WHITE PAPER

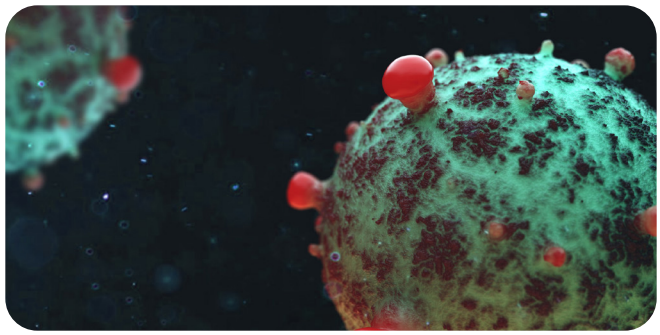
Deciphering the role of neutrophils in virus-induced respiratory disease

The ability to pharmacologically modulate neutrophils, known to be important in many types of inflammatory lung disease, has been called the therapeutic "Holy Grail". However, much remains to be understood before we can curtail the detrimental effects of neutrophils without compromising their vital ability to defend against pathogens.

What is known about neutrophilic response to viral infection?

Extensively studied as bacterial-responsive immune cells, neutrophils are innate immunity effector cells with well-established microbe-killing activities. Though long assumed to be simple, expendable killers adept at clearing bacterial microbes, neutrophils have recently been found to be more heterogeneous than was traditionally thought, sparking renewed interest in the study of neutrophil biology.[1] The role of neutrophils in viral infection is less well understood, though it is evolving rapidly as an important area of study.

Neutrophil proinflammatory defense tactics are known to include degranulation, which releases a variety of proteases at the site of infection, reactive oxygen species and cytokines, and Neutrophil Extracellular Traps (NETs), which are ejected networks of extracellular fibers capable of binding and destroying pathogens. Neutrophil Elastase (NE), a major product of neutrophils, is an especially potent proteolytic enzyme, normally stored in vesicles in an inactive form prior to degranulation or to the release of NETs, which are dotted with NE as part of their arsenal. Effective at clearing infection, dysregulation of neutrophils can cause severe or life-threatening inflammation and tissue damage to the host.



Only recently has it been found that the functional diversity of neutrophils enables them to specialize and assume previously unsuspected tasks, including modulating the activity of other immune cells, such as either boosting or suppressing T-cell activation. Given that neutrophils are important first responders in many instances of respiratory infection both bacterial and viral, a better understanding of neutrophils and their functions, especially as they pertain to viral host defense, may be key to better clinical outcomes. Within the range of viral responses from mild to life-threatening, the factors that determine neutrophilic response and the resulting benefit or detriment to the host have yet to be clearly understood. For example, neutrophils are capable of deploying their defense mechanisms, including degranulation and netosis, in response to virus detection, but it remains unclear to what extent these responses are destructive or even beneficial to viruses such as influenza, which may take advantage of the inflammatory environment and the damage caused to the host tissues.[1] An excessive immune response, which may exacerbate the situation, may be the result of insufficient or incorrect information about the specific cause(s) of infection, thus the separate and combined contributions of the host innate immune response and the influenza virus itself to respiratory disease progression require further study.[2]

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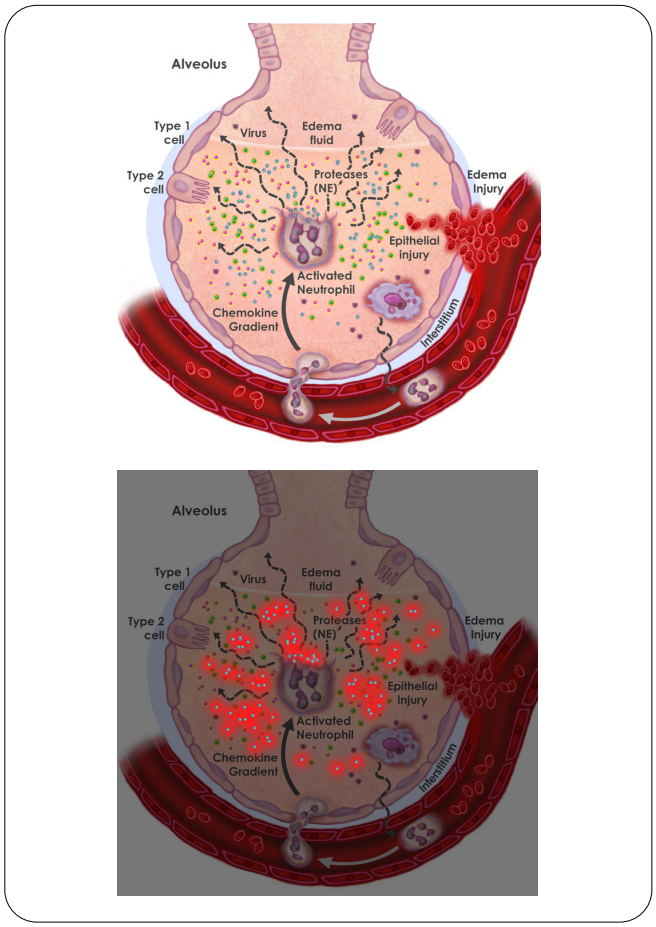

