BIOMOLECULES & THERAPEUTICS

Review
Biomol Ther 31(4), 388-394 (2023)

Nitric Oxide Signal Transduction and Its Role in Skin Sensitization

Jong Hun Kim[1] and Min Sik Choi[2,*]

[1]Department of Food Science and Biotechnology, and Institute for Basic Sciences, Sungshin Women's University, Seoul 01133,
[2]Lab of Pharmacology, College of Pharmacy, Dongduk Women's University, Seoul 02748, Republic of Korea

Abstract

Nitric oxide (NO) is a signaling molecule that plays a crucial role in numerous cellular physiological processes. In the skin, NO is produced by keratinocytes, fibroblasts, endothelial cells, and immune cells and is involved in skin functions such as vasodilation, pigmentation, hair growth, wound healing, and immune responses. NO modulates both innate and adaptive immune responses. As a signaling molecule and cytotoxic effector, NO influences the function of immune cells and production of cytokines. NO is a key mediator that protects against or contributes to skin inflammation. Moreover, NO has been implicated in skin sensitization, a process underlying contact dermatitis. It modulates the function of dendritic cells and T cells, thereby affecting the immune response to allergens. NO also plays a role in contact dermatitis by inducing inflammation and tissue damage. NO-related chemicals, such as nitrofatty acids and nitric oxide synthase (NOS) inhibitors, have potential therapeutic applications in skin conditions, including allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD). Further research is required to fully elucidate the therapeutic potential of NO-related chemicals and develop personalized treatment strategies for skin conditions.

Key Words: Nitric oxide, Skin sensitization, Contact dermatitis

INTRODUCTION

Nitric oxide (NO) is a small gaseous molecule that plays crucial roles in numerous physiological and pathological processes within cells (Namkoong and Kim, 2010; Gantner *et al*., 2020; Lundberg and Weitzberg, 2022). The primary significance of this molecule in cellular processes stems from its ability to function as a signaling molecule, thereby facilitating communication among cells. It is produced from the amino acid L-arginine by a family of enzymes known as nitric oxide synthases (NOS) (Król and Kepinska, 2020). Once produced, NO diffuses across cell membranes because of its lipophilic nature. It exerts its effects by binding to the heme moiety of soluble guanylyl cyclase (sGC), thereby producing cyclic guanosine monophosphate, which acts as a secondary messenger and regulates several cellular processes, including smooth muscle relaxation, inhibition of platelet aggregation, and neurotransmission(Lehners *et al*., 2018; Garthwaite, 2019; Degjoni *et al*., 2022). However, NO can also react with superoxide to form peroxynitrite, a potent oxidant that causes cellular damage (Lim, 2013; Piacenza *et al*., 2022). Hence, the biological effects of NO can be both beneficial and harmful depending on its concentration and cellular context. At the cellular level, NO participates in signal transduction pathways. NO is involved in regulating a variety of enzymes and proteins through a process called S-nitrosylation, in which NO attaches to the thiol group of the cysteine residues in these molecules (Nakamura and Lipton, 2016). This can alter the function of these proteins by affecting processes, such as gene expression, protein aggregation, apoptosis, and mitochondrial respiration (Choi *et al*., 2014; Nakamura and Lipton, 2020; Nakamura *et al*., 2021; Oh *et al*., 2022).

The role of NO in maintaining the vascular tone by promoting vasodilation has been extensively documented in the cardiovascular system (Vanhoutte *et al*., 2016). The NO generated by endothelial cells diffuses into the neighboring smooth muscle cells, triggering a series of responses that lead to muscle relaxation and vasodilation, which, in turn, helps regulate blood pressure and blood flow. NO prevents platelet aggregation, thereby inhibiting blood clot formation. NO also plays a significant role in the immune system (Xue *et al*., 2018). Immune cells, such as macrophages, produce NO as part of the body's defense mechanisms against pathogens. NO exhibits antimicrobial activity and kills or inhibits the growth of bacteria, viruses, and other pathogenic organisms. Furthermore, NO modulates immune responses and influences inflammation

Open Access https://doi.org/10.4062/biomolther.2023.101

Received May 29, 2023 **Revised** Jun 5, 2023 **Accepted** Jun 9, 2023
Published Online Jun 22, 2023

***Corresponding Author**
E-mail: mschoi@dongduk.ac.kr
Tel: +82-2-940-4518, Fax: +82-2-940-4195

Table 1. NO donors used for the purpose of mimicking NO

Chemical class	Examples	Donating mechanism
Diazeniumdiolate	DEA NONOate	Spontaneous-release
Syndnonimine	SIN-1	
Organic nitrates	Nitroglycerin, Glycerol trinitrate	Enzymatic oxidation
Inorganic nitroso compound	Sodium nitroprusside (SNP)	
S-nitrosothiol	SNAP, S-nitrosoglutathione (GSNO)	Chemical reaction

and immune cell function (García-Ortiz and Serrador, 2018; Thwe and Amiel, 2018). In the nervous system, NO acts as a neurotransmitter and is involved in various aspects of neurobiology, including neural development, synaptic plasticity induction, and neurovascular coupling regulation (Contestabile, 2012; Nakamura *et al*., 2013; Lipton, 2022). These findings have implications in learning, memory, and other cognitive functions.

