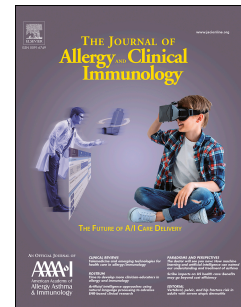


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**Neutrophils in Neutrophilic Dermatoses: Emerging Roles and Promising
Targeted Therapies**

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Neutrophilic dermatoses (NDs) are a heterogeneous group of cutaneous inflammatory diseases characterized by predominant and sterile neutrophilic infiltrates in lesional skin. This neutrophil infiltration varies in degree, leading to manifestations including pustules, abscesses, papules, nodules, and ulcers, with possible systemic or other organ involvement. NDs are subdivided into four groups based on histology (1): (1) epidermal type with pustular psoriasis, which is representative, (2) dermal type, including Sweet's syndrome, (3) hypodermal type with pyoderma gangrenosum (PG) as the paradigm, and (4) overlapping type (epidermal/dermal/hypodermal) referring to emerging PG-associated autoinflammatory syndromes (Table 1).

Genetic and immunological pathogenesis

The pathogenesis of NDs is thought to involve the interplay between genetic mutations and immunological factors (Table 1). These studies have revealed genetic abnormalities such as deficiency of interleukin (IL)-1 receptor antagonist (DIRA), deficiency of IL-36 receptor antagonist (IL36RN) (DITRA), mutations in proline-serine-threonine phosphatase interacting protein 1 (PSTPIP1) and NOD-like receptor family pyrin domain containing (NLRP)s, activate key players of the skin immune system, thus exacerbating downstream autoinflammatory responses in some patients with NDs (2). For example, the most well-defined ND is generalized pustular psoriasis, in which mutations in IL36RN, caspase recruitment domain-containing protein 14 (CARD14), and adaptor-related protein complex 1 subunit sigma 3 (AP1S3) enhance the activation of nuclear factor kappa-B signaling and increase the production of neutrophil-related chemokines and cytokines (e.g., IL-1 α , IL-36, and CXCL1/2/8), leading to autoinflammatory cascades; moreover, myeloperoxidase (MPO) mutations cause neutrophil dysregulation, modulating the recruitment and activation of additional neutrophils and macrophages (Table 1). Therefore, identifying the genetic mechanisms underlying NDs could provide important insights into the mechanisms that augment neutrophil accumulation and activation.

Apart from the abovementioned overproduction of cytokines caused by genetic mutations, epigenetic factors such as mental stress, metabolic states, smoking, and environmental triggers may increase the risk of NDs and aggravate the inflammation march. Dysregulation of innate immune responses, particularly the overactivation of the IL-1 family, IL-17, and tumor necrosis factor (TNF), is considered to be the prominent molecular mechanism of NDs. It is recently identified that granulocyte-colony stimulating factor (G-CSF), the main regulator of neutrophil biology, is strongly activated in hidradenitis suppurativa lesions and enhances neutrophil responses (3). Notably, the successful use of biologics targeting TNF, IL-17A, and IL-36 γ in several refractory cases supports the fundamental roles of these cytokines in NDs.

Therefore, these genetic alterations, inflammatory mediators, and initiating factors synergistically potentiate and maintain the predisposition of lesional skin, attracting and activating more neutrophils to exacerbate the inflammatory process of NDs.

The emerging role of neutrophils

Recent progress has highlighted the critical role of neutrophils in autoinflammation and tissue damage in some NDs. In physiological conditions, neutrophils are released from the bone marrow into the circulation to surveil skin homeostasis as a “patrol” (left panel, Figure 1); however, these cells are recruited by chemoattractants and transmigrate through vessel barriers into sterile inflammatory sites, where they produce proinflammatory mediators through degranulation, neutrophil extracellular traps (NETs), or extracellular vesicles (EVs) (right panel, Figure 1). Our group has shown that neutrophil-derived EVs can be taken up by keratinocytes, upregulating the level of neutrophil-related chemokines and triggering an autoinflammatory loop in pustular psoriasis (4). Neutrophil priming and aberrant NET formation have been reported in most NDs (PG, PAPA syndrome, Sweet’s syndrome, hidradenitis

suppurativa, and pustular psoriasis) (5). NETs can exert proinflammatory effects on many cells, including plasmacytoid dendritic cells, macrophages, and keratinocytes, or can release neutrophil proteases to activate IL-1/IL-36 cytokines within skin lesions (6). Moreover, NETs and the accompanying overproduction of reactive oxygen species also damage the vascular endothelial barrier, causing vasculitis in NDs. Indeed, neutrophil participation is dynamic and may vary depending on the immune imbalance.

