

validated PCR techniques, the presence of either picornavirus (probably human rhinovirus), influenza A virus, or hMPV. To our knowledge, we have described the first cases of documented postviral VCD. The identification of hMPV is of importance. This recently discovered virus from the paramyxovirus family has a spectrum of disease ranging from mild respiratory symptoms to severe cough, wheeze, and pneumonia.⁹ VCD has not previously been described as a mode of presentation of hMPV infection and might account for a proportion of wheezing, cough, and dyspnea associated with this virus.

The pathophysiology of postviral paradoxical vocal cord adduction is poorly understood. Viruses infect the upper airway epithelium, causing a cytopathic effect, mucosal inflammation, and neural activation through nonadrenergic and noncholinergic nerve endings. The resulting sensory activation can lead to known reflex responses that include cough, bronchial hyperresponsiveness, and increased neutrophil recruitment.¹⁰ This manifests in the lower airways as postviral bronchial hyperresponsiveness and asthma exacerbation. It is tempting to postulate that postviral VCD might represent the consequence of epithelial damage and subsequent neural activation and inflammation in the larynx and the extrathoracic airways.

We also demonstrated in 2 cases that VCD can be completely reversible after recovery from an acute viral infection. These findings highlight the heterogeneity of VCD that can clinically present as a transitory response to a viral infection or as a recurrent illness.

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Available online October 22, 2004.

doi:10.1016/j.jaci.2004.08.038

Loss of receptors and IgE *in vivo* during treatment with anti-IgE antibody

To the Editor:

How long does it take for IgE to be removed from mast cells and basophils, for example, under the influence of a therapeutic like omalizumab, and how does one interpret *in vivo* studies that address this question? *In vitro* studies of the dissociation of IgE from human basophils demonstrate a T_{1/2} of approximately 10 days.¹ This slow dissociation is unaltered by the presence of an IgE trap such as E25 (Omalizumab). Studies in last month's journal² examine the loss of receptor expression on tissue mast cells following treatment with Omalizumab, and these studies also suggest that dissociation of IgE is very slow. Indeed, on the basis of the *in vitro* studies, one would expect that significant loss of receptor from tissue mast cells would require many weeks. Ten half-lives—a typical rule-of-thumb length of time for a dissociative process—would be 100 days. Recent studies using Omalizumab and measuring functional changes *in vivo* indirectly suggest that loss of mast cell function is relatively slow.^{3,4} With these results in mind, the measurements of the loss of receptor on circulating basophils⁵ after treatment with Omalizumab—as noted in phase I trials—initially appear puzzling. These measurements showed a T_{1/2} of 3 days, considerably shorter than one would expect. However, a distinguishing characteristic between circulating basophils and tissue mast cells (or basophils in *in vitro* culture) is the brief transit time of basophils in circulation. The brief transit time may represent death or margination to tissues, but in either case, the cell is lost from observation. As a consequence, the apparent rapid loss of receptor expression (or cell surface IgE) is not the result of dissociation of IgE but the appearance of basophils in circulation that express less receptor. In essence, the plotted curve of receptor loss is a mirror reflection of the accumulation of receptor during maturation, or the apparent downregulation is really a reflection of the rate of upregulation.

The argument for this seemingly counterintuitive conclusion is as follows. Our previous studies have demonstrated that accumulation of FcεRI on the surface of basophils (and mast cells) results from IgE stabilizing the constitutive loading of receptor to the plasma membrane.⁶ In the absence of IgE, receptor that initially appears on the cell surface is rapidly lost. The loading of receptor on the