NO plays significant and diverse roles in various cellular processes. It is a versatile molecule that participates in a wide range of biological processes and contributes to the overall homeostasis of the body. However, it is worth noting that imbalances in NO production or activity can lead to pathological conditions, highlighting the need for precise regulation of NO in the body. Additionally, in the skin, diseases associated with NO stem from an imbalance in its levels, caused by deficiency or abundance. At low concentrations, NO acts as a signaling molecule, performing regulatory and homeostatic roles, including vasodilation, melanogenesis, and protection against environmental stressors (Cals-Grierson and Ormerod, 2004). Negative effects of increased NO levels can be observed in immune-related inflammatory conditions, including psoriasis, cutaneous lupus erythematosus, and potentially allergic skin lesions (Ormerod *et al*., 1997; Oates, 2010; Pleńkowska *et al*., 2020). Therefore, in this study, we review whether this feature of NO applies to the skin, particularly in relation to skin sensitization.

NO AND REACTIVE NITROGEN SPECIES (RNS)

The primary method of NO production in the body involves an enzymatic process employing the nitric oxide synthase (NOS) family of enzymes. There are three known NOS isoforms: neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3) (Cinelli *et al*., 2020; Cyr *et al*., 2020; Solanki *et al*., 2022). These enzymes are found in different tissues and have different regulatory mechanisms. All three isoforms of NOS catalyze the same reaction, converting L-arginine into L-citrulline and NO. This reaction requires several cofactors including NADPH, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and (6R-)5,6,7,8-tetrahydrobiopterin (BH4). The enzymatic process of NO synthesis is tightly regulated, with each NOS isoform being subjected to different regulatory mechanisms. For example, eNOS and nNOS are constitutively expressed, and their activity is primarily regulated by intracellular calcium levels, whereas iNOS is expressed in response to inflammatory signals and produces NO in a calcium-independent manner.

In addition to the enzymatic synthesis by NOS, NO can be generated via non-enzymatic pathways. These pathways are generally less significant under physiological conditions but can get activated under certain pathological conditions or in the presence of exogenous nitrite or nitrate (Ma *et al*., 2018). One such nonenzymatic pathway involves the reduction of inorganic nitrite to NO (Carlström *et al*., 2018). This can occur in the stomach under acidic conditions or in other tissues in the presence of certain reductases. Another nonenzymatic pathway involves the reduction of nitrate to nitrite by commensal bacteria in the mouth and gut, followed by further reduction to NO (Vanhatalo *et al*., 2018). In addition, certain drugs and dietary supplements can generate NO non-enzymatically. For example, nitroglycerin, a medication used to treat heart conditions, releases NO into the body, leading to vasodilation (Zhou and Parker, 2019). Nitrate- and nitrite-rich foods such as beetroot and leafy green vegetables can also contribute to NO production via non-enzymatic pathways (Ocampo *et al*., 2018). Various NO donors have been used to simulate NO production (Table 1).

Nitric oxide is a highly reactive molecule that undergoes various chemical reactions to form nitrogen species (Ford and Miranda, 2020). These reactions are influenced by the local environment, including the presence of other molecules, oxygen levels, and pH. A major pathway for NO conversion is its reaction with superoxide (O_2^-), which results in the formation of peroxynitrite ($ONOO^-$) (Jourd'heuil *et al*., 1999; Szabó *et al*., 2007). It is a potent oxidant that can nitrate tyrosine residues in proteins, thereby altering their structure and function. Peroxynitrite can also produce other reactive nitrogen species (RNS) such as nitrogen dioxide (NO_2) and dinitrogen trioxide (N_2O_3). In O-rich environments, NO reacts with oxygen to form nitrogen dioxide (NO_2). It is a potent oxidant that can cause cellular damage. NO can also react with itself to form dinitrogen trioxide (N_2O_3), particularly under acidic conditions and in the presence of certain catalysts. N_2O_3 reacts with amines to form nitrosamines, several of which are carcinogenic. NO can also be converted back to nitrite (NO_2^-) and nitrate (NO_3^-) in a process known as denitrification (Kuypers *et al*., 2018). This process is primarily performed by certain bacteria under anaerobic conditions. Nitrate can also be reduced to NO by nitrate reductases through nitrate respiration or dissimilatory nitrate reduction. Each of these reactions has significant biological implications, because the various nitrogen species formed can affect cellular processes in different ways. Understanding the pathways for the conversion of NO to other nitrogen species and the factors that influence these pathways is, therefore, an important area of research for understanding numerous roles of NO in biology and medicine.

NITRIC OXIDE SYNTHASE (NOS) IN SKIN

The three isoforms of NOS are expressed in various skin cell types: keratinocytes, fibroblasts, endothelial cells, and immune cells (Qureshi *et al*., 1996; Sakai *et al*., 1996; Wang *et al*., 1996; Shimizu *et al*., 1997). Their differential expression and regulation contribute to the spatial and temporal control of NO production in the skin, allowing for the fine-tuned regulation of various skin functions. nNOS and eNOS are considered constitutive NOS isoforms because they are constantly produced under normal physiological conditions. nNOS and eNOS regulate skin pigmentation, hair growth, and wound healing, among other functions (Horikoshi *et al*., 2000; Sowden *et al*., 2005; Man *et al*., 2022b). For example, the NO generated by eNOS in endothelial cells exerts vasodilatory effects that promote blood flow to the skin (Quillon *et al*., 2015). This is crucial for temperature regulation, nutrient delivery, and waste removal. Additionally, eNOS-derived NO stimulates angiogenesis, which is important for wound healing (Chen *et al*., 2020; Xu *et al*., 2023). In contrast, iNOS is an inducible isoform that is usually silent under normal conditions but can be upregulated in response to inflammatory signals or cellular stress. In the skin, iNOS-derived NO plays a crucial role in immune response. For example, NO produced by iNOS in macrophages and other immune cells is involved in killing pathogens and modulating inflammation(Xue *et al*., 2018). Aberrant iNOS activity and NO production have been implicated in various skin pathologies including psoriasis and Alzheimer's disease (Man *et al*., 2022a).