Figure 1. Top: Neutrophils migrate to the site of infection and deploy proinflammatory defenses, including degranulation which releases neutrophil elastase (NE). Dysregulated neutrophil recruitment may cause severe injury to host tissues. Figure 1, Bottom: IVISense™ Neutrophil Elastase 680 FAST fluorescent imaging of NE is represented.

Infections which involve highly pathogenic respiratory viruses, including SARS-CoV and MERS-CoV, are thought to cause Acute Lung Injury (ALI) via infiltration and activation of neutrophils which can progress to Acute Respiratory Distress Syndrome (ARDS). It has been shown that severe cases of SARS-CoV-2 tended to have a higher neutrophil count but lower lymphocyte count compared with non-severe patients, suggesting that the inflammatory response stimulates production of neutrophils and speeds up the apoptosis of lymphocytes - a dysregulation of the immune response that is believed to play a decisive role in the severity of virus-induced disease, resulting in excessive inflammation, and potentially death.[3]

Within the context of studying overall innate immune response and dysregulation, understanding the role neutrophilic invasion plays in relation to other immune cells, such as macrophages, could be useful. A preclinical fluorescent *in vivo* imaging strategy that uses a combined probe approach to measure immune cell activity in lung tissue of affected patients could provide meaningful information on the total immune response. A broadly selective fluorescent probe, that detects all inflammatory cells, can be paired with a highly specific probe such as IVISense™ Neutrophil Elastase 680 FAST, which detects active NE in the extra cellular matrix. Such an approach can provide more targeted information on neutrophil activity, quantified in the context of the broader inflammatory response.

What is IVISense™ Neutrophil Elastase 680 FAST?

IVISense™ Neutrophil Elastase 680 FAST is a Near Infrared (NIR) fluorescent *in vivo* imaging probe that can detect NE post-degranulation or netosis. Using IVISense™ Neutrophil Elastase 680 FAST to image NE from activated neutrophils in affected lung tissue of a mouse model, along with a probe such as IVISense™ Pan Cathepsin 750 FAST (an NIR *in vivo* fluorescent imaging probe for the detection all inflammatory cells), will give a measure of total inflammatory cells and neutrophilic response. This type of *in vivo* study of neutrophils requires a full understanding of the animal model and the kinetics of degranulation. It is important to note that there are situations in which neutrophils are present, but degranulation has not yet occurred. In some models, neutrophil degranulation is a coordinated occurrence, while in others the degranulation is sporadic. This means one should plan their study design carefully, as it can be easy to miss the optimal imaging time for elastase activity.

Monitoring neutrophils as part of the overall innate immune response may provide a true sense of the ratio of neutrophils involved, possibly helping researchers gain a deeper understanding of the pathological process of lung injury after SARS-CoV-2 infection, with the ultimate goal of regulating neutrophil recruitment in an attempt to minimize lung injury for better clinical outcomes.

How is IVISense™ Neutrophil Elastase 680 FAST used?

Here we show a case study demonstrating a possible application of IVISense™ Neutrophil Elastase 680 FAST fluorescent probe.

Case study	Acute respiratory distress syndrome

Noninvasive In Vivo Quantification Of Neutrophil Elastase Activity In Acute Experimental Mouse Lung Injury[4]

Acute neutrophilic inflammation is a hallmark of acute lung injury and ARDS. Of the various enzymes secreted by neutrophils, NE is implicated in wide range of pathological conditions, including ALI/ARDS. To detect and quantify NE activity *in vivo*, mice were challenged intranasally with lipopolysaccharide (LPS, E. coli) in PBS, and later with the chemotactic peptide N-Formyl-Met-Leu-Phe (fMLP) together with IVISense™ Neutrophil Elastase 680 FAST (NE680). The fluorescent non-invasive imaging results indicate that NE680 was cleaved *in vivo* in a murine model of ALI, as fluorescence was readily detectable in the lung region of all mice with lung inflammation, but not in control healthy mice. To analyze the relative contribution of NE to the activation of NE680 *in vivo*, groups of mice were treated with or without the NE inhibitor sivelestat. Total fluorescence was significantly higher in mice with ALI as compared to healthy controls or to mice treated with sivelestat, thereby demonstrating that NE680 is capable of quantifying increased NE activity in lung inflammation and the efficacy of selective therapy *in vivo*.

