preliminary results indicate that, under these test conditions, the presence of DFR depends on the presence of intact fibers in the CST ($P < 0.0001$; Fisher's exact probability test) and that as few as 1% of the CST fibers may be sufficient for DFR.

To investigate the functional potential of the regenerating CST axons induced by OEC transplants, we examined DFR in seven rats at 2 to 3 months after OECs had been transplanted into the lesion sites immediately after lesioning. In "free" tests, four of these seven rats used both paws. When the preferred paw was restrained, each of these four rats switched to using the unrestrained paw for the 50 daily reaches over 5 days (that is, scored as DFR present). This behavior was indistinguishable from the normal, unoperated control animals. Subsequent histology showed that although all four rats had complete unilateral destruction of the CST, the transplants had formed a continuous column of OECs with their associated peripherally myelinated axons, which bridged the entire rostrocaudal length of the lesion in the host CST.

The remaining three rats with OEC transplants used only one paw in the "free" tests, and when that paw was restrained, they never reached through the aperture with the other paw over a period of 5 days, during which time we observed 50 failed reaches per day by the restrained paw (that is, scored as DFR absent). Subsequent histology showed that these three rats also had complete unilateral destruction of the CST on the side of the nonreaching paw. However, in two of these three rats there were no OECs or peripherally myelinated axons in the lesioned CST, and in the third rat OECs and axons were present but only in the caudal part of the CST lesion and did not bridge the full rostrocaudal extent of the defect in the CST. Thus, in this pilot group of seven animals with complete CST lesions, the presence of DFR was correlated ($P = 0.029$; Fisher's exact probability test) with the presence of a continuous bridge of OECs and axons across the lesion.

The suggestions arising from these preliminary behavioral findings will need numerical confirmation and an investigation of the wider behavioral consequences of CST lesions. However, the suggestion that transplanted cultured OECs are able to induce functionally useful regeneration of adult axons in the experimental situation provided by these acute and highly defined rat spinal tract lesions opens up the prospect that in the future they may also have a role in treatment of the more complex lesions caused by human spinal cord injury. The fact that OECs can be obtained from adult donors raises the possibility of autotransplantation for clinical situations.

