

At Last! A Realistic Animal Model of Severe Asthma

At last! In this issue of the *Journal* (pp. 261–268), Bates and colleagues describe an animal model of asthma in which unlimited, potentially fatal, bronchoconstriction occurs (1). Until now, no animal model has demonstrated this essential feature of asthma. Healthy humans are protected against unlimited airway narrowing by a plateau on the dose–response curve (2). If this protective mechanism were present in asthma, the disease would only be a minor nuisance; but it is lost and, as a result, asthmatic attacks can lead to severe symptoms, emergency hospital admissions requiring mechanical ventilation, and death. In contrast, “airway hyperresponsiveness” in many, if not most, animal models of asthma is pathetically small compared with the human disease.

There is no uniform agreement as to what the terms airway hyperresponsiveness, hypersensitivity, and hyperreactivity mean. Hyperresponsiveness has been variously linked to the dose of agonist that produces a given mechanical response, the slope of the dose–response curve, or an increased mechanical response to a given dose of an agonist. In my opinion, the last is a step in the right direction, but a 50% increase in the resistance to flow in and out of the lung in response to a given dose of agonist can lead to an animal being called hyperresponsive. In a human, this would mean flow resistance increasing from approximately $1.4 \text{ cm H}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s}$ to $2.1 \text{ cm H}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s}$, a trivial degree of airway obstruction almost within the error of measurement. We badly need to define these terms carefully, with criteria by which they can be used.

I suggest that hyperresponsiveness means an increased response to a given dose of agonist leading to excessive, unrestricted airway narrowing. The lower than normal dose that produces a given response by definition is not hyperresponsiveness, it is hypersensitivity. An increase in the slope of the dose–response curve is also hypersensitivity; it does not describe excessive airway narrowing either. The former could be called hypersensitivity, type 1, and the latter hypersensitivity, type 2. The term hyperreactivity should be discarded altogether.

Once we get the definitions and criteria established, we can understand what we are talking about. Bates and colleagues do define what they mean by hyperresponsiveness and also synergy (1), an important new concept underlying the mechanism of hyperresponsiveness. They showed that mice, sensitized and challenged with ovalbumin to produce airway inflammation, and then injected intratracheally with the cationic protein poly-L-lysine (PLL), had excessive unrestricted bronchoconstriction when challenged with methacholine. There was a marked increase in lung stiffness and parenchymal energy dissipation by tissue viscance. This was more than additive: allergic inflammation and intratracheal PLL resulted in a response to methacholine that was greater than the sum of either intervention alone. Pharmacologically, this is what synergy means: the combined effect of two drugs is greater than the sum of their individual effects. Interestingly, there was no synergy for flow resistance. Nevertheless, the synergy between inflammation and PLL pro-

duced the breakthrough resulting in an animal model of asthma that mimics the dangerous human disease.

PLL is thought to act by breaking down the epithelial barrier to agonist, thereby increasing the dose of agonist reaching airway smooth muscle. When it is administered alone, it results in hypersensitivity (3). Flow resistance increases at a lower dose of agonist, but the maximal response is not increased and hyperresponsiveness does not occur (3). This is in keeping with the plateau seen on the normal human dose–response curve: the response remains unchanged with increasing dose of agonist (2). This plateau strongly suggests that airway smooth muscle is supramaximally stimulated. If so, then excessive unrestricted bronchoconstriction cannot result from an excess of agonist. The problem becomes postjunctional, that is within or beyond the muscle itself (4), so that the force it develops is increased, and/or the load imposed on it is decreased. There is powerful evidence that either or both can abolish the protection of the plateau (5–7).

The failure of agonist after PLL to cause excessive airway narrowing at high methacholine dose (3) is presumably because the agonist in the absence of PLL supramaximally stimulated smooth muscle to shorten maximally. If so, synergy cannot result from smooth muscle behavior, which has already done its worst after the highest methacholine dose regardless of PLL's effect on epithelial permeability. Yet, Bates and colleagues attribute synergy to the combined effect of “increased” smooth muscle shortening and inflammatory mucosal thickening (1). However, PLL did not lead to increased shortening at the highest methacholine dose (3). Thus, the interaction between shortening and mucosal thickening should have occurred in the absence of PLL in ovalbumin-sensitized animals. Yet, the authors found that PLL was essential to produce synergy. What, then, is going on that explains the synergy between allergic inflammation and increased epithelial permeability?

