

## Reply

### To the Editor:

We thank Dr Wanderer for his insightful comments<sup>1</sup> on our report,<sup>2</sup> and we agree that there is a need for improved methods to objectively evaluate patients with acquired cold urticaria (ACU) for their disease severity and response to therapeutic interventions. We also agree with Dr Wanderer's view (and generally held view) that testing patients with ACU for thresholds of critical cold stimulation times and temperatures can be used to do this. However, evidence obtained in the context of controlled studies to support the value of these tests in characterizing the severity and course of ACU is limited<sup>3,4</sup> (for time thresholds) or absent (for temperature thresholds). This is not surprising, because both tests are difficult to perform and standardize, especially in the case of temperature threshold testing.<sup>2</sup>

TempTest (emo systems, Berlin, Germany), the Peltier effect-based electronic device introduced in our original report,<sup>2</sup> allows for exposing skin to thermal elements with defined temperatures. Thus, TempTest is ideally suited to test thresholds of both stimulation temperatures and times for their respective sensitivity and specificity in identifying patients with severe ACU. Furthermore, TempTest can be used to assess time/temperature threshold changes during the course of ACU in individual patients, including patients in spontaneous remission and patients receiving suppressive medications.

Ongoing and future studies using TempTest will clarify the value of both threshold tests in identifying patients with severe ACU (who could then be advised to avoid cold exposure). Most importantly, TempTest will help to identify the best objective parameter (that can be determined by temperature challenges) for assessing the efficacy of ACU treatment. This will facilitate multicenter clinical trials to evaluate the benefit of existing suppressive and curative therapies. In addition, TempTest can be used in such trials to provide standardized test settings that yield precise and reproducible results. Finally, TempTest could promote the development of novel treatment options for patients with ACU, which may ultimately benefit patients with other forms of physical urticaria.

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## Mast cell degranulation after endobronchial adenosine challenge

### To the Editor:

We read with great interest the article by Crummy et al.<sup>1</sup> The findings of their work appear to be somewhat different from those reported in an earlier article from our group.<sup>2</sup> In this letter, we discuss the potential explanations for the apparently different conclusions.

(1) Differences in severity and medication history of patients. On the basis of the reported treatment regimen, the patients studied by Crummy et al<sup>1</sup> are patients with mild asthma probably on  $\beta$ 2-agonists and inhaled corticosteroids (ICSs) alone. It has been shown that a single inhalation of ICS had highly significant broncho-protection against AMP challenge up to 12 hours.<sup>3,4</sup> It is likely that the observed limited response to AMP in the airways of the subjects with asthma studied by Crummy et al<sup>1</sup> is a result of previous use of ICS. In our study,<sup>2</sup> subjects with asthma had not taken ICSs for at least 24 hours before bronchoscopy.

(2) Differences in timing of bronchoalveolar lavage fluid (BALF) collection. In the article by Crummy et al,<sup>1</sup> the timing for BALF collection after AMP challenge was not stated. In our study,<sup>2</sup> as soon as a visible airway response to instilled AMP was observed, the bronchial segment was lavaged 3 minutes after the last active dose. Our experience in the nasal model of adenosine provocation<sup>5,6</sup> indicates that mast cell mediator release after AMP exposure is transient with a peak at 3 to 5 minutes, and therefore it is critical to control the timing of BALF collection to obtain meaningful results. If BALF collection was performed later, the peak values of mediator release could have been missed.

(3) Differences in the normalization of BALF. Measuring soluble components in BALF is not an easy matter because of the lack of a reliable denominator for normalization. It is particularly challenging to compare the mediator levels in healthy and asthmatic airways when study groups are exposed to high doses of adenosine (degraded from AMP), which is known to act as a potent vasodilator. The issue of standardization with the lavage procedures is critical for comparisons with data from different laboratories. In our study,<sup>2</sup> the concentration of albumin was calculated to account for the dilution by the instilled lavage fluid (and by the exuded plasma that follows vasodilatation in response to endobronchial challenge with AMP). It appears that Crummy et al<sup>1</sup>

standardized baseline and postchallenge lavage by volume. In our opinion, this method of standardization is inappropriate and might have caused important defects in their findings (particularly in the bronchoalveolar lavage data obtained from their healthy nonatopic subjects, for whom very high doses of AMP were instilled endobronchially).