The dual nature of NO as a beneficial and potentially harmful molecule in the skin is largely dictated by the activity of NOS enzymes. Insufficient NO production can negatively affect normal skin function, whereas excessive NO can lead to tissue damage and contribute to the development of diseases. Understanding the complex roles of NOS enzymes in the skin may provide new therapeutic avenues for managing a range of skin conditions.

NO AND IMMUNE RESPONSES IN SKIN

Role of NO in innate and adaptive immune responses

NO plays a crucial role in both the innate and adaptive immune responses and acts as a signaling molecule and cytotoxic effector (Bogdan, 2015). In the innate immune system, NO is primarily produced by immune cells such as macrophages and neutrophils, in response to pathogenic invasion (Kashfi *et al*., 2021; Sadaf *et al*., 2021). These cells express the inducible form of nitric oxide synthase (iNOS), which is activated upon recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) (Fitzgerald and Kagan, 2020). Once activated, it catalyzes the production of high levels of NO. This molecule exerts direct antimicrobial effects against various pathogens, including bacteria, viruses, fungi, and parasites. Its cytotoxic action is because of its ability to cause DNA damage, protein modification, and disruption of metabolic pathways in these microorganisms. Additionally, NO modulates the inflammatory response by regulating the expression and function of various cytokines, chemokines, and adhesion molecules, thereby influencing the recruitment and activation of immune cells at the infection site (Guzik *et al*., 2003; Qian and Fulton, 2012).

In the adaptive immune response, NO influences the activity of both T and B cells, the primary effectors of the adaptive immune system (Bogdan, 2015; García-Ortiz and Serrador, 2018). Studies have revealed that NO can inhibit T cell proliferation and cytokine production and induce T cell apoptosis, thereby acting as a negative regulator of the T cell-mediated immune response (Cripps *et al*., 2010; Giordano *et al*., 2014). However, under certain conditions, NO promotes T cell activation and function (Nagy *et al*., 2003). In contrast, B cells are influenced by NO during antibody production. Dugas *et al*. suggested that NO can enhance antibody production by B cells (Dugas *et al*., 2000), whereas others have shown inhibitory effects (Jayasekera *et al*., 2006; Sammicheli *et al*., 2016), indicating the context-dependent role of NO.

Thus, NO contributes significantly to the orchestration of the immune response, providing a dynamic response to various pathogenic challenges. However, several aspects of NO involvement in immune regulation remain to be fully elucidated. Further research on the precise mechanisms and contexts of NO action in immunity may uncover novel therapeutic strategies for various immune-related disorders.

NO as a mediator of skin inflammation

Skin inflammation was significantly influenced by NO (Man *et al*., 2022a). NO is synthesized by different skin cell types, including keratinocytes, fibroblasts, and immune cells, each of which plays a role in the inflammatory response. Macrophages, a type of immune cells, produce large amounts of NO in response to inflammatory stimuli. NO produced by macrophages has antimicrobial properties that protect the skin from infection (Bath *et al*., 2021). However, the role of NO in skin inflammation is not entirely protective. It also contributes to the development and progression of inflammatory skin conditions. For example, the overproduction of NO has been implicated in conditions such as psoriasis and atopic dermatitis (Pleńkowska *et al*., 2020; Yu and Li, 2022). In these cases, excessive NO levels can lead to skin damage and exacerbate inflammation. NO is a key mediator of skin inflammation; once released, NO diffuses into the surrounding tissues and exerts its effects by modulating blood vessel dilation, immune cell activation, and the release of other proinflammatory molecules (Man *et al*., 2022a). Consequently, NO contributes to the initiation, propagation, and resolution of inflammatory responses in the skin, making it an important mediator of skin homeostasis (Ormerod *et al*., 1997; Oates, 2010; Pleńkowska *et al*., 2020). The role of NO is multifaceted with its capacity to both protect against and contribute to skin inflammation. The influence on immune cells adds an additional layer of complexity, highlighting the intricate balance of skin immunity.

NO AND SKIN SENSITIZATION

Skin sensitization

Skin sensitization is the process by which the immune system learns to react to a particular substance, leading to an allergic response upon subsequent exposure. This is an important mechanism underlying allergic contact dermatitis (ACD), a common skin inflammatory condition (Aquino and Rosner, 2019). It is important to note that skin sensitization does not typically occur upon the first exposure to an allergen. Instead,

the initial exposure triggers a process known as sensitization, which primes the immune system to react to future exposure.

Skin sensitization can be divided into several stages: induction, elicitation, and resolution (Li and Li, 2021; Johansen *et al*., 2022; Patel and Nixon, 2022). During the induction phase, small reactive chemicals known as haptens penetrate the skin and form complexes with proteins. These hapten–protein complexes are then taken up by Langerhans cells (a type of dendritic cell located in the skin), which process these complexes and present them to T cells in the lymph nodes. This process "teaches" the immune system to recognize a hapten as a threat. The elicitation phase begins upon re-exposure to the same hapten. Memory T cells primed during the induction phase recognize haptens and initiate an immune response. This response involves the release of inflammatory cytokines, causing redness, swelling, and itching, leading to the characteristic symptoms of ACD. The resolution phase involves the mechanisms that alleviate inflammation and repair tissue damage. This phase is not as well understood as the induction or elicitation phases.

