antibodies against these nucleases in HS and PAPA sera. The levels of anti-DNase 1 and anti-DNase 1L3 antibodies were significantly elevated in HS serum samples but not in PAPA serum samples compared with those in control samples (Figure 3c and d). When we stratified the HS serum samples by Hurley stage, we found that the levels of anti–DNase 1 and –DNase 1L3 antibodies were significantly higher in samples from patients with Hurley stage II (Figure 3c, blue panel, and D, red panel). A negative trend was found between antibodies to DNase 1 and DNase 1 activity measured in HS sera (Figure 3e). DNase 1 activity was negatively associated ($r = -0.5958, P = 0.0266$) with body mass index (BMI) (Figure 3f). Antibodies to DNase 1L3 ($r = 0.6043, P = 0.0144$) were significantly associated with BMI (Figure 3g), whereas a positive trend was found for anti–DNase 1 antibodies and BMI (Figure 3h). These results suggest that antibodies against nucleases DNase 1 and DNase 1L3 are present in HS and may contribute to the reduced DNase activity exhibited in HS sera.

Anti–DNase 1 and –DNase 1L3 antibodies are present in HS skin lesions

We have previously reported enhanced NET formation in HS skin lesions, which may suggest a possible impairment in NET clearance in HS skin (Byrd et al., 2019). Therefore, we decided to further investigate whether antibodies against DNase 1 and DNase 1L3 can be detected in HS skin. The demographics of patients with HS who underwent skin biopsies are displayed in Table 1. HS and control protein homogenates purified from HS skin were tested by ELISA for these autoantibodies. Indeed, antibodies against DNase 1 and DNase 1L3 were significantly elevated in HS skin

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**Figure 1. HS serum samples display impaired NET degradation.** (a) Ctrl (n = 8) and HS (n = 18) serum samples were analyzed for their ability to degrade PMA-generated NETs from control neutrophils, according to Material and Methods. PMA + no serum (NETs) was used as a reference. Each circle corresponds to one individual donor. DNA was stained using the fluorescent dye, Sytox green. Results are the mean ± SEM. **$P < 0.01$ and ***$P < 0.001$. Mann–Whitney U test analysis was used. (b) Representative microphotographs of NETs stained with Sytox green in the presence or absence of Ctrl or HS serum. Bar = 100 μm. (c) Pie charts depicting the percentage of Ctrl and HS serum samples that either degraded (black) or did not degrade NETs (red). Ctrl, control; HS, hidradenitis suppurativa; NET, neutrophil extracellular trap; PMA, phorbol myristate acetate.
Figure 2. Exogenous nucleases partially rescue NET degradation ability by HS serum. (a) HS serum samples (n = 18) were spiked with exogenous DNase 1 or MNase and then analyzed for their ability to degrade NETs. The dashed line represents the mean of the control degradation. Results are the mean ± SEM. **P < 0.01. Kruskal–Wallis analysis was performed. Representative microphotographs of NETs in the presence of HS serum and (b) exogenous DNase 1 or (c) MNase for 16 h. Bar = 100 μm. (d) Pie charts depicting the percentage of the sample that degraded (black) NETs. Correlation analysis of levels of anti-NET antibodies
samples compared with those in the control (Figure 4a and b). Stratification of HS skin samples by Hurley stage showed significant levels of anti-DNase 1 and −DNase 1L3 antibodies in Hurley stage III HS skin compared with those in the control (Figure 4, blue panel, and b, red panel), suggesting a dysregulation of the immune system during chronic stages of the disease. A significant reduction in DNase 1 activity was detected in HS skin lesions (Figure 4c). This reduction in DNase 1 activity was negatively associated with the presence of antibodies to DNase 1 (r = −0.4185, P = 0.0418) (Figure 4d) and BMI (r = −0.4779, P = 0.0245) (Figure 4e). In addition, BMI was significantly associated (r = 0.4421, P = 0.0269) with the presence of antibodies to DNase 1L3 (Figure 4f). In contrast, no correlation was found between BMI and antibodies to DNase 1 measured in HS skin lesions (Figure 4g). Taken together, antibodies against nucleases are present in HS skin samples and correlate with the chronic stages of HS and decreased DNase 1 activity.