Furthermore, neutrophil heterogeneity and their pathogenic functions have been gradually revealed. The most extensively characterized neutrophil subset is low-density granulocytes (LDGs), which exhibit an intermediate level of maturity and a proinflammatory phenotype with enhanced synthesis of proinflammatory cytokines and NETs, thus promoting autoinflammation in some NDs (5). Recently, one study has preliminarily shown that mature neutrophils are infiltrated in the skin lesions of subcorneal pustular dermatosis and PG, and high frequency of immature neutrophils in pustular psoriasis, hidradenitis suppurativa, and Sweet's syndrome (7), suggesting that the local environment may reprogram neutrophil subsets and cause different outcomes. Consistently, single-cell RNA sequencing analysis has shown that neutrophils are reprogrammed in target tissues; therefore, their lifespan and properties vary across multiple tissues (8). Targeting pathogenic neutrophil subsets may be a promising treatment strategy for NDs in the future that does not disrupt the protective effects of neutrophils.

Neutrophil-targeting therapy in the future

NDs are typically treated with systemic steroids or immunosuppressants and have recently been successfully treated by granulocyte and monocyte adsorptive apheresis in some cases. Biologic agents targeting the pathogenic factors of the disease, such as IL-1, TNF, and IL-17A, have also been reported in several refractory cases; however, high-quality comparative data are lacking. The immune imbalance of NDs is not caused by a single cytokine but involves multiple factors. Therefore, correcting

neutrophil dysfunctions may provide an alternative strategy for patients with refractory NDs, and these strategies may include inhibiting neutrophil recruitment (anti-CXCR1/2/4) or cytokines that induce neutrophil chemoattractants (i.e., anti-IL-1, IL-36, TNF, IL-17A.), preventing NET-induced inflammation (peptidylarginine deiminase 4 (PAD4) inhibitor, DNase I, or disrupting cellular crosstalk) or activation (anti-neutrophil elastase or MPO), or manipulating neutrophil-derived EVs (9, 10). Moreover, rationally depleting or targeting those proinflammatory neutrophil subsets (e.g., LDGs) is another strategy for treating NDs. However, LDGs are also heterogeneous and lack a specific marker, which calls for further studies to classify neutrophil subsets and their pathogenic roles. As neutrophils exhibit distinct gene expression profiles or metabolic processes depending on their developmental stage and tissue microenvironment, it is also advisable to develop stage-specific therapeutic strategies to modulate neutrophil functions in NDs. Overall, the safety and efficacy of these neutrophil-specific therapeutic strategies *in vivo* remain to be determined in future human clinical trials.

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Figure 1. Neutrophils induce local hyperinflammation in neutrophilic dermatoses

Mutations in several genes (e.g., IL36RN, CARD14, PSTPIP1) regulate innate immunity and increase critical proinflammatory cytokines, including IL-1, IL-36, and IL-17, chemokines, and other effector molecules that responsible for neutrophil chemotaxis and activation. In healthy states, neutrophils are released from the bone marrow into the circulation to surveil skin homeostasis, while in NDs, neutrophils are attracted into lesional skin and activated to release inflammatory mediators, extracellular vesicles, and NETs. These effector molecules and structures may activate resident and immune cells such as keratinocytes, pDCs, and macrophages, catalyze the cytokines pro-IL-1/IL-36, and damage the vascular endothelium, thus promoting vascular damage and autoinflammation in some NDs. Furthermore, different neutrophil subsets, including LDGs, may show distinct proinflammatory effects during this process. (Hyperinflammation is the inflammatory responses generated by the overproduction of proinflammatory cytokines, chemokines, and NETs that contributes to disease severity; local inflammation is the immune responses in lesional skin.) AP1S3, adaptor-related protein complex 1 subunit sigma 3; CARD, caspase recruitment domain; EC, endothelial cell; EVs, extracellular vesicles; G-CSF, granulocyte colony-stimulating factor; IFN, interferon; IL, interleukin; IL36RN, interleukin-36 receptor antagonist; KC, keratinocyte; LCN2, lipocalin-2; LDG, low-density granulocyte; NET, neutrophil extracellular trap; NOD, nucleotide-binding oligomerization domain; pDC, plasmacytoid dendritic cell; PSTPIP1, proline-serine-threonine phosphatase interacting protein.