It is important to mention that not all substances will cause skin sensitization in all individuals. The potential of a substance to cause sensitization depends on a variety of factors, including an individual's genetic profile, concentration of the substance, and duration of exposure. The process of skin sensitization involves an intricate interplay among immune cells, epidermal and dermal skin cells, and signaling molecules such as nitric oxide.

Role of NO in contact dermatitis (CD)

Allergic contact dermatitis and irritant contact dermatitis (ICD) are skin conditions caused by exposure to allergens and irritants, respectively (Li and Li, 2021). These conditions trigger an immune response resulting in inflammation and rashes. NO plays a role in both these conditions, although its exact contribution has multiple aspects (Ormerod *et al*., 1997; Sahin *et al*., 2001; Mehling *et al*., 2021).

In ACD, the immune response is driven by T cells. When the skin comes into contact with an allergen, antigen-presenting cells, such as dendritic cells, capture the allergen and present it to T cells, triggering an immune response. NO plays a role in this process by modulating the functions of both dendritic and T cells (García-Ortiz and Serrador, 2018). NO can affect dendritic cell migration and maturation, which are critical for the presentation of allergens to T cells (Thwe and Amiel, 2018). NO can regulate the proliferation of T cells and the production of cytokines and molecules that drive the immune response (Mahidhara *et al*., 2003). Therefore, alterations in the NO signaling pathway could potentially affect the severity and course of ACD. For example, UV phototherapy stimulates the production of NO through NOS, leading to disease improvement. This commonly employed treatment for inflammatory skin conditions is believed to enhance the development of Tregs possessing the cutaneous lymphocyte-associated antigen. In this study, NO promoted the skin-homing Treg phenotype via an sGC-GMP-dependent pathway (Yu *et al*., 2017). It has been reported that when tested in mice with contact dermatitis, NCX 1022, a hydrocortisone compound that releases NO, has faster and stronger protective effects than hydrocortisone alone (Hyun *et al*., 2004). In contrast, it has been suggested that low NO levels facilitate the attraction of neutrophils, whereas high levels of NO are anti-inflammatory (Ross and Reske-Kunz, 2001).

On the other hand, ICD is caused by direct skin damage caused by irritants, leading to inflammation and activation of the innate immune system. Irritants can lead to the production of reactive oxygen species and NO in keratinocytes, which are the predominant cell types in the skin epidermis (Mehrotra *et al*., 2005). This increase in NO levels can lead to protein modification and DNA damage, thereby contributing to skin inflammation. Moreover, NO can dilate blood vessels and increase vascular permeability, leading to redness and swelling, common symptoms of ICD. Interestingly, the NOS inhibitor L-NAME inhibited irritant patch test reactions, mimicking ICD in human skin (Wallengren and Larsson, 2001). Thus, NO mediates inflammation and tissue damage during ICD implantation.

Further studies are required to elucidate the role of NO in ACD and ICD. Understanding these pathways could lead to the development of novel therapeutic strategies that target the NO signaling pathway to treat skin conditions.

POTENTIAL THERAPEUTIC APPLICATIONS OF NO-RELATED CHEMICALS IN CD

NO-related chemicals have potential therapeutic applications for various skin conditions, including ACD and ICD. It has been reported that nitro-oleic acid, an electrophilic nitro-fatty acid obtained from the reactions among NO, nitrite, and unsaturated fatty acids, significantly inhibits the infiltration of inflammatory cells and production of inflammatory cytokines in the skin of ACD mice (Mathers *et al*., 2017). In this study, nitro-fatty acids (NO2-FAs) reduced IL-1β and IL-6, which are the essential pro-inflammatory cytokines for the differentiation of Th17 cells involved in ACD. It has also been demonstrated that nitrofatty acids inhibit vascularization in an ACD setting. Another recent study discovered that NO2-FAs exhibit anti-inflammatory effects, suggesting their possible use as beneficial lipid molecules in regulating detrimental immune responses mediated by T cells (Bago *et al*., 2023). This study discovered that nitro-oleic acid can target and affect the Ser/Thr phosphatase calcineurin through nitroalkylation. Specifically, nitroalkylation of calcineurin at Cys372 decreases the transcriptional activity of nuclear factor of activated T-cell (NFAT) and alters the production of pro-inflammatory cytokines in activated T cells.

Irritant contact dermatitis is a non-immune-mediated inflammatory skin condition caused by irritant exposure. NOS inhibitors have been studied for their potential in alleviating ICD symptoms (Shen *et al*., 2007; Ali and Sultana, 2012). Aminoguanidine, an inhibitor of NOS, decreases the mRNA and protein levels of NOS and the production of NO in skin keratinocytes. As NO is involved in vasodilation and increased vascular permeability, inhibiting its synthesis may help reduce the inflammation and edema associated with ICD. Furthermore, NO inhibition can modulate the release of pro-inflammatory mediators such as prostaglandins and leukotrienes, which play a role in the pathogenesis of ICD. NOS inhibitors offer a potential approach to manage the inflammatory response in ICD by targeting the NO pathway. In addition, a role for arginase1 (Arg1) and iNOS in ICD immunity has been suggested (Suwanpradid *et al*., 2017). Elevated iNOS levels are associated with increased 2,4-dinitrofluorobenzene treatment and ICD-associated inflammation. In this study, the iNOS inhibi-

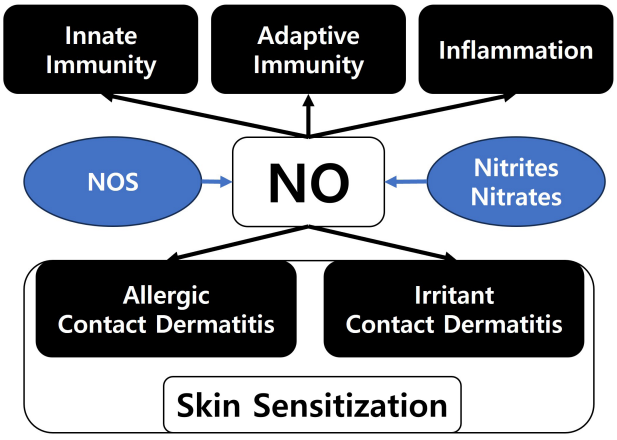

Fig. 1. Diagram showing the involvement of NO in various skin functions.

tor, N6-(1-iminoethyl)-l-lysine (l-NIL), significantly ameliorated ICD responses in mice. Therefore, modulating iNOS in macrophages may be a novel avenue for the future therapeutic targeting of ICD.