**Purified HS IgGs reduce DNase 1 activity**

To determine whether the antibodies against DNase 1 found in HS sera directly impact DNase 1 activity, we purified total IgG from HS sera. DNase 1 activity was monitored in control serum samples in the presence or absence of HS IgGs. DNase 1 activity was significantly reduced in control serum samples incubated with HS IgGs (Figure 5a). This suggests that anti-DNase 1 antibodies present in HS can affect the function of DNase 1. To further corroborate the specificity of the antibodies against DNase 1, recombinant DNase 1 was incubated in the presence or absence of HS IgGs. A significant reduction in DNase 1 activity was observed after incubation with HS IgGs (Figure 5b), suggesting that HS IgGs can neutralize DNase 1 in the serum, impairing its ability to degrade NETs. To support this hypothesis, phorbol myristate acetate–induced NETs were generated from control neutrophils and treated with control sera preincubated with or without HS IgGs. Fluorescence analysis showed a significant increase in DNA in the control samples incubated with HS IgG compared with that in the control alone (Figure 5c). This result indicates that HS IgGs impair NET degradation by affecting serum DNase 1 activity. Taken together, these results show the presence of neutralizing anti-DNase 1 antibodies in HS that impair NET degradation.

**DISCUSSION**

Impaired NET degradation has been documented in autoimmune and inflammatory diseases (Hakkim et al., 2010; Mistry et al., 2018), and it has been associated with clinical manifestations, such as nephritis in patients with lupus (Hakkim et al., 2010). Neutrophils from patients with HS display an enhanced ability for NET formation, and these structures are abundant in HS skin lesions (Byrd et al., 2019). We now describe that HS serum samples display a reduced ability to degrade NETs. Furthermore, although the addition of exogenous DNase 1 and MNase partially restored this ability, a subset of patients’ samples did not improve degradation capabilities. Accordingly, we found decreased DNase 1 activity and the presence of circulating and tissue anti-DNase antibodies, with HS IgGs inducing reduced DNase 1 activity.

Immune dysregulation has been reported in HS and is proposed as a significant component of disease pathogenesis (Lowe et al., 2020). In other inflammatory conditions, NETs have been suggested to drive inflammation and immune responses through the externalization and modification of autoantigens (Carmona-Rivera et al., 2017; Khandpur et al., 2013). Furthermore, NETs can be internalized by phagocytic cells and other nonprofessional antigen-presenting cells and trigger aberrant immune responses (Carmona-Rivera et al., 2017). Improper NET degradation can also promote or be associated with endothelial damage, organ dysfunction, inflammation, and autoimmunity. The decreased capability of HS serum samples to degrade NETs may contribute to immunogenicity and trigger aberrant innate and adaptive immune responses and autoantibody generation in this disease. In support of this, we previously confirmed the presence of autoantibodies against DNA, nuclear proteins, and NET components in patients with HS (Carmona-Rivera et al., 2022).

Genetic alterations in DNASE genes have been linked to autoimmune diseases with organ damage involvement (Hartl et al., 2021; Yasutomo et al., 2001). Genetic variants in DNASE1 have been described in SLE presenting with low levels of circulating DNase 1 and high levels of anti-DNA antibodies (Yasutomo et al., 2001). A loss-of-function alteration in DNASE1L3 results in the pediatric onset of familial SLE characterized by high levels of circulating anti-DNA antibodies and renal involvement (Ozçakar et al., 2013). A polymorphism in DNASE1L3 resulting in an R206C substitution causing loss of function of the nuclease has been associated with less severe autoimmune manifestation (Al-Mayouf et al., 2011). Finally, mutations in DNASE2 resulting in loss-of-nuclease activity have been associated with fluctuating levels of anti-DNA antibodies alongside increased levels of type I IFNs and glomerulonephritis in pediatric patients with SLE (Rodero et al., 2017). HS skin lesions are characterized by a type I IFN signature and feature an extensive presence of NETs, suggesting that pathways similar to those found in IFN-mediated inflammatory diseases may be involved in patients with HS.

Decreased DNase 1 activity has been described in SLE and microscopic polyangiitis, in association with the presence of neutralizing antibodies (Nakazawa et al., 2014). More recently, high levels of circulating NETs were described in patients with SLE with lupus nephritis, which correlated with elevated circulating levels of anti-DNA antibodies or low complement C3 activity. DNase 1 activity was found to be selectively associated with patients with lupus nephritis compared with association with those with SLE and controls despite similar DNase 1 serum levels (Napirei et al., 2005).
Figure 3. DNase 1 activity is reduced, and antinucleases antibodies are present in HS serum. (a) DNase 1 activity and (b) levels of G-actin were measured in the sera samples from ctrl (n = 7), HS (n = 18), and/or PAPA (n = 7). Sera were tested for the presence of autoantibodies against (c) DNase 1 and (d) DNase 1L3. Results are the mean ± SEM. **P < 0.01 and ****P < 0.0001. Mann–Whitney U test analysis was used. Autoantibodies present in HS sera were stratified by Hurley stage (stage I, n = 4; stage II, n = 8; stage III, n = 6). Results are the mean ± SEM. Kruskal–Wallis analysis was used. Correlation analysis of DNase 1
IgG depletion was able to restore DNase 1 efficiency, suggesting the presence of neutralizing antibodies (Nakazawa et al., 2014). Moreover, antibodies against DNase 1L3 have been reported in patients with SLE with lupus nephritis and are associated with decreased DNase 1L3 activity (Hartl et al., 2021). Similar to patients with SLE, we report that patients with HS display a significant decrease in DNase 1 activity that can be recapitulated by adding HS IgG to recombinant nucleases in vitro. The ability of exogenous DNase 1 to restore NET degradation by HS serum samples suggests that modulation of DNase1 activity may represent an opportunity to restore impaired NET clearance in HS and its associated complications.

