The Journal of Immunology

Turning on the Lights inside Neutrophils

Robert L. Fairchild

J Immunol 2016; 197:681-682; ;
doi: 10.4049/jimmunol.1600985
http://www.jimmunol.org/content/197/3/681

This information is current as of May 19, 2022.

Supplementary Material **http://www.jimmunol.org/content/suppl/2016/07/19/197.3.681.DC1**

References This article **cites 7 articles**, 3 of which you can access for free at:
http://www.jimmunol.org/content/197/3/681.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

**average*

Subscription Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2016 by The American Association of Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Downloaded from http://www.jimmunol.org/ by guest on May 19, 2022

Pillars of Immunology

The Journal of Immunology

Turning on the Lights inside Neutrophils

Robert L. Fairchild

1916 100 years of The JI 2016

A Century of Excellence

Selections from the 100 most highly cited articles in The JI

Cells of the innate immune compartment provide critical functions in protective responses to infectious agents as well as in other types of immune responses. Responses to infections and tissue inflammation are initiated by innate cell receptors that sense conserved pathogen- and/or inflammation-derived molecular patterns and trigger functions that are critical to early control of the infection and to subsequent activation of the adaptive immune response (1, 2). Importantly, the adaptive immune response does not function in isolation, but rather guides the trafficking and function of neutrophils and macrophages to ingest and destroy bacterial and fungal pathogens. The major mechanism used by these phagocytes to destroy ingested pathogens is through the NADPH oxidase–mediated generation of reactive oxygen radicals and downstream mediators, including H_2O_2 and HOCl. The critical role of neutrophils and macrophages and their machinery in ridding the host of pathogens is best exemplified in humans who have mutations in one of the enzymatic components comprising NADPH oxidase. These mutations prevent the formation and/or function of the NADPH oxidase enzyme system in generating the oxidative burst and its downstream products, so that ingested pathogens are not destroyed by the phagocytes. The resulting chronic granulomatous disease renders the afflicted individuals susceptible to chronic infections that are dealt with by formation of characteristic granulomas to sequester the ingested pathogens in tissue, thereby preventing their spread.

Recognition of the importance of neutrophil and macrophage ingestion and destruction of bacteria compelled active investigation to identify the components and formation of the NADPH oxidase complex and the substrates and products of the oxidative burst. These studies also established that following neutrophil and macrophage phagocytosis of bacteria, the delivery of stimulatory signals through agents later identified as IFN-γ and TLR ligands was required to induce the formation and activity of the NADPH oxidase enzyme complex. During the course of these early studies, major questions arose as to whether all neutrophils in cells isolated from the peripheral blood could express NADPH oxidase activation or whether this bactericidal function was restricted to specific cells within the neutrophil population. This necessitated the development of new approaches to identify and quantify the production of H_2O_2 and/or other products of the NADPH oxidase complex on a per cell basis within a large and complex population of cells. The solution to this problem was revealed in the 1983 paper by Bass et al. (3), which is the subject of this *Pillars of Immunology* commentary.

Two technical developments that were critical to the development of this work warrant a brief discussion. First, work by Rotman and Papermaster (4) used fluorescence microscopy to show cultures of lymphoma cells could be loaded with fluorogenic substrates that were almost immediately cleaved into fluorescent products that were retained in viable, but not dead, cells. The enzymatic reactions generating fluorescent products from the fluorogenic substrates within loaded cells were subsequently identified several years later (5). Second, one of the major advances in science arose with the development of flow cytometry, in which such fluorochrome-labeled cells were rapidly separated from nonlabeled cells in a liquid stream when excited by a beam of blue light emitted from a mercury arc source (6). Also note that in these early experiments the mouse spleen cells separated from Chinese hamster ovary cells were shown to contain the cells producing Ab, demonstrating an important application of this technology. Although the development of flow cytometry advanced rapidly to the use of lasers and more sophisticated photomultiplier tubes, early application focused on the use of fluorochrome-labeled mAbs to detect lymphoid cell populations expressing specific phenotypic markers, first using single color (fluorescein)-labeled Abs and then in 1983, two-color flow cytometric analysis of lymphoid cells with the development of additional fluorochromes such as Texas Red (7).

Bass et al. (3) had been aware of the work detailing the intracellular enzymatic cleavage of fluorogenic substrates to generate fluorescent products and tested these principles to assess generation of these products in individual cells using the flow cytometry technology. Their seminal work extended the use of flow cytometry from the phenotypic analysis of cell surface markers on lymphoid populations to the intracellular analysis of NADPH oxidase function in human neutrophils. The polymorphonuclear leukocytes (i.e., neutrophils) were isolated from peripheral blood by centrifugation through Isolymph gradients and then loaded with a dye, 2′,7′-dichlorofluorescin diacetate (DCFH-DA), which was rapidly deacetylated by intracellular esterases to DCFH, a

Department of Immunology, Cleveland Clinic, Cleveland, OH 44195; and Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH 44106

Address correspondence and reprint requests to Dr. Robert L. Fairchild, NB3-79, Lerner Research Institute, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195. E-mail address: fairchr@ccf.org

Abbreviations used in this article: DCF, dichlorofluorescein; DCFH-DA, 2′,7′-dichlorofluorescin diacetate.