REFERENCES AND NOTES

1. M. Vidal-Sanz *et al.*, *J. Neurosci.* **7**, 2894 (1987).
2. T. J. Neuberger, C. J. Cornbrooks, L. F. Kromer, *J. Comp. Neurol.* **315**, 16 (1992); C. N. Montero-Menei *et al.*, *Brain Res.* **570**, 198 (1992); X. M. Xu, A. Chen, V. Guénard, N. Kleitman, M. B. Bunge, *J. Neurocytol.* **26**, 1 (1997).
3. Y. Li and G. Raisman, *J. Neurosci.* **14**. 4050 (1994).
4. ———, *Exp. Neurol.* **134**, 102 (1995).
5. K. R. Kuhlengel, M. B. Bunge, R. P. Bunge, H. Burton, *J. Comp. Neurol.* **293**, 74 (1990).
6. D. G. Moulton, *Ann. N.Y. Acad. Sci.* **237**, 52 (1974); P. C. Barber and G. Raisman, *Brain Res.* **141**, 57 (1978); K. C. P. Wilson and G. Raisman, *ibid.* **205**, 245 (1981); P. P. C. Graziadei and G. A. Montigraziadei, *J. Neurocytol.* **8**, 1 (1979).
7. P. C. Barber and R. M. Lindsay, *Neuroscience* **7**, 3077 (1982); R. Devon and R. Doucette, *Brain Res.* **589**, 175 (1992).
8. A. Ramón-Cueto and F. Valverde, *Glia* **14**, 163 (1995).
9. I. A. Franceschini and S. C. Barnett, *Dev. Biol.* **173**, 327 (1996).
10. G. Raisman, *Neuroscience* **14**, 237 (1985).
11. A. Ramón-Cueto and M. Nieto-Sampedro, *ibid.* **47**, 213 (1992).
12. Unilateral lesions of the CST were produced (on the side that offered access with minimal damage to pial vessels) by a current of 10 μA for 8 to 10 min passed through a stainless steel electrode inserted to a vertical depth of 1 mm, at 1 mm caudal to the obex, and 0.3 mm from the midline in 71 young adult female AS rats (6 to 8 months old, body weights 200 to 220 g). Immediately after withdrawing the lesioning electrode, 50 rats received 5 μl of a suspension containing about 125,000 cultured OECs injected into the lesion site over a period of 1 to 2 min through a glass micropipette (*3*). In 30 rats 10% BD was injected stereotaxically into the contralateral medullary pyramid (*4*) 6 days before they were killed to allow time for anterograde labeling of the CST axons. At survival times of 6 days ($n = 6$), 10 days ($n = 9$), 3 weeks ($n = 4$), 4 weeks ($n = 7$), and 9 weeks ($n = 4$) after the lesioning and transplantation, the rats were killed and perfusion fixed (*4*). Longitudinal Vibratome sections 100 μm thick were used to identify the OECs by immunofluorescence for p75 low-affinity neurotrophin receptor and the CST axons by immunofluorescence for BD, and the results were recorded by confocal microscopy of serial longitudinal sections through the operated area. The remaining 20 rats [after survivals of 10 days ($n = 3$), 20 days ($n = 3$), 4 weeks ($n = 7$), and 2 to 3 months ($n = 7$)] were killed and perfusion fixed for electron microscopy (*4*).
13. I. Q. Whishaw, R. B. O'Connor, S. B. Dunnett, *Brain* **109**, 805 (1986).
14. Perfusion-fixed spinal cords of all 28 tested rats were divided coronally, from the obex caudally, into 20 consecutive 0.7-mm-thick blocks; three blocks corresponded to one cervical segment (as ascertained from surface observation of the spinal nerve roots). The lesions damaged an area of tissue about 0.5 mm in cross section and 1 to 2 mm in a rostrocaudal direction. In semithin sections, the descending CST axons were distinguished from the larger ascending sensory fibers of the dorsal columns by their position as a well-delineated, compact bundle in the mediodorsal edge of the dorsal columns, by their smaller fiber diameter (about 1 μm), and by the denser packing of the interfascicular glial cells. The extent of the lesions, the position of the transplants, and the presence of transplanted OECs and OEC-myelinated axons were recorded in camera lucida drawings of 1-μm-thick coronal sections. The area of any remaining part of the CST was measured.

15. We thank D. Li for consultation and collaboration. Y. Ajayi adapted the method for culturing the OECs. Supported by the Medical Research Council, the British Neurological Research Trust, the International Spinal Research Trust, the Barnwood House Trust, and Smith's Charities.

2 July 1997; accepted 21 August 1997

Requirement for Macrophage Elastase for Cigarette Smoke–Induced Emphysema in Mice

R. Dean Hautamaki, Dale K. Kobayashi, Robert M. Senior, Steven D. Shapiro*

To determine which proteinases are responsible for the lung destruction characteristic of pulmonary emphysema, macrophage elastase–deficient ($MME^{-/-}$) mice were subjected to cigarette smoke. In contrast to wild-type mice, $MME^{-/-}$ mice did not have increased numbers of macrophages in their lungs and did not develop emphysema in response to long-term exposure to cigarette smoke. Smoke-exposed $MME^{-/-}$ mice that received monthly intratracheal instillations of monocyte chemoattractant protein–1 showed accumulation of alveolar macrophages but did not develop air space enlargement. Thus, macrophage elastase is probably sufficient for the development of emphysema that results from chronic inhalation of cigarette smoke.

Pulmonary emphysema is a major component of the morbidity and mortality of chronic obstructive pulmonary disease (COPD), a condition that afflicts more than 14 million persons in the United States and has become the country's fourth leading cause of death (*1*). Cigarette smoking is the main risk factor for the development of COPD. With the rapid rise in cigarette smoking occurring now in many countries, COPD may become epidemic worldwide in years to come.