Antibodies against NETs have been described in autoimmune and inflammatory conditions and associated with impaired NET degradation (Zuo et al., 2021). In HS, we found that anti-NET antibody negatively correlated with decreased NET degradation. This suggests that anti-NET antibodies may have a minimal role in the poor NET degradation capacity displayed in patients with HS. Perhaps this
Figure 5. HS IgGs decrease DNase 1 activity. (a) DNase 1 activity was measured in Ctrl (n = 5) sera in the presence or absence of 25 μg of total IgG isolated from HS sera (n = 5). Results are the mean ± SEM. *P < 0.05. Wilcoxon matched-pairs test analysis was used. (b) The activity of human recombinant DNase 1 (10 units) was assessed in the presence or absence of total HS IgGs. (c) Ctrl serum samples (n = 5) were tested for their ability to degrade NETs in the absence or presence of HS IgG. NETs were stained with Sytox green, and fluorescence values are expressed as RFUs. Results are the mean ± SEM. *P < 0.05. Mann-Whitney U test analysis was used. Ctrl, control; NET, neutrophil extracellular trap; HS, hidradenitis suppurativa; RFU, relative fluorescence unit.

Effect is driven by protecting NET fibers from recognition by nucleases but is likely not the main driver of this phenotype.

Overall, we report the detection of anti-DNase 1 and anti-DNase 1L3 antibodies in circulation and skin lesions from patients with HS. Antibody levels were primarily elevated in patients with chronic HS with Hurley stages II and III, whereas those from Hurley stage I displayed low titers of these antibodies. This may suggest that disease progression is tied to increased DNA release, decreased DNA clearance by nucleases or other mechanisms, and dysregulation of the adaptive immune system. These elements should be explored in future studies. Whether anti-DNase antibodies can define specific Hurley stages and be used as a biomarker of severity remains unknown.

Limitations of the study are primarily derived from the limited clinical information of the cohort studied. Furthermore, the cohort examined in this study was primarily African American. Although HS is more prevalent in those of African American descent, it is important to confirm these findings in patients with other ethnic backgrounds. The impact of impaired NET formation in extradermal complications of HS remains to be determined. In addition, we did not have access to paired serum and skin samples from each patient to determine whether these antibodies were detected both systemically and locally at the level of the lesions. Finally, we were technically limited in our ability to assess the activity of DNase 1L3 in an accurate way, which would allow the determination of the specific impact of DNase 1L3 itself on NET degradation in HS samples. Despite these limitations, we provide evidence that DNase 1 activity in HS is impaired owing to the presence of neutralizing antibodies, contributing to poor ability to degrade NETs.

Given the findings of this study and the apparent contributions of immunological dysregulation in HS, expansion of the clinical assessment in patients with HS beyond dermatology may be recommended, and a multidisciplinary approach to HS disease management should be considered. Furthermore, DNase 1 activity may be modulated by a mosaic of factors beyond those discussed in this study. For instance, not all patients with reduced DNase 1 activity displayed anti-DNase 1 antibodies or anti-DNase 1L3 antibodies. As such, the findings of this study warrant further genetic investigations, which may help to explain the mechanisms involved in these abnormalities in those patients with HS with low DNase 1 activity but no measurable antibodies.

In summary, our findings support a link between dysregulated NET formation and clearance and the presence of neutralizing antibodies against DNases in patients with HS. These findings provide a better understanding of the immunological mechanisms underlying HS pathogenesis and further suggest potential therapeutic developments in targeting dysfunctional DNase 1 or accounting for the potential utility of autoantibodies as clinically relevant biomarkers of disease progression and risk. Because the biological significance of NETs in HS is still not completely understood, future studies should continue to investigate the potentially deleterious role of NETs in the pathophysiology of this condition.

