mans. Thus, only two experimental avenues have been open: the impact of iNOS inhibitors on the mycobactericidal activity of human macrophages *in vitro*, and a search for iNOS at sites of tuberculosis. Studies of human macrophages *in vitro* have been more frustrating than informative, because the macrophages tested to date have rarely exerted a bactericidal effect against *M. tuberculosis*, precluding a determination as to the contribution of iNOS to killing. Both mouse (5) and human macrophages (9) that lack iNOS can exert a bacteriostatic effect against *M. tuberculosis* by an unknown mechanism, but with respect to iNOS these cells do not model human macrophages at sites of infection or inflammation, which are often intensely iNOS-positive. Thus, one of the major unfulfilled goals for research in the immunology of tuberculosis is to learn how to obtain or culture human macrophages that are as iNOS-positive *in vitro* as they are *in vivo* and to test whether they kill *M. tuberculosis* and use iNOS to do so.

With so many experimental avenues blocked, intense interest devolves on the one remaining: whether iNOS is expressed in human tuberculosis. The answer has been affirmative for cells obtained by bronchoalveolar lavage. Macrophages lavaged from each of 11 patients with tuberculosis, but not those from normal subjects, expressed active iNOS as assessed by immuno- and cytochemistry (10). Tuberculosis patients exhaled more nitric oxide than healthy control subjects, and their bronchoalveolar macrophages contained iNOS and released nitric oxide *in vitro* (11, 12). Choi and coworkers (1) have now carried this inquiry into lung specimens resected from eight patients with tuberculosis. Immunohistochemistry demonstrated iNOS in the inflammatory zone of granulomas and surrounding pneumonitic regions. The enzyme was abundant in epithelioid macrophages, multinucleated giant cells, alveolar macrophages, and epithelial cells (1).

Nitrotyrosine was detected in the same cells (1). Tyrosine residues become nitrosated when peroxynitrite arises in their immediate vicinity from the interaction of nitric oxide and superoxide, or when nitrite (arising from the spontaneous oxidation of nitric oxide) is oxidized by hydrogen peroxide through the agency of myeloperoxidase. By whichever of these routes tyrosine residues became nitrated, the cellular colocalization of nitrotyrosine and iNOS implies that at some point in the development of the granulomas, there must have been enough oxygen to sustain the catalytic activity of iNOS.

Finally, Choi and coworkers (1) detected macrophage expression of NOS3, an enzyme first cloned from endothelial cells. NOS3 has rarely been detected in macrophages and never implicated in their function. In endothelial cells the enzyme is intermittently active in accord with its regulation by transient elevations in intracellular Ca^{2+} or stimulus-induced serine phosphorylation. The amounts of nitric oxide made by NOS3 in endothelial cells are probably too low to exert mycobactericidal activity. What might activate NOS3 in infected macrophages, and to what effect? Perhaps the Chan laboratory will complete the circle, testing the significance of the observation they made in humans by studying *M. tuberculosis* infection in $NOS3^{-/-}$ mice (13).

The work of Choi and colleagues (1) does not establish, but strongly supports, the possibility that iNOS may be functionally important in human tuberculosis. Their report is timely, because we urgently need to understand the biochemistry of the dialog between the macrophage and this peristent pathogen.

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The Paranasal Sinuses and a Unique Role in Airway Nitric Oxide Production?

Enzymes responsible for nitric oxide (NO) production have been demonstrated both in the nose and in the paranasal sinuses. In the sinuses, NO levels have been reported to be several-fold higher than in the nose. This has led to the suggestion that the paranasal sinuses are the main site for NO production within the airways (1). Until the report by Weitzberg and Lundberg (2) in the current issue of *AJRCCM* (pp. 144–145), there have been no attempts to investigate if NO found in the paranasal sinuses could enter the nasal cavity and perhaps spread further down in the lower airways. Since the first report by Lundberg and colleagues in *Nature Medicine* (1), there have been few papers published in the area. So far, only 15