169 **Table 1. Classification of neutrophilic skin diseases (examples)**

Diseases	Genetics	Immunological factors	References (digital object identifier)
Pustular psoriasis/palmoplantar pustulosis	IL36RN	IL-36/IL-1	1. Marrakchi et al. 10.1056/NEJMoa1013068
	CARD14	NF- κ B-activated cytokines	2. Jordan et al. 10.1016/j.ajhg.2012.03.013
	AP1S3	TLR3 signaling	3. Kaffetzi et al. 10.1016/j.ajhg.2014.04.005
	MPO	Neutrophil dysfunctions	4. Haskamp et al. 10.1016/j.ajhg.2020.07.001
AGEP	IL36RN	IL-36/IL-1	5. Navarini et al. 10.1001/jamadermatol.2014.3848
	CARD14	NF- κ B-activated cytokines	6. Podlipnik et al. 10.1111/ajd.12669
Sweet's syndrome	MEFV	Inflammasome activation	7. Ishikawa et al. 10.1002/acn3.50937
	UBA1	Activation of innate immune pathways	8. Beck et al. 10.1056/NEJMoa2026834
	IDH1	Not identified	9. Snyder et al. 10.21873/anticancerres.12462
Erythema elevatum diutinum	Not identified	Not identified	
Pyoderma gangrenosum	MEFV	Inflammasome activation	10. Marzano et al. 10.1111/bjd.15226
	NLRP3		
	NLRP12		11. Campbell et al. 10.3390/ijms17050725
	PSTPIP1		
	NOD2	Caspase-1-IL-1	
	LPIN2	Not identified	
	MTHFR		12. DeFilippis et al. 10.1111/bjd.13493
Hidradenitis suppurativa	JAK2		
	PSENEN	γ -secretase-Notch pathway	13. Wang et al. 10.1126/science.1196284
	NCSTN		
	PSEN1		
Behçet's disease	POFUT1		14. Villanueva et al. 10.1111/bjd.16264
	MEFV	Inflammasome activation	7. Ishikawa et al. 10.1002/acn3.50937

	CARD15	Not identified	15.	Ahmad	et	al.
						10.1080/03009740510018714
Neutrophilic panniculitis	Not identified	Not identified				
PAPA	PSTPIP1	Inflammasome activation	10.	Marzano	et	al.
						10.1111/bjd.15226
PASH	MEFV	Inflammasome activation	10.	Marzano	et	al.
	NLRP3					10.1111/bjd.15226
	NLRP12		11.	Campbell	et	al.
	PSTPIP1					10.3390/ijms17050725
	NOD2	Caspase-1-IL-1				
	LPIN2	Not identified				
	NCSTN	Not identified	16.	Duchatelet	et	al.
						10.1111/bjd.13668
	PSENEN	γ -secretase-Notch pathway	17.	Zhang	et	al.
						10.1111/1346-8138.15527
PAPASH	PSTPIP1	Inflammasome activation	18.	Kotzerke	et	al.
						10.1111/jdv.17171
	MEFV		19.	Serrano	et	al.
						10.1016/j.medcli.2020.03.018

Abbreviations: AGEp, acute generalized exanthematous pustulosis; AP1S3, adaptor-related protein complex 1 subunit sigma 3; CARD, caspase recruitment domain; IDH1, isocitrate dehydrogenase 1; IL36RN, interleukin-36 receptor antagonist; JAK, Janus kinases; LPIN2, lipin 2; MEFV, Mediterranean fever; MTHFR, methylenetetrahydrofolate reductase; MPO, myeloperoxidase; NCSTN, nicastrin; NLRP, NOD-like receptor family pyrin domain containing; NOD, nucleotide-binding oligomerization domain; PAPA, pyogenic arthritis, pyoderma gangrenosum and acne conglobate; PAPASH, pyogenic arthritis, acne, PG and suppurative hidradenitis; PASH, pyoderma gangrenosum, acne and suppurative hidradenitis; PG, pyoderma gangrenosum; POFUT1, Protein O-fucosyltransferase 1; PSEN1, presenilin 1; PSENEN, presenilin enhancer; PSTPIP1, proline-serine-threonine phosphatase interacting protein 1.

