

taking metformin had a similarly high rate of compliance with the medication regimen; the mean compliance rate was 79.3% (interquartile range, 70.0 to 92.8) in the metformin group and 79.7% (interquartile range, 71.0 to 92.3) in the group that received metformin and clomiphene. In addition, the combination-therapy group had a rate of diarrhea (60.3%) that was similar to that in the metformin group (64.9%), although dropout, pregnancy, and live-birth rates were significantly higher and similar to those in the clomiphene group. We attribute the higher dropout rates in the metformin group to patients' dissatisfaction with the diminished rates of ovulation and pregnancy.

Richard S. Legro, M.D.

Pennsylvania State University College of Medicine
Hershey, PA 17033
rsl1@psu.edu

Evan R. Myers, M.D., M.P.H.

Duke University School of Medicine
Durham, NC 27710

for the Cooperative Multicenter Reproductive
Medicine Network

1. Wadden TA, Berkowitz RI, Womble LG, et al. Randomized trial of lifestyle modification and pharmacotherapy for obesity. *N Engl J Med* 2005;353:2111-20.
2. Tsagareli V, Noakes M, Norman RJ. Effect of a very-low-calorie diet on in vitro fertilization outcomes. *Fertil Steril* 2006;86:227-9.
3. Morris SN, Missmer SA, Cramer DW, Powers RD, McShane PM, Hornstein MD. Effects of lifetime exercise on the outcomes of in vitro fertilization. *Obstet Gynecol* 2006;108:938-45.
4. Schwartz S, Fonseca V, Berner B, Cramer M, Chiang YK, Lewin A. Efficacy, tolerability, and safety of a novel once-daily extended-release metformin in patients with type 2 diabetes. *Diabetes Care* 2006;29:759-64.
5. Fujioka K, Brazg RL, Raz I, et al. Efficacy, dose-response relationship and safety of once-daily extended-release metformin (Glucophage XR) in type 2 diabetic patients with inadequate glycaemic control despite prior treatment with diet and exercise: results from two double-blind, placebo-controlled studies. *Diabetes Obes Metab* 2005;7:28-39.

GM-CSF Autoantibodies in Pulmonary Alveolar Proteinosis

TO THE EDITOR: Uchida et al. (Feb. 8 issue)¹ report that in patients with pulmonary alveolar proteinosis, neutrophil functions are impaired because of autoantibodies against granulocyte-macrophage colony-stimulating factor (GM-CSF). Although this may be true, neutralizing GM-CSF autoantibodies are found at high levels in a small proportion (<5%) of healthy persons. The Gamma-gard immune globulin used as a "negative" control by Uchida et al. contains these autoantibodies.^{2,3} Pulmonary alveolar proteinosis has been described in patients without autoantibodies against GM-CSF,⁴ and pathological specimens from patients with this condition may respond to preparations of immunoglobulin containing these autoantibodies.⁵ When primed with 10 ng of GM-CSF per milliliter, whole-blood neutrophils from patients with pulmonary alveolar proteinosis were severely impaired, presumably because most GM-CSF adhered to autoantibodies in the blood. However, without investigating whether GM-CSF at high autoantibody-saturating doses can salvage neutrophil functions in pulmonary alveolar proteinosis, one cannot exclude the possibility that neutrophil dysfunction in pulmonary alveolar proteinosis involves additional factors.

Klaus Bendtzen, M.D., D.M.Sc.

Morten Svenson, Ph.D.

Morten B. Hansen, M.D., D.M.Sc.

Rigshospitalet National University Hospital
DK-2100 Copenhagen, Denmark
kben@mail.dk

1. Uchida K, Beck DC, Yamamoto T, et al. GM-CSF autoantibodies and neutrophil dysfunction in pulmonary alveolar proteinosis. *N Engl J Med* 2007;356:567-79.
2. Svenson M, Hansen MB, Ross C, et al. Antibody to granulocyte-macrophage colony-stimulating factor is a dominant anti-cytokine activity in human IgG preparations. *Blood* 1998;91:2054-61.
3. Bendtzen K, Hansen MB, Ross C, Svenson M. High-avidity autoantibodies to cytokines. *Immunol Today* 1998;19:209-11.
4. Lin FC, Chang GD, Chern MS, Chen YC, Chang SC. Clinical significance of anti-GM-CSF antibodies in idiopathic pulmonary alveolar proteinosis. *Thorax* 2006;61:528-34.
5. Cho K, Nakata K, Ariga T, et al. Successful treatment of congenital pulmonary alveolar proteinosis with intravenous immunoglobulin G administration. *Respirology* 2006;11:Suppl: S74-S77.

TO THE EDITOR: Uchida and colleagues postulate the use of a humanized monoclonal GM-CSF antibody for the treatment of the acute respiratory distress syndrome (ARDS), which they regard as a chronic inflammatory disorder. We believe this proposal is incorrect. First, ARDS has an acute

onset and is related to sepsis in most cases.¹ Second, elevated concentrations of GM-CSF in bronchoalveolar-lavage specimens have been found to correlate positively with survival,² suggesting a beneficial effect of GM-CSF in ARDS. In patients with severe sepsis and acute respiratory dysfunction (40% of patients with ARDS), the administration of GM-CSF at low doses over a period of 5 days improved gas exchange in parallel with a reduction in alveolar neutrophils.³ In light of these findings, the use of human recombinant GM-CSF rather than its antibody might offer a valuable new option for ARDS treatment.

