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Human GM-CSF Autoantibodies and Reproduction of Pulmonary Alveolar Proteinosis

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To the Editor

Idiopathic pulmonary alveolar proteinosis is a rare disease in which surfactant lipids and proteins accumulate in pulmonary alveolar macrophages and alveoli, resulting in respiratory insufficiency and, in severe cases, respiratory failure.[1] Granulocyte–macrophage colony-stimulating factor (GM-CSF) autoantibodies occur in these patients[2] and may mediate the pathogenesis of this disease, but they are also present in healthy persons and in immune globulin prepared from plasma obtained from healthy persons. Since GM-CSF is required for surfactant catabolism by alveolar macrophages in mice, we hypothesized that high levels of GM-CSF autoantibodies (i.e., levels sufficient to eliminate endogenous GM-CSF priming of myeloid cells) could cause idiopathic pulmonary alveolar proteinosis by impairing surfactant clearance by alveolar macrophages.[3] We previously found that high levels of GM-

No other potential conflict of interest relevant to this letter was reported.

CSF autoantibodies are specifically associated with idiopathic pulmonary alveolar proteinosis[4] and can be isolated in pure form from these patients.[5]

We administered highly purified GM-CSF autoantibodies derived from a patient with idiopathic pulmonary alveolar proteinosis to healthy nonhuman primates (*Macaca fascicularis*). These autoantibodies were administered intravenously, and serum levels of 40 μg per milliliter or more were maintained for 10 months. A marked reduction in levels of GM-CSF–stimulated CD11b in blood leukocytes indicated that GM-CSF signaling was blocked; these results were identical to those in patients with idiopathic pulmonary alveolar proteinosis.[5] A diffuse, patchy distribution of lung lesions composed of well-preserved alveoli filled with eosinophilic, lipoproteinaceous material and enlarged, foamy alveolar macrophages developed in the macaques that received GM-CSF autoantibodies (Fig. 1A). Alveolar macrophages and intraalveolar material stained positively for surfactant protein B (Fig. 1B) and lipid (Fig. 1C). Ultrastructural evaluation revealed that alveolar macrophages were engorged with lipid droplets and lamellar inclusion bodies (Fig. 1D), the numbers of which were both significantly increased as compared with those of a control primate that was injected with saline (Fig. 1E). The bronchoalveolar-lavage fluid had a milky appearance and increased amounts of surfactant phospholipids and surfactant proteins as compared with the control fluid, which was normal in appearance and composition (not shown). GM-CSF autoantibodies from a patient with idiopathic pulmonary alveolar proteinosis or from a primate injected with patient-derived GM-CSF autoantibodies blocked the GM-CSF–stimulated increase in cell-surface CD11b levels in leukocytes (Fig. 1F).[5] Together, these results show that GM-CSF autoantibodies reproduce the pathologic manifestations of idiopathic pulmonary alveolar proteinosis and provide strong evidence of causality in human idiopathic pulmonary alveolar proteinosis, including disease association, isolation in pure form, reproduction of disease in healthy macaques, and reisolation from previously healthy macaques who were injected with GM-CSF autoantibodies. The term “autoimmune pulmonary alveolar proteinosis” can now be used instead of “idiopathic pulmonary alveolar proteinosis” to describe this disease. These observations have potential therapeutic implications for pulmonary alveolar proteinosis and for the potential use of GM-CSF autoantibodies to treat inflammatory and autoimmune disorders.