Owing to the intricate characteristics of skin conditions and the various processes implicated in their development, it is crucial to thoroughly assess these possible therapeutic approaches. Moreover, personalized treatment strategies and specialized delivery systems for NO-based therapies may amplify their efficacy and reduce adverse effects.

CONCLUSION

NO plays significant and diverse roles in various cellular processes, including immune responses and skin inflammation (Fig. 1). The differential expression and regulation of NOS isoforms contribute to the spatial and temporal control of NO production in the skin, allowing for the fine-tuned regulation of proper skin functions. NO acts as a signaling molecule and cytotoxic effector in both innate and adaptive immune responses, modulating the activity of immune cells and influencing cytokine production. However, imbalances in NO production or activity can lead to pathological conditions, such as psoriasis and atopic dermatitis. Furthermore, NO plays a complex role in skin sensitization, where it is involved in the induction and elicitation phases of ACD as well as the inflammation associated with ICD. NO-related chemicals, such as nitrofatty acids and NOS inhibitors, are promising as potential therapeutic agents for regulating detrimental immune responses and alleviating inflammation in conditions such as ACD and ICD. However, further research is required to fully elucidate these mechanisms and develop personalized treatment strategies for the effective implementation of NO-based therapies for contact dermatitis.

ACKNOWLEDGMENTS

This work was supported by a National Research Foundation of Korea (NRF) grant (2021R1F1A1064344) and by the Korea Environment Industry & Technology Institute (KEITI) through “the Technology Development Project for Safety Management of Household Chemical Products,” funded by the Korea Ministry of Environment (MOE) (RS-2023-00215856).

REFERENCES

Ali, F. and Sultana, S. (2012) Repeated short-term stress synergizes the ROS signalling through up regulation of NFkB and iNOS expression induced due to combined exposure of trichloroethylene and UVB rays. *Mol. Cell. Biochem.* **360**, 133-145.
Aquino, M. and Rosner, G. (2019) Systemic contact dermatitis. *Clin. Rev. Allergy Immunol.* **56**, 9-18.
Bago, Á., Cayuela, M. L., Gil, A., Calvo, E., Vázquez, J., Queiro, A., Schopfer, F. J., Radi, R., Serrador, J. M. and Íñiguez, M. A. (2023) Nitro-oleic acid regulates T cell activation through post-translational modification of calcineurin. *Proc. Natl. Acad. Sci. U. S. A.* **120**, e2208924120.
Bath, P. M., Coleman, C. M., Gordon, A. L., Lim, W. S. and Webb, A. J. (2021) Nitric oxide for the prevention and treatment of viral, bacterial, protozoal and fungal infections. *F1000Res.* **10**, 536.
Bogdan, C. (2015) Nitric oxide synthase in innate and adaptive immunity: an update. *Trends Immunol.* **36**, 161-178.
Cals-Grierson, M. M. and Ormerod, A. D. (2004) Nitric oxide function in the skin. *Nitric Oxide* **10**, 179-193.
Carlström, M., Lundberg, J. O. and Weitzberg, E. (2018) Mechanisms underlying blood pressure reduction by dietary inorganic nitrate. *Acta Physiol. (Oxf.)* **224**, e13080.
Chen, Z., Haus, J. M., Chen, L., Wu, S. C., Urao, N., Koh, T. J. and Minshall, R. D. (2020) CCL28-induced CCR10/eNOS interaction in angiogenesis and skin wound healing. *FASEB J.* **34**, 5838-5850.
Choi, M. S., Nakamura, T., Cho, S. J., Han, X., Holland, E. A., Qu, J., Petsko, G. A., Yates, J. R., Liddington, R. C. and Lipton, S. A. (2014) Transnitrosylation from DJ-1 to PTEN attenuates neuronal cell death in parkinson’s disease models. *J. Neurosci.* **34**, 15123-15131.
Cinelli, M. A., Do, H. T., Miley, G. P. and Silverman, R. B. (2020) Inducible nitric oxide synthase: regulation, structure, and inhibition. *Med. Res. Rev.* **40**, 158-189.
Contestabile, A. (2012) Role of nitric oxide in cerebellar development and function: focus on granule neurons. *Cerebellum* **11**, 50-61.
Cripps, J. G., Wang, J., Maria, A., Blumenthal, I. and Gorham, J. D. (2010) Type 1 T helper cells induce the accumulation of myeloid-derived suppressor cells in the inflamed Tgfb1 knockout mouse liver. *Hepatology* **52**, 1350-1359.
Cyr, A. R., Huckaby, L. V., Shiva, S. S. and Zuckerbraun, B. S. (2020) Nitric oxide and endothelial dysfunction. *Crit. Care Clin.* **36**, 307-321.
Degjoni, A., Campolo, F., Stefanini, L. and Venneri, M. A. (2022) The NO/cGMP/PKG pathway in platelets: the therapeutic potential of PDE5 inhibitors in platelet disorders. *J. Thromb. Haemost.* **20**, 2465-2474.
Dugas, N., Dereuddre-Bosquet, N., Goujard, C., Dormont, D., Tardieu, M. and Delfraissy, J. F. (2000) Role of nitric oxide in the promoting effect of HIV type 1 infection and of gp120 envelope glycoprotein on interleukin 4-induced IgE production by normal human mononuclear cells. *AIDS Res. Hum. Retroviruses* **16**, 251-258.
Fitzgerald, K. A. and Kagan, J. C. (2020) Toll-like receptors and the control of immunity. *Cell* **180**, 1044-1066.
Ford, P. C. and Miranda, K. M. (2020) The solution chemistry of nitric oxide and other reactive nitrogen species. *Nitric Oxide* **103**, 31-46.
Gantner, B. N., LaFond, K. M. and Bonini, M. G. (2020) Nitric oxide in cellular adaptation and disease. *Redox Biol.* **34**, 101550.
García-Ortiz, A. and Serrador, J. M. (2018) Nitric oxide signaling in T cell-mediated immunity. *Trends Mol. Med.* **24**, 412-427.
Garthwaite, J. (2019) NO as a multimodal transmitter in the brain: discovery and current status. *Br. J. Pharmacol.* **176**, 197-211.
Giordano, D., Draves, K. E., Li, C., Hohl, T. M. and Clark, E. A. (2014) Nitric oxide regulates BAFF expression and T cell-independent antibody responses. *J. Immunol.* **193**, 1110-1120.
Guzik, T. J., Korbut, R. and Adamek-Guzik, T. (2003) Nitric oxide and