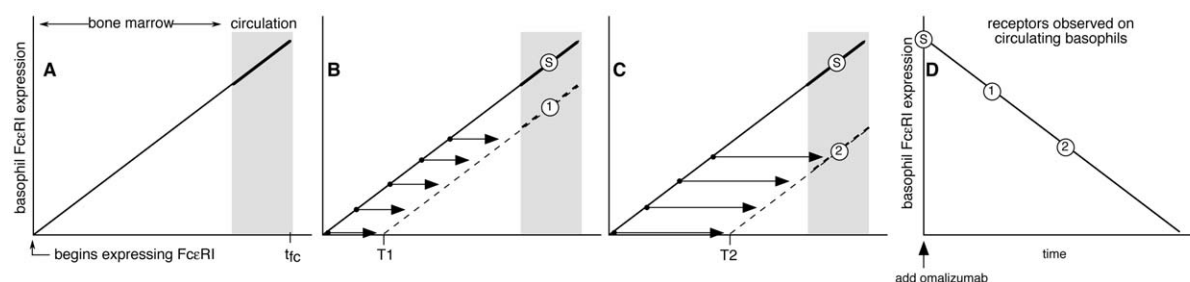


FIG 1. Receptor expression on basophils as they mature in the bone marrow and appear in the circulation. **A**, Natural progression of receptor expression in the presence of IgE. **B**, Introduction of anti-IgE at $t = 0$ and the shift in the population by $t = T_1$. **C**, Same as Fig 1, **B**, but at $t = T_2$. **D**, Kinetics of basophil expression as it appears in the circulation over the same time frame.

plasma membrane follows a line whose slope is a function of the IgE concentration. Therefore, the moment a basophil progenitor turns on the genes necessary for FcεRI expression, receptor begins to accumulate on the cell surface in a linear fashion (Fig 1, A). For practical purposes, the concentration of free IgE drops to zero once Omalizumab is administered. Receptor stops accumulating on the cell surface, stopping at the level achieved shortly after Omalizumab is administered (arrows in Fig 1, B and C, carry over the level of expression that existed at the time antibody was given). If the length of time between the beginning of FcεRI expression on basophils in the bone marrow and the leaving of basophils from circulation (call this T^{fc}) is reasonably shorter than the dissociation rate of IgE from the receptor, then for the purposes of this argument, IgE dissociation does not occur to a meaningful extent. The basophil circulates for only a fraction of the total time it is expressing FcεRI; most estimates are approximately 1 day. In other words, the observational window occurs at the end of the period of receptor accumulation, and this is likely a small fraction of time FcεRI is present. When these conditions are applied to the problem, the predicted downward line of receptor loss after Omalizumab is administered (Fig 1, D) is the mirror image of the line of upregulation that occurred before Omalizumab administration (Fig 1, A). This happens because the downward curve reflects the slow emergence of basophils from the bone marrow arrested at progressively lower levels of FcεRI expression. Two pieces of information come from this curve: the slope reflects the rate receptor is loaded, and the time when FcεRI expression reaches near zero is the age of basophils from the time they begin expressing FcεRI.

Before discussing some assumptions, it is possible to make a few calculations. In the phase I studies of Omalizumab, the antibody was given at a dose that reduced free IgE to less than 5 ng/mL in 1 hour. This low concentration of free IgE is—from the standpoint of receptor upregulation—essentially zero. In these studies, receptor expression was decreased by 50% in 3 days. This leads to the conclusion that the transit time of the basophils was 6 days (T^{fc}), and receptor loaded on these cells at the rate of 42,000 per day. Because the free IgE before

treatment was quite high, the synthetic rate of receptor in basophils was also near 42,000 per day.

There are potentially important caveats. For this analysis to be meaningful, the dissociation rate of IgE from receptor must be considerably longer than T^{fc} , and although all evidence suggests that this is true, it has not been explicitly determined for cells *in vivo*. A second underlying assumption is that no changes occur to the orderly maturation and egress of mature basophils from the bone marrow. Furthermore, one must assume that the IgE concentration in the bone marrow equals that of the circulation. There are conditions that can shorten leukocyte transit times in the bone marrow as well as conditions that increase the number of progenitors that find their way into the circulation. It is not yet known whether administration of Omalizumab is one of these conditions. Finally, because receptor accumulation on the plasma membrane of basophils is the balance of constitutive synthesis and loss of unoccupied receptors, a steady state is reached in the complete absence of IgE that is above zero. From studies *in vitro*, this number is approximately 10,000, and the phase I studies suggest a similar steady-state value. Therefore, the loss curve settles down to this value rather than zero. At a synthetic rate of 42,000 per day, only a few hours is needed to approximate this steady state.

If this analysis proves correct, the knowledge provides a tool for assessing FcεRI loading rates and, more than likely, the *in vivo* synthetic rates of FcεRI. The transit time (T^{fc}) of the basophil can also be determined. A similar analysis might be applied to other circulating cell types or cells rapidly transiting tissues that express FcεRI.⁷

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Available online October 14, 2004.
doi:10.1016/j.jaci.2004.07.064

Method of nebulizer calibration and methacholine airway responsiveness

To the Editor:

Direct bronchoprovocation challenges are commonly conducted by using one of 2 methods: the 2-minute tidal breathing method or the 5-breath dosimeter method. Both methods are described in the current American Thoracic Society guidelines.¹ We recently compared these 2 methods,² and in doing so, we realized that nebulizer calibration for the 5-breath dosimeter method could be conducted in several ways. Current recommendations are that dosimeter nebulizer systems be calibrated while in use by a subject to deliver 9 μ L per breath.¹ However, calibration guidelines are not well described, and 2 plausible methods meet American Thoracic Society guidelines. We have documented that measured output is greater with a standard (exhalation to the room) compared with a rebreathe (exhalation into the nebulizer) calibration method.³ We therefore investigated the effect of calibration method on methacholine PC₂₀ values.