Copyright © 2016 by The American Association of Immunologists, Inc. 0022-1767/16/$30.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1600985

Downloaded from http://www.jimmunol.org/ by guest on May 19, 2022

nonfluorescent substrate for oxidation products. PMA-mediated stimulation of protein kinase C in neutrophils activated the NADPH oxidase to generate H_2O_2 that oxidized the DCFH substrate to the highly fluorescent product dichlorofluorescein (DCF). The fluorescent product was retained in the stimulated neutrophils and detected at the single cell level during flow cytometric analysis using an argon laser to excite the generated fluorescent product. H_2O_2 and the fluorescent DCF product were not generated in the DCFH-DA–loaded neutrophils in the absence of PMA stimulation of NADPH oxidase. As an important control, the authors took advantage of the naturally occurring human mutations in NADPH oxidase; neutrophils isolated from the blood of chronic granulomatous disease patients took up the DCFH-DA that was converted to DCFH by intracellular esterases, but PMA stimulation of these neutrophils failed to activate the nonfunctional NADPH oxidase and the generation of the H_2O_2 to oxidize the substrate to the fluorescent DCF product. The authors also used a spectrofluorometer to construct a DCF standard curve to quantitate the DCF fluorescent product generated on a per cell basis in PMA-stimulated neutrophils, determined to be 160 $\pm$ 13 versus 6.9 $\pm$ 0.7 attomol/cell in unstimulated cells. This experimental approach also allowed the investigators to test whether the PMA-mediated activation of neutrophil NADPH oxidase and generation of H_2O_2 occurred as an all-or-none or graded response. By performing a PMA dose stimulation experiment, the fluorescence intensity produced by the generation of H_2O_2 and the fluorescent product increased as a uniform shift in the entire test neutrophil population, indicating a graded response to produce H_2O_2 in response to increasing PMA stimulation.

The major advance provided in this seminal work comes in the development of a novel use for flow cytometry: in the quantitative detection of metabolically generated fluorescent products within a test cell population and in analysis directed to immune cells other than lymphocytes. The importance of this development is reflected in the >1500 times the paper has been cited, including at least 14 citations already this year and >30 last year. The development of this experimental strategy allowed for accurate and rapid analysis of the constituents required for NADPH oxidase formation and activation, assessment of inhibitors of NADPH oxidase enzyme complex, and analysis of neutrophils from patients with mutations in various constituents of NADPH oxidase that prevent its formation and critical function in clearing viral and bacterial infections. With the present focus on the development of macrophages and neutrophils to different functional phenotypes during various states of inflammation and tissue homeostasis, it is easy to see the continued use of this approach to monitor the function of NADPH oxidase or other metabolic functions in cells in combination with mAbs defining cell phenotypes to provide new mechanistic insights into the initiation of tissue pathology and the resolution of inflammation.

Disclosures

The author has no financial conflicts of interest.

References

1. Kawai, T., and S. Akira. 2011. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* 34: 637–650.
2. Iwasaki, A., and R. Medzhitov. 2015. Control of adaptive immunity by the innate immune system. *Nat. Immunol.* 16: 343–353.
3. Bass, D. A., J. W. Parce, L. R. Dechatelet, P. Szejda, M. C. Seeds, and M. Thomas. 1983. Flow cytometric studies of oxidative product formation by neutrophils: a graded response to membrane stimulation. *J. Immunol.* 130: 1910–1917.
4. Rotman, B., and B. W. Papermaster. 1966. Membrane properties of living mammalian cells as studied by enzymatic hydrolysis of fluorogenic esters. *Proc. Natl. Acad. Sci. USA* 55: 134–141.
5. Sengbusch, G. V., C. Couwenbergs, J. Kühner, and U. Müller. 1976. Fluorogenic substrate turnover in single living cells. *Histochem. J.* 8: 341–350.
6. Hulett, H. R., W. A. Bonner, J. Barrett, and L. A. Herzenberg. 1969. Cell sorting: automated separation of mammalian cells as a function of intracellular fluorescence. *Science* 166: 747–749.
7. Parks, D. R., R. R. Hardy, and L. A. Herzenberg. 1983. Dual immunofluorescence—new frontiers in cell analysis and sorting. *Immunol. Today* 4: 145–150.

Downloaded from http://www.jimmunol.org/ by guest on May 19, 2022