phagocytic process was responsible [6]. It was also thought that because the disease occurred in boys and was familial that the defect was X-linked. However, reports of the disease in females began to emerge, thereby revealing an autosomal recessive inheritance of the same phenotype [7,8]. It was then that Nathan and Baehner [9] showed that leukocytes from CGD patients, unlike normal human leukocytes, did not reduce nitroblue tetrazolium (NBT), a compound that converts to insoluble blue formazan product upon reduction by the superoxide anion ($O2^{-\cdot}$). This phenomenon was rapidly established as a sensitive clinical screening test for CGD that is still used today.

Over the next twenty years, over 150 scientific papers had been published reporting cases of patients with CGD describing their symptoms and similarities to other granuloma forming diseases. It was observed that CGD leucocytes could ingest micro-organisms but failed to kill the organisms responsible for the infections. Phagocytosis is normally accompanied by a marked increase in oxidative metabolism and studies had shown that NADPH oxidase was the respiratory enzyme responsible for bactericidal activity [10]. The hexose monophosphate shunt is responsible for generating reduced NADPH [10]. The critical deficiency in CGD cells is to generate $O2^{-\cdot}$ and other reactive oxygen species such as hydrogen peroxide (H_2O_2) [11]. The importance of H_2O_2 was illustrated by the fact that some bacterial species, such as *Streptococci*, that produces its own H_2O_2, could be killed by CGD leucocytes [12]. Following further corroboration that the deficiency in CGD was caused by a defect in the NADPH oxidase system in CGD patients, scientists began to research the formation of the free radical form of oxygen, $O2^{-\cdot}$, produced by NADPH oxidase during its respiratory burst, and showed that whereas normal leucocytes generated $O2^{-\cdot}$ during phagocytosis, CGD phagocytes were not able to do this [13-15].

Speculation that a b-type cytochrome may also be involved in this $O2^{-\cdot}$ generating activity began in 1979 from observations that a cytochrome-b associated with a particulate fraction of normal neutrophils, was absent from the neutrophils from some, but not all, patients with CGD [16]. Described as the heme-containing protein, cytochrome-b_{558} was proposed as a primary component of the microbicidal oxidase system of phagocytes. A multicenter European evaluation of its incidence and relevance was conducted in London [17] where it was found to be undetectable in all 19 of the men studied in whom the defect appeared to be located on the X chromosome. Thus cytochrome-b_{558} was hailed an important component of the microbicidal NADPH oxidase system and provided insight into its role in the enzyme complex. Borregaard and colleagues [18] demonstrated that approximately 90% of the cytochrome-b_{558} resides in the membrane of the specific granules of unstimulated human neutrophils and that the cytochrome-b_{558} translocates to the plasma membrane when the cells are stimulated. The authors speculated that the observed translocation was essential to the formation of an electron transport chain which generates $O2^{-\cdot}$, the single precursor from which all microbicidal oxidants ultimately arise.

Unravelling the NADPH oxidase enzyme

The 1980s saw the formation of a disease-gene relationship. Linkage analysis using cloned, polymorphic DNA probes suggested a proximal location (Xp21) within CGD families [19]. Cytochrome-b_{558} was, so far, the only clearly defined component of this oxidase system and its absence provided the molecular basis of X-linked CGD, in which a profound predisposition to infection resulted from complete failure of this respiratory burst. Within a month of each other in March 1987, three separate groups working on the constituents of the phagocyte NADPH oxidase (phox) enzyme published their findings. Segal was the first to find that cytochrome-b_{558} had two subunits – a 23 K protein and the previously described 76–92 K glycoprotein. Reporting that the subunits were closely linked and remained associated with the heme of cytochrome-b_{558}, neither protein was detected in the cells of five patients with X-linked CGD, whereas both were present in two with the autosomal recessive inheritance form of this disease. This was the first finding substantiating what we now know are the smaller and larger subunits of the phagocyte cytochrome-b_{558} heterodimer (p22phox and gp91phox respectively) [20]. Umei and colleagues then discovered a 66 kDa component [21] particulate in oxidase fractions obtained from patients with CGD, regardless of whether they contained cytochrome-b_{558} or not, and Curnutte and Scott [22] also found a soluble activation factor that was localized entirely to the cytosolic fraction with a mass of approximately 40 kDa. Later studies confirmed the exact molecular weight to be 47 kDa (p47phox).

In 1981, a variant or atypical X-linked form of CGD was described [23] whereby the gp91phox subunit was found in normal levels but only able to function partially. Curnutte [24] suggested that these rare type of CGD cases were allelic variants and patients with this uncommon cytochrome-b_{558}-positive X-linked form of CGD have been reported by others since [25,26]. Today, classical X-linked CGD refers to cases where there is no respiratory burst activity demonstrable in a patient's neutrophils and the gp91phox subunit is absent. Autosomal recessive CGD is diagnosed when burst activity is abnormal and one of the other NADPH oxidase subunits is deficient. Variant or atypical CGD is diagnosed when a patient's neutrophils have demonstrable amounts of NADPH oxi-