Thilo Busch, Ph.D.

Sven Bercker, M.D.

Udo Kaisers, M.D.

University of Leipzig
D-04103 Leipzig, Germany

1. Rubenfeld GD, Caldwell E, Peabody E, et al. Incidence and outcomes of acute lung injury. *N Engl J Med* 2005;353:1685-93.
2. Matute-Bello G, Liles WC, Radella F II, et al. Modulation of neutrophil apoptosis by granulocyte colony-stimulating factor and granulocyte/macrophage colony-stimulating factor during the course of acute respiratory distress syndrome. *Crit Care Med* 2000;28:1-7.
3. Presneill JJ, Harris T, Stewart AG, Cade JF, Wilson JW. A randomized phase II trial of granulocyte-macrophage colony-stimulating factor therapy in severe sepsis with respiratory dysfunction. *Am J Respir Crit Care Med* 2002;166:138-43.

THE AUTHORS REPLY: With regard to the comments by Bendtzen and colleagues, their previous report showed increased autoantibodies against GM-CSF in 4 of 1258 apparently healthy donors, and another study¹ showed non-neutralizing autoantibodies in 38 patients and neutralizing autoantibodies in 3 among 425 patients who had autoimmune diseases without evidence of pulmonary alveolar proteinosis. We calculate that the levels of total autoantibodies in these latter three patients were 2.9, 8.6, and 17.3 μg per milliliter, respectively. Our data are consistent with previous reports that autoantibody levels are markedly elevated in patients with primary pulmonary alveolar proteinosis (mean \pm SE value in a total of 158 patients, 113 ± 7 μg per milliliter)^{2,3} and are very low (≤ 1 μg per milliliter) in patients with secondary pulmonary alveolar proteinosis, congenital pulmonary alveolar proteinosis, or other lung diseases and in healthy controls.³ The autoantibody level is only 11 μg per milligram of protein in Gamma-gard (equivalent to approximately 2 to 3 μg per milliliter in serum). In pulmonary alveolar protein-

osis, polyclonal autoantibodies are both neutralizing and non-neutralizing, and levels of autoantibodies do not correlate with disease severity.^{2,4}

These puzzling observations are explained by the concept of a "critical threshold" level of neutralizing autoantibodies (not total autoantibodies) that is required to reduce GM-CSF bioactivity to a level that impairs myeloid-cell functions.² Assuming that 50% of autoantibodies are neutralizing, we estimate that the critical threshold level is between 8 and 22 μg per milliliter. Our hypothesis is supported by the dose-dependent rescue of neutrophil function (the CD11b stimulation index) at higher concentrations of GM-CSF. In a group of five patients with pulmonary alveolar proteinosis, all had an increase in the CD11b stimulation index, reaching $93 \pm 11\%$ of the control value at 100 ng per milliliter or more.

With regard to the comments by Busch and colleagues, GM-CSF has both short-term and long-term effects on myeloid cells, as well as direct effects on respiratory epithelium. ARDS is a heterogeneous syndrome with distinct phases, including exudative, proliferative, and fibrotic phases. Similarly, the distinct phases of sepsis are the at-risk, hyperinflammatory, and immunoparalytic phases. We propose that although a subgroup of patients with ARDS may benefit from augmentation of GM-CSF bioactivity,⁵ another subgroup may benefit from neutralization of GM-CSF bioactivity.

Kanji Uchida, M.D., Ph.D.

David C. Beck, M.D., Ph.D.

Bruce C. Trapnell, M.D.

Cincinnati Children's Hospital Medical Center
Cincinnati, OH 45229
bruce.trapnell@cchmc.org

1. Meager A, Wadhwa M, Bird C, et al. Spontaneously occurring neutralizing antibodies against granulocyte-macrophage colony-stimulating factor in patients with autoimmune disease. *Immunology* 1999;97:526-32.
2. Uchida K, Nakata K, Trapnell BC, et al. High-affinity autoantibodies specifically eliminate granulocyte-macrophage colony-stimulating factor activity in the lungs of patients with idiopathic pulmonary alveolar proteinosis. *Blood* 2004;103:1089-98.
3. Trapnell BC, Whitsett JA, Nakata K. Pulmonary alveolar proteinosis. *N Engl J Med* 2003;349:2527-39.
4. Seymour JF, Doyle IR, Nakata K, et al. Relationship of anti-GM-CSF antibody concentration, surfactant protein A and B levels, and serum LDH to pulmonary parameters and response to GM-CSF therapy in patients with idiopathic alveolar proteinosis. *Thorax* 2003;58:252-7.
5. Trapnell BC. Granulocyte macrophage-colony stimulating factor augmentation therapy in sepsis: is there a role? *Am J Respir Crit Care Med* 2002;166:129-30.