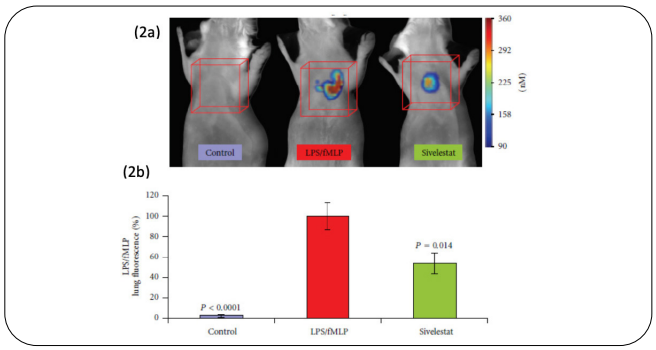

Figure 2. Imaging and quantification of NE680 activation *in vivo*. CD-1 mice were challenged i.n. with LPS and fMLP. A subset of mice was also treated with the NE inhibitor sivelestat 15 min prior to fMLP and NE680 and mice imaged 5 h later by FMT 2500. (a) Representa-tive volume rendering projections taken at the same color gating from control, LPS/fMLP and LPS/fMLP mice which had been treated with sivelestat. (b) The mean concentration of fluorescence was quantified in specific ROIs for the lung area in control mice, mice with ALI, and mice with ALI treated with sivelestat.

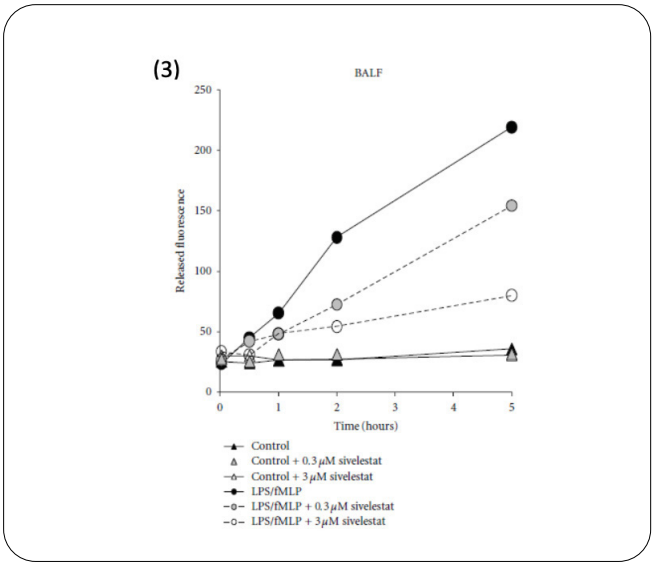

Figure 3. Bronchoalveolar cellular infiltration 24 h after LPS challenge. Mice were challenged i.n. with LPS followed 18h later by fMLP. Five hours later, mice were sacrificed, and bronchoalveolar lavage collected. (c) NE activity in the Bronchoalveolar Lavage Fluid (BALF) was measured at various time points using a fluorescence microplate reader.

Use IVISense™ Neutrophil Elastase as part of a complete experimental solution package

Revvity provides complete *in vivo* imaging solutions including reagents, instrumentation, and support expertise that can help you monitor and design experiments to understand the progression of diseases and their related processes, or to evaluate the potential therapeutic efficacy of drugs targeting the underlying mechanisms involved in disease.

Table 1. hSTING mutations introduced in the CDN binding domain

CAT #	Product	
NEV11169	IVISense™ Neutrophil Elastase 680 FAST	Selectively cleaved by elastase produced by activated neutrophils during acute inflammation. Optimum imaging time is 3-6 hours after injection.

References

1. Carlos, Rosales. Neutrophil: A Cell with Many Roles in Inflammation or Several Cell Types? *Frontiers in Physiology,* Vol. 9, 2018.

2. Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. Into the eye of the cytokine storm. *Microbiol Mol Biol Rev* (2012) 76:16–32. doi:10.1128/MMBR. 05015-11.

3. Yuwei Liu, et. Al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. *Journal of Infection,* April 10, 2020. https://doi.org/10.1016/j.jinf.2020.04.002.

4. Kossodo S, et. Al. Noninvasive *In Vivo* Quantification of Neutrophil Elastase Activity in Acute Experimental Mouse Lung injury. ***International Journal of Molecular Imaging***. Volume 2011, Article ID 581406, doi:10.1155/2011/581406.

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