

## Degranulation deconstructed

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### Hindsight

Pediatricians first described the clinical features of chronic granulomatous disease (CGD) in 1959. Almost a decade later, in a collaborative effort that crossed disciplines, we participated in the discoveries that defined the cellular deficiencies of CGD, specifically finding that improper degranulation of leukocytes did not explain their failure to fight pathogens, rather that the fundamental defect was due to problems in the unique NADPH oxidase system of phagocytizing leukocytes. In the years that followed, the subunit components and structure of NADPH oxidase and their translocation during leukocyte phagocytosis to form the active enzyme were well described, leading to the identification of the component genes, the mapping of their chromosomal locations, and their subsequent cloning. This remarkable progress has led to effective therapies, including bone marrow transplants and gene therapy, that would have been unimaginable when we began.

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**Pediatricians first described the clinical features of chronic granulomatous disease (CGD) in 1959. Almost a decade later, in a collaborative effort that crossed disciplines, we participated in the discoveries that defined the cellular deficiencies of CGD, specifically finding that improper degranulation of leukocytes did not explain their failure to fight pathogens, rather that the fundamental defect was due to problems in the unique NADPH oxidase system of phagocytizing leukocytes. In the years that followed, the subunit components and structure of NADPH oxidase and their translocation during leukocyte phagocytosis to form the active enzyme were well described, leading to the identification of the component genes, the mapping of their chromosomal locations, and their subsequent cloning. This remarkable progress has led to effective therapies, including bone marrow transplants and gene therapy, that would have been unimaginable when we began.**

Over 40 years ago, we were privileged to play a role in studies designed to help unravel the pathogenesis of chronic granulomatous disease (CGD) (1). Pediatricians first described the clinical features in 1959 (2), and non-caseous granulomas and pigmented lipid histiocytes were noted in the lungs, livers, lymph nodes, and other organs obtained from male children who had died from chronic and recurrent bacterial infections (3). Ten years later, R.A. Good and colleagues at the University of Minnesota documented that CGD blood leukocytes failed to kill bacteria *in vitro* (4). In 1966, I (R.L. Baehner) was a post-doctoral fellow in David G. Nathan's Division of Hematology at Children's Hospital Boston. We had just attended Problem Rounds, which focused on a boy with anemia, lymphadenopathy, and lung granulomas. Nathan suggested to me that the basis for this boy's disease could be due to a defect in his leukocytes. He introduced me to Manfred Karnovsky in the Department of Biochemistry at Harvard Medical School. Karnovsky and his group had published that guinea pig peritoneal leukocytes displayed a remarkable burst of oxygen consumption and stimulation of the hexose monophosphate shunt during phagocytosis (5). Following this model, we studied our patient's blood leukocytes and the blood leukocytes of patients with

infections. We observed that, similar to guinea pig peritoneal leukocytes, blood leukocytes from patients with acute infections demonstrated the oxidative burst and related biochemical reactions during phagocytosis. In addition, we found that these oxidative reactions could be monitored and quantitated by addition of the redox dye nitroblue tetrazolium (NBT) (6). One of my most exciting research moments and one I'll never forget was when I held two test tubes of phagocytizing blood leukocytes: the left hand held control cells, and the right hand held the patient's blood cells. In the patient's sample, I could clearly see the unchanged yellow hue of the unreduced NBT, indicating a lack of the oxidative response! Quantitation of the NBT reaction in blood leukocytes from parents as well as other children with CGD showed that the genetic transmission was usually x-linked but that an autosomal form of the disease occurred as well (6).

R.A. Good and colleagues also noted an oxidative defect as well as diminished autophagic and phagocytic vacuole formation in CGD leukocytes (7). This latter observation led us to hypothesize that degranulation during phagocytic vacuole formation was abnormal in CGD leukocytes. We set off to prove this. It was then that I discovered that Harvard Medical School employed two professors with the surname Karnovsky, the other was Morris Karnovsky – Manfred's brother – who worked in the Department of Pathology. I had the good fortune for the next six

months of my fellowship to collaborate with the Karnovsky brothers, and they helped me design and carry out the experiments necessary to either confirm or deny my hypothesis. We used both quantitative biochemical analysis of several granule-associated enzymes, including peroxidase, as well as electron microscopic study of the shift of the latter enzyme into phagocytic vacuoles both from control patients with infection and 5 patients with CGD. Both techniques showed that degranulation was normal in CGD blood leukocytes (1), and our findings were confirmed by another group that same year (8).

It was now clear that the bactericidal defect in CGD leukocytes could not be explained by defective degranulation. This changed the thrust of research efforts on CGD toward a closer examination of the products of the oxidative burst, which underpinned the normal bactericidal reactions as well as studies designed to understand the complexities of the NADPH oxidase reaction in phagocytizing leukocytes. The critical importance of hydrogen peroxide in bactericidal processing became clearer when it was noted that those bacteria capable of producing their own hydrogen peroxide were killed by CGD leukocytes *in vitro* and never caused infection in patients with CGD (9).

Subsequently, several reactive oxygen species formed during the reduction of oxygen by NADPH oxidase were identified. The superoxide anion was highly unstable in phagocytizing leukocytes and dismutated to hydrogen peroxide, forming a more stable hypochlorous acid, which was thought to be the prime natural bactericidal agent in the cells. Oxidized proteases and alterations in pH and osmolarity within the phagocytic vacuoles have been shown more recently to be required as well (10). The subunit components and structure of NADPH oxidase and their translocation during leukocyte phagocytosis to form the active enzyme were well described over the next decade. This information led to identification and cloning of the component genes. Although gene therapy is now available, most patients today are identified at an early age before significant chronic infection takes place

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**Decades of progress for CGD**

- 1950s Clinical description of boys dying of recurrent infections, with elevated immunoglobulins and granuloma formation in lymph nodes, lungs, liver, and skin. Basic science studies of the oxidative burst in phagocytizing guinea pig leukocytes.
- 1960s Leukocyte oxidase and bactericidal defect documented in CGD. Diagnostic tests became available. X-linked and autosomal recessive forms of disease transmission identified.
- 1970s NADPH oxidase-catalyzed reaction defined in human blood leukocytes. Reduced oxygen by-products linked to the bactericidal reaction in leukocytes.
- 1980s NADPH oxidase subunit structure and its assembly during phagocytic activation defined biochemically. Classification of CGD described based on subunit mutations. Treatment protocols employing prophylactic antibiotics, fungicides, and interferon-gamma significantly improve survival of patients with CGD.
- 1990s Gene therapy experiments initiated. Cloning of subunit component genes completed. CGD registry mutation database established. Bone marrow transplantation successful in HLA-matched patients.
- 2000s Gene therapy clinical trials ongoing.

and are managed on a protocol employing prophylactic antibiotics, fungicides, and interferon-gamma. Bone marrow transplantation has been successful in those patients when a suitable donor is available. We look back today with amazement at the remarkable progress that has been made for this disease over the past six decades (11) (see *Decades of progress for CGD*).

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His brother, Morris J. Karnovsky, and I dedicate this article to the late Manfred L. Karnovsky, who was the senior author of the original article.

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