superoxide in inflammation and immune regulation. *J. Physiol. Pharmacol.* **54**, 469-487.
Horikoshi, T., Nakahara, M., Kaminaga, H., Sasaki, M., Uchiwa, H. and Miyachi, Y. (2000) Involvement of nitric oxide in UVB-induced pigmentation in guinea pig skin. *Pigment Cell Res.* **13**, 358-363.
Hyun, E., Bolla, M., Steinhoff, M., Wallace, J. L., Del Soldato, P. and Vergnolle, N. (2004) Anti-inflammatory effects of nitric oxide-releasing hydrocortisone NCX 1022, in a murine model of contact dermatitis. *Br. J. Pharmacol.* **143**, 618-625.
Jayasekera, J. P., Vinuesa, C. G., Karupiah, G. and King, N. J. C. (2006) Enhanced antiviral antibody secretion and attenuated immunopathology during influenza virus infection in nitric oxide synthase-2-deficient mice. *J. Gen. Virol.* **87**, 3361-3371.
Johansen, J. D., Bonefeld, C. M., Schwensen, J. F. B., Thyssen, J. P. and Uter, W. (2022) Novel insights into contact dermatitis. *J. Allergy Clin. Immunol.* **149**, 1162-1171.
Jourd'heuil, D., Miranda, K. M., Kim, S. M., Espey, M. G., Vodovotz, Y., Laroux, S., Mai, C. T., Miles, A. M., Grisham, M. B. and Wink, D. A. (1999) The oxidative and nitrosative chemistry of the nitric oxide/superoxide reaction in the presence of bicarbonate. *Arch. Biochem. Biophys.* **365**, 92-100.
Kashfi, K., Kannikal, J. and Nath, N. (2021) Macrophage reprogramming and cancer therapeutics: role of iNOS-derived NO. *Cells* **10**, 3194.
Król, M. and Kepinska, M. (2020) Human nitric oxide synthase—its functions, polymorphisms, and inhibitors in the context of inflammation, diabetes and cardiovascular diseases. *Int. J. Mol. Sci.* **22**, 56.
Kuypers, M. M. M., Marchant, H. K. and Kartal, B. (2018) The microbial nitrogen-cycling network. *Nat. Rev. Microbiol.* **16**, 263-276.
Lehners, M., Dobrowinski, H., Feil, S. and Feil, R. (2018) cGMP signaling and vascular smooth muscle cell plasticity. *J. Cardiovasc. Dev. Dis.* **5**, 20.
Li, Y. and Li, L. (2021) Contact dermatitis: classifications and management. *Clin. Rev. Allergy Immunol.* **61**, 245-281.
Lim, S. C. (2013) Interrelation between expression of ADAM 10 and MMP 9 and synthesis of peroxynitrite in doxorubicin induced cardiomyopathy. *Biomol. Ther. (Seoul)* **21**, 371-380.
Lipton, S. A. (2022) Hidden networks of aberrant protein transnitrosylation contribute to synapse loss in Alzheimer's disease. *Free Radic. Biol. Med.* **193**, 171-176.
Lundberg, J. O. and Weitzberg, E. (2022) Nitric oxide signaling in health and disease. *Cell* **185**, 2853-2878.
Ma, L., Hu, L., Feng, X. and Wang, S. (2018) Nitrate and nitrite in health and disease. *Aging Dis.* **9**, 938-945.
Mahidhara, R. S., Hoffman, R. A., Huang, S., Wolf-Johnston, A., Vodovotz, Y., Simmons, R. L. and Billiar, T. R. (2003) Nitric oxide-mediated inhibition of caspase-dependent T lymphocyte proliferation. *J. Leukoc. Biol.* **74**, 403-411.
Man, M. Q., Wakefield, J. S., Mauro, T. M. and Elias, P. M. (2022a) Regulatory role of nitric oxide in cutaneous inflammation. *Inflammation* **45**, 949-964.
Man, M. Q., Wakefield, J. S., Mauro, T. M. and Elias, P. M. (2022b) Role of nitric oxide in regulating epidermal permeability barrier function. *Exp. Dermatol.* **31**, 290-298.
Mathers, A. R., Carey, C. D., Killeen, M. E., Diaz-Perez, J. A., Salvatore, S. R., Schopfer, F. J., Freeman, B. A. and Falo, L. D., Jr. (2017) Electrophilic nitro-fatty acids suppress allergic contact dermatitis in mice. *Allergy* **72**, 656-664.
Mehling, R., Schwenck, J., Lemberg, C., Trautwein, C., Zizmare, L., Kramer, D., Müller, A., Fehrenbacher, B., Gonzalez-Menendez, I., Quintanilla-Martinez, L., Schröder, K., Brandes, R. P., Schaller, M., Ruf, W., Eichner, M., Ghoreschi, K., Röcken, M., Pichler, B. J. and Kneilling, M. (2021) Immunomodulatory role of reactive oxygen species and nitrogen species during T cell-driven neutrophil-enriched acute and chronic cutaneous delayed-type hypersensitivity reactions. *Theranostics* **11**, 470-490.
Mehrotra, P., Mishra, K. P., Raman, G. and Banerjee, G. (2005) Differential regulation of free radicals (reactive oxygen and nitrogen species) by contact allergens and irritants in human keratinocyte cell line. *Toxicol. Mech. Methods* **15**, 343-350.
Nagy, G., Koncz, A. and Perl, A. (2003) T cell activation-induced mitochondrial hyperpolarization is mediated by Ca2+- and redox-dependent production of nitric oxide. *J. Immunol.* **171**, 5188-5197.