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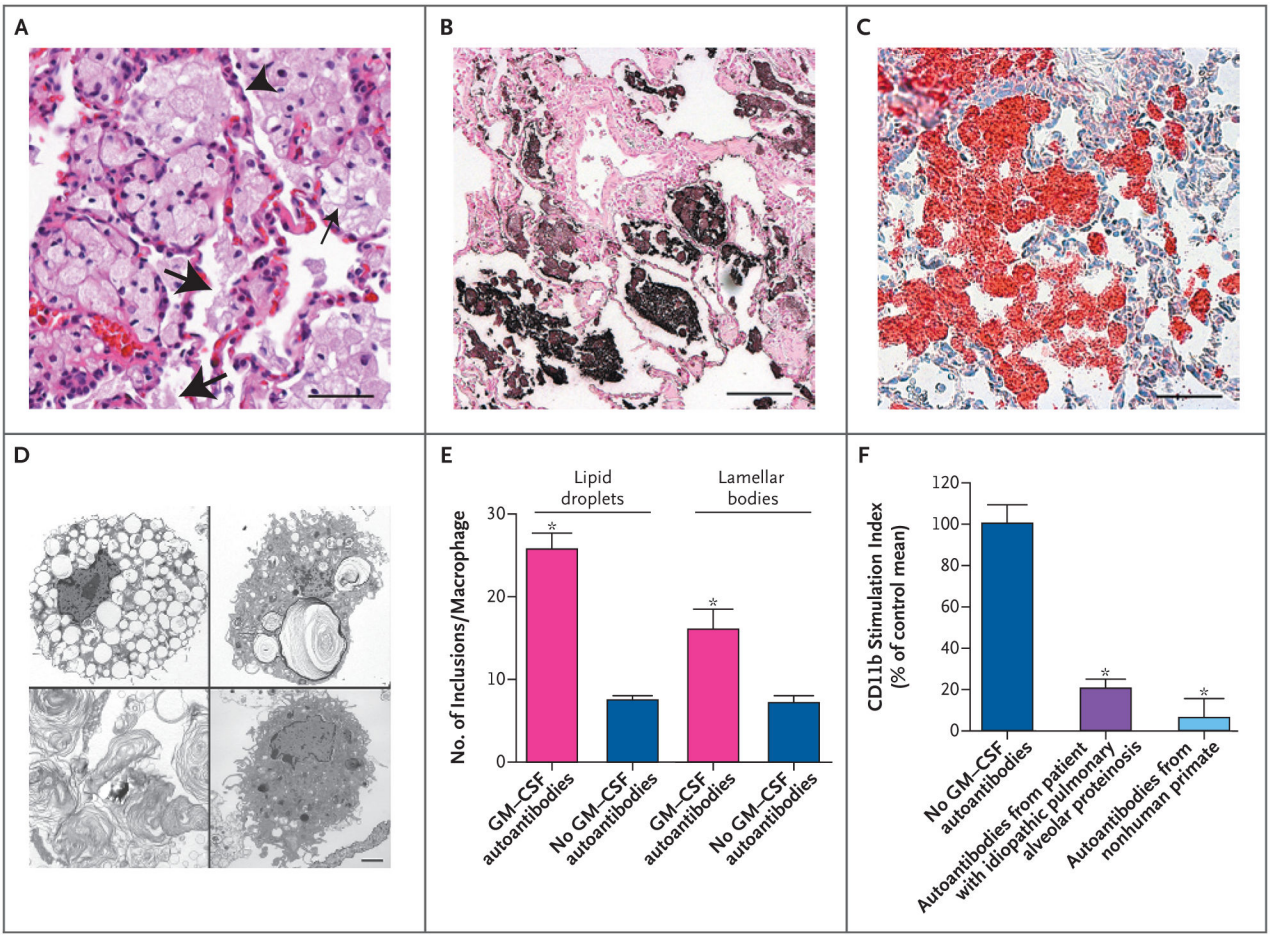

Figure 1. Effects of Human GM-CSF Autoantibodies in Nonhuman Primates

Healthy nonhuman primates (*Macaca fascicularis*) were pretreated with rituximab and cyclophosphamide to block the immune response to human immunoglobulins and were injected with human granulocyte–macrophage colony-stimulating factor (GM-CSF) autoantibodies derived from a patient with idiopathic pulmonary alveolar proteinosis (purified as previously described[5]) or saline, as a control. Panel A shows the histologic appearance of the lung of a macaque injected with GM-CSF autoantibodies (hematoxylin and eosin). Well-preserved alveolar walls (arrowhead), granular eosinophilic material (thick arrows), and numerous foamy alveolar macrophages filling alveoli (thin arrow) are shown. The scale bar represents 50 μm. Panel B shows immunostaining of the lung for surfactant protein B (brown areas), with nuclear fast red counterstaining. The scale bar represents 100 μm. Panel C shows oil red O staining of lipid in alveolar macrophages and alveolar spaces. The scale bar represents 50 μm. Panel D shows the ultrastructure of alveolar macrophages (top panels) and lung-lavage sediment (bottom left panel) from a passively immunized macaque and a typical normal alveolar macrophage from a control macaque (bottom right panel). Alveolar macrophages from the immunized macaque are engorged with lipid droplets (top left panel) and lamellar bodies (top right panel), and the lung-lavage sediment contains copious amounts of lamellar material that is typical of lung surfactant (uranyl acetate–lead citrate staining for all four panels). The scale bar represents 2 μm. Panel E

shows the number of inclusions of lipid droplets and lamellar bodies in alveolar macrophages obtained from bronchoalveolar-lavage specimens from macaques injected with GM-CSF autoantibodies and from a control. Inclusion bodies were counted by means of electron photomicrographs of at least 100 randomly selected macrophages from each macaque. The asterisks indicate $P \leq 0.01$ for the comparison with the corresponding control that did not receive antibodies, calculated with the use of Student's t-test. Panel F shows the neutralizing capacity of GM-CSF autoantibodies isolated directly from a patient with idiopathic pulmonary alveolar proteinosis and from a passively immunized macaque; these autoantibodies were measured according to the CD11b stimulation index.[5] Neither of the autoantibodies had any effects on mouse neutrophils, in contrast to anti-mouse GM-CSF antibodies that blocked GM-CSF stimulation completely; this shows that specificity was also retained during passive immunization (not shown). The asterisks indicate $P \leq 0.01$ for the comparison with the corresponding control that did not receive antibodies, calculated with the use of analysis of variance. In Panels E and F, numeric data were normally distributed, the bars represent the mean of triplicate determinations, and the T bars indicate the standard error.