subjects have been studied using intrasinusal NO measurements (1, 3, 4). In a report by Haight and colleagues, nearly 90% of nasal NO in the one subject investigated, the principal author himself, was derived from the nose; they found that high NO levels could develop in the nasal cavity during a period of stagnation even if the ostia connecting the nasal cavity with the sinuses were occluded (4). These data challenged the viewpoint of Lundberg and coworkers that the sinuses are the main source of NO in the upper respiratory tract (1, 3). The report presented in this issue further supports the original idea by demonstrating that humming caused a more than tenfold increase in NO levels in the nasal cavity. The authors state that this form of continuous phonation causes the air to oscillate, which in turn increases the exchange of air between the sinuses and nasal cavity (2). Although the extent of such gas exchange in humans is largely unexplored, it is known that the oscillation of air occurring normally within the respiratory cycle facilitates the exchange of gases between the sinuses and the nasal cavity (5); this observation supports the presently introduced theory.

The proposed role for the sinuses as mere reservoirs for NO as put forward by Haight (4) requires further discussion and analysis. In a recent set of experiments, we found that continuous measurements of NO in air sample from the cannulated maxillary sinuses always displayed a high initial peak before returning to a much lower steady state level (6). The finding of a very high concentration of NO in air trapped in the human maxillary sinus and a much lower steady state level in air sampled from the nose appears to fit somewhere between the contradictory reports of Lundberg and Haight (1, 4). The peak values are in line with the values reported in the Lundberg papers (1) and support the idea of a special role for the paranasal sinuses in the airway production of NO. However, they also emphasize the role of the sinuses as reservoirs enabling NO to reach extremely high levels. In the latter aspect, our data are in line with Haight (4) and stress the fact that the chamber construction of the sinuses is important for NO accumulation. This conclusion is also partly supported by data derived from nasal NO sampling after unilateral maxillectomy (7). Additional confirmation for a central role for the sinuses in NO production comes from studies demonstrating that during ostial occlusion, which might occur in acute sinusitis and severe nasal polyposis, nasal NO levels are reduced (8). Further support for this interpretation is provided in the finding of large differences between the nasal and sinus response to local administration of L-NAME, a well-characterized inhibitor of NO production (3). The special role of the paranasal sinuses in the airway production of NO is underscored by comparing the original work on NO presented in *Nature Medicine* and recent data derived from intrasinusal sampling of carbon monoxide (1, 9). As with NO, carbon monoxide is produced in both the upper and lower airways, but when sampling from the maxillary sinuses, the obtained levels of carbon monoxide do not differ from the levels sampled directly from the nose (9).

It is presently not known how the production of NO in the sinus is regulated. The finding of inducible nitric oxide synthase in the mucosa suggests an enzymatic component in this process. In the present issue of *AJRCCM*, Weitzberg and Lundberg propose that measuring NO during humming could function as an easy noninvasive method for evaluation of sinus ostial patency, and that such a test could aid in identifying subjects at risk of developing sinusitis (2). Their suggestion is based on the assumption that the ostium is a key factor in the pathogenesis of sinusitis. It is also intriguing to speculate that the ostium has a direct role in the regulation of intrasinusal NO production. During pathologic conditions, the sinus can be sealed off through an obstruction of the ostium. Similar to what happens in the sealed off middle ear, a negative pressure is then formed in the sinus through the resorption of gases into the mucosa (10). In parallel with lowering of the pressure, relative hypoxia develops inside the sinus cavity. Hypoxia is one of the most powerful inducers of NO production, and hypoxic induction of inducible nitric oxide synthase activity could therefore be one consequence of ostial occlusion. In support for this idea are recent data from pressure chamber experiments demonstrating that a decrease of sinus pressure, such as seen in upper airway allergy or infection, can cause an increase in NO production (11). If there is a way for the high amounts of NO present in the paranasal sinuses to enter the nasal cavity in large quantities, as suggested by Weitzberg and Lundberg (2), this could serve an important role in the nasal defense against invading microorganisms (12). There might also be, at least in theory, a pressure-hypoxia related mechanism enabling an increase in sinus production of NO when inflammatory or infectious diseases cause swelling of the adjacent mucosa and temporary ostial occlusion.

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