(4) In Table III, if one compares the pre-AMP versus post-AMP challenge in patients with asthma, the levels of histamine, tryptase, and prostaglandin D<sub>2</sub> appear to be increased. It would have been helpful to perform additional statistical analysis on these data and re-evaluate the conclusion that there was no significant increase in patients with asthma.

(5) As for the reasons for the increases in mediator release in normal subjects, it might be worth pointing out that there was a clear outlier for the levels of histamine and tryptase. By taking it out (this outlier has a >12-fold increase in histamine levels), it is likely that the statistical significance will be lost.

In conclusion, we propose the following explanations for the apparent discrepancy between the article by Crummy et al<sup>1</sup> and our previous publication.<sup>2</sup> First, it is likely that the severity of asthma and medication history of patients were very different between the 2 studies. Second, methods of normalization of mediator release were different, and the time point of BALF collection could also have been different. Finally, there seems to be an outlier in the level of histamine and tryptase in normal subjects in the article by Crummy et al,<sup>1</sup> which could have biased the conclusion.

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## Reply

To the Editor:

We thank Drs Lauretta and Polosa<sup>1</sup> for their interest in our article and the opportunity to respond to their comments. They have enumerated their points, and we therefore respond in the same order.

With regard to their first point about the medication our subjects were taking and the effect on the response to endobronchial adenosine, we have clearly stated that subjects with asthma were specifically excluded from the study: "...if they had taken antihistamines, long-acting  $\beta_2$  agonists, theophyllines, leukotriene antagonists or inhaled or oral steroids in the six months preceding the study..." (Methods, p. 34, paragraph 1, line 3).<sup>2</sup> In addition, in subjects maintained on short-acting  $\beta_2$ -agonists, these were withheld for 8 hours before the procedure because of the known mast cell stabilizing effects of these agents.<sup>3</sup> Therefore, it is extremely unlikely that treatment requirements had a significant effect on the results of the study.

As stated in our article,<sup>2</sup> the endobronchial challenge protocol was based on the protocol used by Dr Polosa et al<sup>4</sup> in their previous article, with minor modifications, which are again outlined in our article. Thus, lavage was undertaken either 3 minutes after a response to endobronchial adenosine was noted or 3 minutes after the third aliquot was administered. This timing, based on the previously published nasal model of adenosine challenging and on the study by Dr Polosa et al, should have been able to detect peak values of mediators.<sup>4,5</sup>

Standardizing for soluble mediators is a perennial problem in lavage studies, and there is no universally accepted technique that is not open to possible confounding factors. Consensus would suggest that acellular components should be standardized for volume of bronchoalveolar lavage.<sup>6</sup> More significantly, consensus would suggest that standardization with albumin may be of value in those with normal lung permeability, but in those with abnormal permeability, its use is negated, and it simply becomes a marker of permeability. Therefore, in the situation of an airway challenge, where the challenge through either direct effects or indirect effects is likely to cause intraluminal increase in albumin (as with AMP challenge), we disagree with the supposition that standardization for lavage albumin is appropriate. We would argue that correction for albumin is more likely to confound rather than standardize, because this is a dynamic variable dependent on many factors as part of the challenge protocol. We regard the change in protein as significant and related to the permeability effects of the adenosine challenge because it did not occur in the placebo challenge site.

We performed the same appropriate statistical comparison on the subjects with asthma that we performed on the other groups. We agree that the trend for the postchallenge values is higher in the subjects with asthma, but despite this trend, these are not statistically different. We are not sure which "additional statistical tests" Drs Polosa and