Nakamura, T. and Lipton, S. A. (2020) Nitric oxide-dependent protein post-translational modifications impair mitochondrial function and metabolism to contribute to neurodegenerative diseases. *Antioxid. Redox Signal.* **32**, 817-833.
Nakamura, T. and Lipton, S. A. (2016) Protein S-nitrosylation as a therapeutic target for neurodegenerative diseases. *Trends Pharmacol. Sci.* **37**, 73-84.
Nakamura, T., Oh, C. K., Zhang, X. and Lipton, S. A. (2021) Protein S-nitrosylation and oxidation contribute to protein misfolding in neurodegeneration. *Free Radic. Biol. Med.* **172**, 562-577.
Nakamura, T., Tu, S., Akhtar, M. W., Sunico, C. R., Okamoto, S. and Lipton, S. A. (2013) Aberrant protein s-nitrosylation in neurodegenerative diseases. *Neuron* **78**, 596-614.
Namkoong, S. and Kim, Y. M. (2010) Therapeutic application of nitric oxide in human diseases. *Biomol. Ther. (Seoul)* **18**, 351-362.
Oates, J. C. (2010) The biology of reactive intermediates in systemic lupus erythematosus. *Autoimmunity* **43**, 56-63.
Ocampo, D. A. B., Paipilla, A. F., Marín, E., Vargas-Molina, S., Petro, J. L. and Pérez-Idárraga, A. (2018) Dietary nitrate from beetroot juice for hypertension: a systematic review. *Biomolecules* **8**, 134.
Oh, C. K., Dolatabadi, N., Cieplak, P., Diaz-Meco, M. T., Moscat, J., Nolan, J. P., Nakamura, T. and Lipton, S. A. (2022) S-nitrosylation of p62 inhibits autophagic flux to promote α-synuclein secretion and spread in Parkinson's disease and lewy body dementia. *J. Neurosci.* **42**, 3011-3024.
Ormerod, A. D., Dwyer, C. M., Reid, A., Copeland, P. and Thompson, W. D. (1997) Inducible nitric oxide synthase demonstrated in allergic and irritant contact dermatitis. *Acta Derm. Venereol.* **77**, 436-440.
Patel, K. and Nixon, R. (2022) Irritant contact dermatitis - a review. *Curr. Dermatol. Rep.* **11**, 41-51.
Piacenza, L., Zeida, A., Trujillo, M. and Radi, R. (2022) The superoxide radical switch in the biology of nitric oxide and peroxynitrite. *Physiol. Rev.* **102**, 1881-1906.
Pleńkowska, J., Gabig-Cimińska, M. and Mozolewski, P. (2020) Oxidative stress as an important contributor to the pathogenesis of psoriasis. *Int. J. Mol. Sci.* **21**, 6206.
Qian, J. and Fulton, D. J. R. (2012) Exogenous, but not endogenous nitric oxide inhibits adhesion molecule expression in human endothelial cells. *Front. Physiol.* **3**, 3.
Quillon, A., Fromy, B. and Debret, R. (2015) Endothelium microenvironment sensing leading to nitric oxide mediated vasodilation: a review of nervous and biomechanical signals. *Nitric Oxide* **45**, 20-26.
Qureshi, A. A., Hosoi, J., Xu, S., Takashima, A., Granstein, R. D. and Lerner, E. A. (1996) Langerhans cells express inducible nitric oxide synthase and produce nitric oxide. *J. Invest. Dermatol.* **107**, 815-821.
Ross, R. and Reske-Kunz, A. B. (2001) The role of NO in contact hypersensitivity. *Int. Immunopharmacol.* **1**, 1469-1478.
Sadaf, S., Nagarkoti, S., Awasthi, D., Singh, A. K., Srivastava, R. N., Kumar, S., Srivastava, R. N., Kumar, S., Barthwal, M. K. and Dikshit, M. (2021) nNOS induction and NOSIP interaction impact granulopoiesis and neutrophil differentiation by modulating nitric oxide generation. *Biochim. Biophys. Acta Mol. Cell Res.* **1868**, 119018.
Sahin, S., Önder, M., Sancak, B., Bukan, N. and Gürer, M. A. (2001) The role of nitric oxide in allergic contact dermatitis. *Arch. Dermatol. Res.* **293**, 214-217.
Sakai, M., Shimizu, Y., Nagatsu, I. and Ueda, H. (1996) Immunohistochemical localization of NO synthases in normal human skin and psoriatic skin. *Arch. Dermatol. Res.* **288**, 625-627.
Sammicheli, S., Kuka, M., Di Lucia, P., De Oya, N. J., De Giovanni, M., Fioravanti, J., Cristofani, C., Maganuco, C. G., Fallet, B., Ganzer, L., Sironi, L., Mainetti, M., Ostuni, R., Larimore, K., Greenberg, P. D., de la Torre, J. C., Guidotti, L. G. and Iannacone, M. (2016) Inflammatory monocytes hinder antiviral B cell responses. *Sci. Immunol.* **1**, eaah6789.
Shen, T., Zhu, Q. X., Yang, S., Ding, R., Ma, T., Ye, L. P., Wang, L. J., Liang, Z. Z. and Zhang, X. J. (2007) Trichloroethylene induce nitric oxide production and nitric oxide synthase mRNA expression in cultured normal human epidermal keratinocytes. *Toxicology* **239**,

