

Correspondence

Reply

To the Editor:

We thank Roesler and Rösen-Wolff for their comments¹ regarding our recent publication titled “Restoration of anti-*Aspergillus* defense by neutrophil extracellular traps in human chronic granulomatous disease after gene therapy is calprotectin-dependent” in the *Journal of Allergy and Clinical Immunology*.² They raise several interesting points, questioning our suggestion that formation of neutrophil extracellular traps (NETs) and NETs-mediated killing of *Aspergillus* species might contribute to clearance in healthy individuals. We concluded this, because neutrophils from patients with chronic granulomatous disease (CGD) do not release NETs on contact with *Aspergillus* and as a consequence killing is impaired. In the following text, we discuss the comments of Roesler and Rösen-Wolff.

Data on myeloperoxidase (MPO) deficiency (MIM #254600) were first published in 1966 by the name of Alius-Grignaschi anomaly, which was defined as total hereditary peroxidase deficiency of neutrophils and monocytes in a healthy person in this publication. Epidemiological data on this rare disease are scarce and controversial (reference list available on request)—incidence rates in the United States and Europe range from 1 case in 2,000 (partial MPO deficiency) to 1 in 4,000 (complete MPO deficiency) population and from 1 in 17,500 (partial MPO deficiency) to 1 in 57,135 (complete MPO deficiency) population in Japan. Some studies do not differentiate between partial and complete deficiency, whereas others report results only from screening of a small collective originating from a restricted geographical area; in addition, a uniform definition of complete MPO deficiency is lacking (eg, null mutation with the absence of protein expression vs hypomorphic mutation with impaired enzymatic function). Information on clinical consequences of complete MPO deficiency, especially with regard to infection, is rare, and a review is lacking to date (reference list available on request). Indeed, a more definitive epidemiological survey would be an important contribution to understanding MPO deficiency.

Therefore, it is difficult to draw any conclusion as to susceptibility to infection of patients completely deficient in MPO. In addition, exposure to infectious agents might vary from one geographical region to another. As Roesler and Rösen-Wolff state correctly, *Candida* infections seem common in patients completely deficient in MPO³; however, disseminated infection with *Aspergillus flavus* and pulmonary infection with *Aspergillus fumigatus*^{3,4} as well as invasive bacterial infection with *Staphylococcus aureus* and *Legionella* have been described (references available on request). Thus, the increased susceptibility to infection might be linked to the absence of NETs formation in individuals completely deficient in MPO and in patients with CGD,^{2,3} although definitive *in vivo* evidence is obviously lacking.

Given the inability of completely MPO-deficient neutrophils to form NETs and some published evidence of susceptibility to infection, identification and a detailed clinical workup of further patients with complete MPO deficiency seems to be warranted in order to clarify its status as a primary immunodeficiency according to the WHO classification (Primary immunodeficiency

diseases: an update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee Meeting in Budapest, 2005). Such a study would need to analyze genotype-phenotype correlation and susceptibility to infection and optimally comprise *in vitro* analyses of NETs formation on stimulation with identified disease-relevant pathogens.

Susceptibility in murine models should be interpreted carefully, as clinical phenotypes in mice and men do not always correlate. Interestingly, earlier studies with human phagocytes showed that MPO-deficient as well as CGD neutrophils damage *Aspergillus fumigatus* hyphae equally inefficiently.^{5,6} Notably, hydrogen peroxide (a product of the nicotinamide adenine dinucleotide phosphate [NADPH] oxidase) or hypochlorous acid (a product of MPO) alone damaged *Aspergillus* hyphae only in concentrations ≥ 1 mM,⁵ and extracellular products of MPO did not rescue NETs formation in response to *Candida albicans* stimulation in patients completely deficient in MPO,³ suggesting that MPO acts cell-intrinsically, independent of its enzymatic activity, most likely by driving chromatin decondensation during NETs formation.⁷

To the best of our knowledge, NETs formation and NETs killing of *Aspergillus* species conidia or hyphae have not been analyzed yet in patients completely deficient in MPO. To address Roesler's and Rösen-Wolff's concerns, neutrophils of patients completely deficient in MPO should be analyzed parallelly with those of patients with CGD and healthy controls and by adding products of MPO (eg, hypochlorous acid, histamine chloramines) and/or NADPH oxidase (eg, H₂O₂) to neutrophils.

We therefore conclude that according to current knowledge, both NADPH oxidase and MPO are required for fungus-induced NETs formation. However, the apparent divergence of major pathogens related to CGD and complete MPO deficiency, concluded from scarce, thus potentially unreliable, epidemiological data in complete MPO deficiency, remains to be defined.

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