Twelve subjects (7 women; age, 33 ± 3.1 [$\pm$ SEM] years; height, 67 ± 1.0 in) with mild asthma and mild-to-moderate airway hyperresponsiveness (tidal breathing methacholine PC₂₀, ≤ 8 mg/mL) were recruited to the study. Subjects had an FEV₁ of 70% of predicted value or greater (3.07 ± 0.18 L; $84\% \pm 3.1\%$ of predicted value). As-needed inhaled salbutamol ($n = 12$) was withheld for 6 or more hours, and inhaled corticosteroid ($n = 2$) or cromolyn ($n = 1$) was continued at a stable dose (≥ 4 weeks). There were no respiratory infections or, where applicable, allergen exposure for 4 or more weeks. Ethics approval was granted, and signed informed consent was obtained.

Four DeVilbiss 646 nebulizers (Sunrise Medical HHG Inc, Somerset, Pa) containing 3 mL of sterile isotonic saline were calibrated in triplicate by a single calibrator (research technician) to deliver $90 \mu\text{L} \pm 10\%$ after 10 breath actuations (ie, 9 μL per breath) for each of 2

different methods. The standard method involved inhalation from functional residual capacity to total lung capacity, a 5-second breath hold, and then removal of the mouthpiece from the mouth and exhalation to the room. The rebreathe method involved inhalation from functional residual capacity to total lung capacity, a 5-second breath hold, and exhalation back into the nebulizer through the mouthpiece. The stand-alone KoKo Dosimeter (Pulmonary Data Services, Inc, Louisville, Colo) actuation time for both methods was 0.6 seconds.

Two methacholine challenges were done in the same way as their respective calibrations and in random order within 7 days at the same time of day. The nebulizer output was measured for each subject by using the calibrator's settings before both challenges. If the output differed by greater than 10%, the nebulizer was recalibrated by the subject to administer 9 μL per breath. Baseline spirometry, including full forced vital capacity maneuvers (baseline only), were performed in triplicate before diluent inhalation. Doubling concentrations of methacholine were then administered, beginning with a predetermined concentration and continuing until the FEV₁ measured 30 and 90 seconds after inhalation had decreased by 17% or more compared with that seen after the diluent. The time between the start of inhalation of one concentration to the start of inhalation of the next concentration remained constant at 5-minute intervals. Methacholine PC₂₀ values were then algebraically calculated from the log concentration versus response curves.^{4,5}

Log-transformed methacholine PC₂₀ values were compared with the paired *t* test (STATISTIX 4.1 Analytical Software, Tallahassee, Fla).

Results of the calibrator's studies are as follows. The output of the 4 nebulizers determined by using the rebreathe method with the standard settings was lower by about 30% (6.5 vs 9.3 μL ; $n = 4$; $P = .0008$). With the nebulizer settings increased to obtain the desired output by the rebreathe method, the standard calibration measurement at these settings resulted in a 50% higher measured output (13.3 vs 8.8 μL ; $n = 4$; $P = .003$).

The methacholine PC₂₀ value was a half concentration lower (more responsive) for the rebreathe method versus the standard calibration method. The geometric mean PC₂₀ values were 1.5 mg/mL (95% CI, 1.0-4.9) and 2.3 mg/mL (95% CI, 0.70-3.3), respectively. Individual values are shown in Fig 1.

A secondary observation was that the measured output was closer to the calibrator's for the standard versus rebreathe method ($7.3 \pm 0.5 \mu\text{L}$ vs $3.4 \pm 1.1 \mu\text{L}$, respectively; $P = .001$).

These results lead to 3 important observations. First, the rebreathe method of calibration produces a lower measurement of output by about one third than does the standard method, confirming our previous study.³ Second, increasing the nebulizer settings to achieve the desired 9- μL measured output by means of the rebreathe calibration method results in about a one-half concentration reduction in PC₂₀ value. Third, the standard method of calibration