If PLL increases permeability and this allows agonist freer access to smooth muscle, shouldn't it also work in the reverse direction to allow inflammatory exudate better access to the airway lumen? During bronchoconstriction an appropriate pressure gradient develops between the submucosa and the lumen, and exudates can certainly flow. Indeed, “mucus” plugging seen in fatal asthma is predominantly inflammatory exudate (8). For that matter, there is an even greater pressure gradient between the submucosa and the peribronchial space during bronchoconstriction, where the pressure normally is more negative than pleural pressure (9). Is it conceivable that exudate flows across the smooth muscle to the adventitia and dynamically unloads the muscle (10)? If flow of inflammatory exudate is the mechanism in the synergic mouse model, it should have immediate relevance to the human disease. The inflammatory secretions plugging the airways in fatal asthma also suggest flow from the submucosa to the airway lumen. Morphometric comparisons between fatal asthma and fatal bronchoconstriction in the synergic mouse model are now of utmost importance. At last, crucially important questions about the life-threatening pathophysiology in asthma can be addressed in an animal model. This should lead to exciting new therapeutic possibilities.

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DOI: 10.1164/rccm.200711-1627ED

Waiting to Exhale

Over 75% of new lung cancer diagnoses are in patients who present with distant or regional metastatic disease (1). Early diagnosis remains an elusive goal. Enthusiasm for imaging by computed tomography (CT) scan and by fluorescent bronchoscopy is lowered by concerns of low specificity and overdiagnosis (2, 3). Enthusiasm for proteomic analysis of blood specimens is tempered by challenges presented by blood proteome complexity (4); and the utility of sputum analysis is diminished by difficulties in specimen acquisition.

Compared with other biological specimens, exhaled breath condensate (EBC) is simple to collect and process and is available from all individuals. In a previous study, Carpagnano and colleagues reported that microsatellite alterations representing four loci on chromosome 3p were detected more frequently in the EBC DNA of patients with non-small cell lung cancer compared with EBC acquired from smokers without lung cancer (5). Microsatellites are short, polymorphic, tandem repeat DNA sequences that are dispersed throughout the genome. They are subject to allelic loss (loss of heterozygosity [LOH]) and to changes of microsatellite length (microsatellite instability [MI]) in disease states such as lung cancer (6, 7). LOH and MI are not specific for lung cancer since they are detectable in the airway epithelium of smokers without cancer (8), but taken together these reports and others suggest that the presence of LOH in the lung or blood may be a molecular dosimeter of cigarette carcinogen exposure that correlates with lung cancer risk (8).

In this issue of the *Journal* (pp. 337–341), Carpagnano and colleagues extend their previous findings to now ask if microsatellite alterations in EBC are similar to those in lung tissue from the same individuals (9). Using the same chromosomal 3p microsatellite primers as in the prior study, they examined alterations in EBC, lung tissue, and white blood cell DNA acquired from 41 patients with non-small cell lung cancer and from 18 patients with nonmalignant lung diseases. This analysis presents three important results. First, the study confirms that the prevalence of microsatellite alterations in EBC of patients with cancer is higher than in control subjects. Second, microsatellite alterations appear to be more prevalent in heavy smokers compared with other smokers, although this analysis may be confounded by disease and by classification bias. Third, Table 3 in the article shows the striking finding that, in each instance, microsatellite alterations in EBC matched those detected in lung tissue.

What are we to make of this 100% concordance between EBC and the lung? Carpagnano and colleagues suggest that this result indicates the “organ-specificity of 3p microsatellite alterations.” This is not necessarily true. Rather, the findings suggest that EBC microsatellite 3p alterations mirror those in the lung and may be a *sensitive* indicator of pulmonary DNA alterations. We do not know the source of DNA in EBC within the aerodigestive tract and we do not expect it to be specific for lung cancer. However, lung tumor specificity is not required for this assay to be useful or important. For example, Spira and colleagues recently reported gene expression alterations shared by tumor and by nonmalignant lung that were significantly associated with the presence of cancer within an individual smoker (10). Thus, the promise of the EBC assay is that it may provide a simple, noninvasive test that identifies individuals potentially harboring DNA alterations that signify a high risk for lung cancer.

Much work lies ahead before the promise of this biomarker can be considered for clinical practice. As suggested by Pepe and colleagues, biomarker development and validation are composed of five conceptual phases (11): (1) preclinical exploratory studies to identify biomarkers unique to cancer, (2) clinical assay development and establishment of assay reproducibility, (3) retrospective longitudinal studies, (4) prospective screening studies, and (5) cancer control studies. Thus far, Carpagnano and colleagues have developed a clinical assay that identifies chromosome 3p microsatellite DNA alterations in EBC and in lung tissue of patients with non-small cell lung cancer. The detection of DNA alterations in EBC is a promising approach for lung cancer risk assessment and early diagnosis. However, until the EBC DNA microsatellite alteration results are reproduced and validated, we need to hold our breath.

Conflict of Interest Statement: C.A.P. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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