**Materials and Methods**

**Patient specimens**

Healthy controls were recruited by advertisement. Sera and skin tissue were collected under the NA_00013177 and NA_00031269 studies approved by the Johns Hopkins University Institutional Review Board. All individuals gave written informed consent. Normal (non-HS) and lesional HS skin were obtained from surgical resections and from Johns Hopkins’ tissue bank obtained from patients undergoing surgical removal of HS skin. Samples from patients with PAPA were obtained as previously described (Mistry et al., 2018). Samples were stored at −80 °C.

**Degradation assay**

Phorbol myristate acetate (Sigma-Aldrich, St. Louis, MO)–induced NETs were generated as previously described (Carmona-Rivera et al., 2015). NETs were treated with 5% serum from either healthy controls or subjects with HS for 16 hours at 37 °C. Wells
were stained with 0.2 μM Sytox green (Thermo Fisher Scientific, Waltham, MA) for 5 minutes. The plate was read using a microplate reader (Synergy HT, Bio-Tek, Rockville, MD). Results are presented as relative fluorescence units. Wells were visualized to corroborate the presence or the absence of NETs using a ZOE Fluorescent Cell Imager (Bio-Rad Laboratories, Hercules, CA).

NET isolation
NETs were isolated as previously described (Carmona-Rivera et al., 2019). Purified NETs were transferred to a fresh tube, and protein was quantified using BCA kit (Thermo Fisher Scientific) according to the manufacturer's instructions.

Isolation of protein from tissue
Tissue sections were flash frozen with liquid nitrogen. Protein from tissue was isolated as previously described (Byrd et al., 2019). Proteins were quantified using the BCA kit (Thermo Fisher Scientific) according to the manufacturer's instructions.

Anti–DNase 1 and –DNase 1L3 and NETs antibody detection
A 96-well plate was coated with 10 μg/ml of phorbol myristate acetate–generated NETs, 2.5 μg/ml recombinant DNase 1 (Abcam, Boston, MA), or DNase 1L3 (MyBioSource, San Diego, CA) in PBS overnight at 4 °C. The plate was blocked with 1% BSA for 1 hour at room temperature. A total of 1 μg of total protein from control or HS skin samples was incubated overnight at 4 °C. After washing, horseradish peroxidase–conjugated antihuman IgG secondary antibody (1:5,000, Sigma-Aldrich) was incubated for 1 hour at room temperature. The plate was developed in the presence of 3,3′,5′,5′-tetramethylbenzidine and read at 450 nm using a microplate reader (Synergy HT, Bio-Tek). Results are presented as optical density index (ratio of the optical density in the patient serum to the mean optical density in healthy control serum).

DNase 1 activity assay
DNase 1 activity was measured using a DNase 1 assay kit (Abcam) as per the manufacturer’s instructions. Briefly, 25 μl of the sample (control or HS sera) or 10 U of recombinant human DNase 1 (Abcam) were incubated in the absence or presence of 25 μg of purified HS IgG. DNA probe was added to all samples. Fluorescence (excitation/ emission: 651/681 nm) was measured using a Biotek Synergy H1 plate reader. The standard curve was calculated on the basis of the relative fluorescence units at 90 minutes. The activity was calculated and expressed as nmol/min/ml or nmol/min/Unit.

IgG isolation
IgGs were purified from HS sera with a Melon Gel IgG Spin kit following the manufacturer’s instructions (Thermo Fisher Scientific).

G-actin ELISA
Serum G-actin levels were detected using a commercially available G-Actin ELISA kit (MyBioSource) following the manufacturer’s instructions.

Statistical analysis
Data were analyzed using GraphPad Prism, version 8.1.1 (GraphPad Software, La Jolla, CA). For samples with non-Gaussian distribution, Mann–Whitney U test was used. Kruskal–Wallis test (Dunn’s multiple comparison test) was used to compare parameters among groups, whereas Pearson correlation test was used for all non-categorical statistics, and Spearman correlation test was used for those groups without normal distribution. All analyses were considered statistically significant at P < 0.05.

Data availability statement
All data are available in the manuscript and on request.

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CONFLICT OF INTEREST
ASB is a consultant for Senté, received an honorarium for presenting at the Annual AbbVie-sponsored Symposium on Hidradenitis Suppurativa for the 77th Annual Society for Investigative Dermatology meeting, and is a co-director of the Howard University Skin of Color Postgraduate Research Fellowship (sponsored by Pfizer). GAO is on an advisory board for Pfizer, UCB, Eli Lilly, Abbvie, and Novartis and a consultant for Janssen. MJK is on the scientific advisory Board of Neutrolis. The remaining authors state no conflict of interest.

REFERENCES

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