

## METHODS

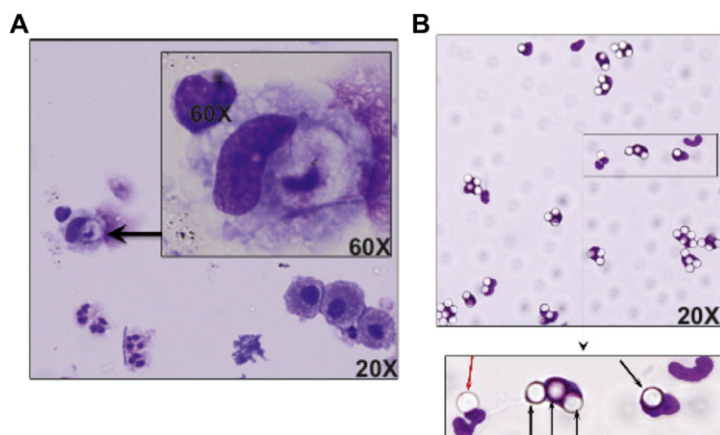
### Inclusion/exclusion criteria

Inclusion criteria for enrollment included: age 18 and older, a clinical history of asthma, FEV<sub>1</sub> >40% predicted, and either (1) beta-agonist reversibility to 4 puffs albuterol  $\geq$ 12% OR (2) PC20 FEV<sub>1</sub> methacholine  $\leq$ 8 mg/mL NOT on an inhaled corticosteroid, or  $\leq$ 16 mg/mL ON an inhaled corticosteroid. Participants also demonstrated a need for daily controller therapy (inhaled corticosteroids, leukotriene modifiers, and/or long-acting beta-agonists) as shown by either: (1) use or prescription of an asthma controller during past year OR (2) symptoms more than twice a week if not on asthma controller. If the participant was using an inhaled steroid (any drug at any dose not exceeding the equivalent of 1000  $\mu$ g fluticasone daily), he or she must have been on a stable dose for at least 2 weeks. All participants were nonsmokers (total lifetime smoking history <10 pack-years, no smoking for at least

1 year), and were required to provide informed consent. Exclusion criteria included use of other asthma medications or contraindicated medications, significant medical illnesses or lung diseases other than asthma, vocal cord dysfunction, respiratory tract infection or significant asthma exacerbation in the previous 4 weeks, history of life-threatening asthma in the past 5 years, pregnancy, lack of use of acceptable birth control methods if of child-bearing potential, hyposensitization therapy other than an established maintenance regimen, and an inability to effectively participate in study-related activities.<sup>E1</sup>

### REFERENCE

- E1. Peters SP, Kunselman SJ, Icitovic N, Moore WC, Pascual R, Ameredes BT, et al. Tiotropium bromide step-up therapy for adults with uncontrolled asthma. *N Engl J Med* 2010;363:1715-26.



**FIG E1.** Efferocytosis by sputum macrophages and blood monocytes. **A**, Sputum was induced and processed as reported in the [Methods](#) section, and efferocytosis was assessed from cytospin preparations by using light microscopy. A representative image is shown. **B**, Efferocytosis of 5- $\mu$ m carboxylated beads by monocytes was assessed by using light microscopy. A representative image is shown. *Black arrows* indicate beads that have been ingested by monocytes compared with beads bound to the surface, as represented by the *red arrow*.

**TABLE E1.** Characteristics of subset study population providing blood monocytes\*

| Subjects                                | Nonobese subjects | Obese subjects | P values |
|-----------------------------------------|-------------------|----------------|----------|
| No.                                     | 15                | 9              |          |
| Age (y)                                 | 41 (10)           | 38 (15)        | .7       |
| Female sex (no.)                        | 7 (53%)           | 7 (70%)        | .5       |
| African American (no.)                  | 6 (28%)           | 3 (33%)        | .8       |
| BMI (kg/m <sup>2</sup> )                | 24.1 (3.1)        | 35.6 (4.6)     | <.01*    |
| FEV <sub>1</sub> (L [before albuterol]) | 3.0 (0.2)         | 2.5 (0.2)      | .8       |
| FEV <sub>1</sub> (L [after albuterol])  | 3.0 (0.8)         | 3.0 (0.8)      | .5       |
| Pre-FVC (L [before albuterol])          | 4.0 (1.1)         | 5.0 (0.8)      | .9       |
| Post-FVC (L [after albuterol])          | 4.0 (1.1)         | 5.0 (0.8)      | .06      |
| FEV <sub>1</sub> /FVC ratio             | 0.6 (0.1)         | 0.7 (0.1)      | .5       |
| PC <sub>20</sub> (mg/mL)                | 2.0 (2.0)         | 1.8 (2.0)      | .8       |
| FENO (ppb)                              | 26 (12)           | 28 (25)        | .6       |
| Adiponectin (μg/mL)                     | 8.5 (5.9)         | 9.5 (5.8)      | .5       |
| Leptin (ng/mL)                          | 9.5 (14.0)        | 28.0 (10.0)    | <.01*    |
| IL-6 (pg/mL)                            | 1.6 (2.0)         | 1.4 (0.6)      | .3       |
| TNF-α (pg/mL)                           | 1.5 (0.9)         | 1.5 (0.7)      | .7       |
| hs-CRP (mg/mL)                          | 3.3 (5.0)         | 4.4 (2.4)      | <.01*    |
| 25(OH)D (ng/mL)                         | 29.0 (11.0)       | 24.4 (8.3)     | .6       |

Data are presented as means (SDs), unless otherwise indicated.

FENO, Fraction of exhaled nitric oxide; hs-CRP, high-sensitivity C-reactive protein.

\*No significant differences were found for these characteristics between nonobese subjects of this subset compared with nonobese subjects of the entire cohort of Table I. Similarly, no significant differences were found comparing obese subjects of this subset with obese subjects of the entire cohort of Table I.