186-194.
Shimizu, Y., Sakai, M., Umemura, Y. and Ueda, H. (1997) Immunohistochemical localization of nitric oxide synthase in normal human skin: expression of endothelial-type and inducible-type nitric oxide synthase in keratinocytes. *J. Dermatol.* **24**, 80-87.
Solanki, K., Rajpoot, S., Bezsonov, E. E., Orekhov, A. N., Saluja, R., Wary, A., Axen, C., Wary, K. and Baig, M. S. (2022) The expanding roles of neuronal nitric oxide synthase (NOS1). *PeerJ* **10**, e13651.
Sowden, H. M., Naseem, K. M. and Tobin, D. J. (2005) Differential expression of nitric oxide synthases in human scalp epidermal and hair follicle pigmentary units: implications for regulation of melanogenesis. *Br. J. Dermatol.* **153**, 301-309.
Suwanpradid, J., Shih, M., Pontius, L., Yang, B., Birukova, A., Guttman-Yassky, E., Corcoran, D. L., Que, L. G., Tighe, R. M. and MacLeod, A. S. (2017) Arginase1 deficiency in monocytes/macrophages upregulates inducible nitric oxide synthase to promote cutaneous contact hypersensitivity. *J. Immunol.* **199**, 1827-1834.
Szabó, C., Ischiropoulos, H. and Radi, R. (2007) Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. *Nat. Rev. Drug Discov.* **6**, 662-680.
Thwe, P. M. and Amiel, E. (2018) The role of nitric oxide in metabolic regulation of Dendritic cell immune function. *Cancer Lett.* **412**, 236-242.
Vanhatalo, A., Blackwell, J. R., L'Heureux, J. E., Williams, D. W., Smith, A., van der Giezen, M., Winyard, P. G., Kelly, J. and Jones, A. M. (2018) Nitrate-responsive oral microbiome modulates nitric oxide homeostasis and blood pressure in humans. *Free Radic. Biol. Med.* **124**, 21-30.
Vanhoutte, P. M., Zhao, Y., Xu, A. and Leung, S. W. S. (2016) Thirty years of saying NO: sources, fate, actions, and misfortunes of the endothelium-derived vasodilator mediator. *Circ. Res.* **119**, 375-396.
Wallengren, J. and Larsson, B. (2001) Nitric oxide participates in prick test and irritant patch test reactions in human skin. *Arch. Dermatol. Res.* **293**, 121-125.
Wang, R., Ghahary, A., Shen, Y. J., Scott, P. G. and Tredget, E. E. (1996) Human dermal fibroblasts produce nitric oxide and express both constitutive and inducible nitric oxide synthase isoforms. *J. Invest. Dermatol.* **106**, 419-427.
Xu, T. Y., Qing, S. L., Zhao, J. X., Song, J., Miao, Z. W., Li, J. X., Yang, F. Y., Zhao, H. Y., Zheng, S. L., Li, Z. Y., Wang, S. N. and Miao, C. Y. (2023) Metrnl deficiency retards skin wound healing in mice by inhibiting AKT/eNOS signaling and angiogenesis. *Acta Pharmacol. Sin.* doi: 10.1038/s41401-023-01090-x [Online ahead of print].
Xue, Q., Yan, Y., Zhang, R. and Xiong, H. (2018) Regulation of iNOS on immune cells and its role in diseases. *Int. J. Mol. Sci.* **19**, 3805.
Yu, C., Fitzpatrick, A., Cong, D., Yao, C., Yoo, J., Turnbull, A., Schwarze, J., Norval, M., Howie, S. E. M., Weller, R. B. and Astier, A. L. (2017) Nitric oxide induces human CLA+CD25+Foxp3+ regulatory T cells with skin-homing potential. *J. Allergy Clin. Immunol.* **140**, 1441-1444.e6.
Yu, L. and Li, L. (2022) Potential biomarkers of atopic dermatitis. *Front. Med. (Lausanne)* **9**, 1028694.
Zhou, K. and Parker, J. D. (2019) The role of vascular endothelium in nitroglycerin-mediated vasodilation. *Br. J. Clin. Pharmacol.* **85**, 377-384.