Emphysema is defined as the enlargement of peripheral air spaces of the lung, including respiratory bronchioles, alveolar ducts, and alveoli, accompanied by destruction of the walls of these structures (*2*, *3*). Before the development of emphysema, smokers' lungs show an accumulation of macrophages, lym-

Departments of Internal Medicine, and Cell Biology and Physiology, Washington University School of Medicine at Barnes-Jewish Hospital, St. Louis, MO 63110, USA.

*To whom correspondence should be addressed. E-mail: sshapiro@imgate.wustl.edu

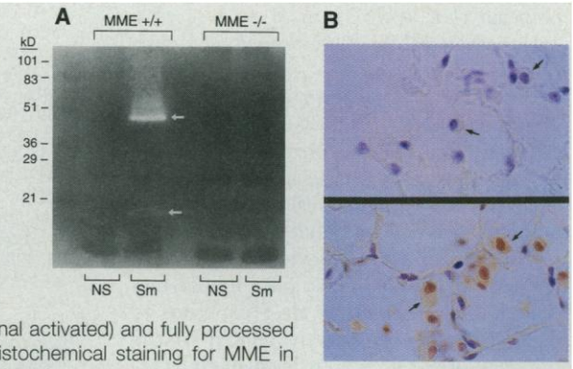

Fig. 1. Cigarette smoke exposure induces MME expression in alveolar and interstitial macrophages. (**A**) Lung tissue homogenates were subjected to κ-elastin zymography as described (*13*). Areas of elastin degradation were visualized as clear zones of lysis after Coomassie staining. Lytic bands were observed only in lungs of $MME^{+/+}$ mice exposed to smoke. These bands were inhibited by EDTA and comigrated in this nondenatured gel with the 45-kD MME (NH_2-terminal activated) and fully processed 22-kD MME (arrows). (**B**) Immunohistochemical staining for MME in lung tissue of $MME^{+/+}$ mice exposed to cigarette smoke for 3 months (bottom) and age-matched controls (top). An affinity-purified polyclonal antibody generated against MME (*9,10*) (0.5 mg/ml) was used at 1:2000 dilution [as in (*17*)]. MME staining is limited to macrophages (arrows). In $MME^{+/+}$ control mice, few macrophages had detectable MME. Exposure of $MME^{+/+}$ mice to cigarette smoke was associated with the induction of MME in most macrophages. No MME was detected in $MME^{-/-}$ mice.

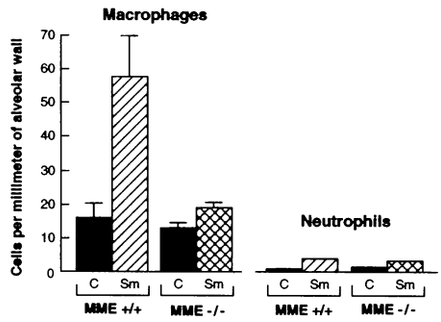

Fig. 2. Macrophage accumulation in the lung in response to cigarette smoke is dependent on MME. Groups of 10 $MME^{+/+}$ and $MME^{-/-}$ mice were exposed to cigarette smoke for 6 months (Sm). Equal numbers of age-matched $MME^{+/+}$ and $MME^{-/-}$ littermates were used as controls (C). Lung tissue was prepared as described (*13*). The number of macrophages and neutrophils were counted (*17*) and normalized per millimeter of alveolar wall. Error bars represent the standard deviation.

phocytes, and neutrophils in the walls and adjacent air spaces of the respiratory bronchioles, alveolar ducts, and alveoli (*4*). Inherited deficiency of α_1-antitrypsin, which is the primary inhibitor of neutrophil elastase, predisposes individuals to early onset emphysema (*5*), and intrapulmonary instillation of elastolytic enzymes in experimental animals causes emphysema (*6*). Together, these findings have led to the hypothesis that emphysema results from proteolytic injury directed especially against elastin, the main component of elastic fibers.

Macrophages, which account for over 90% of the inflammatory cells in smokers' lungs, produce many proteolytic enzymes that are capable of degrading elastic fibers and other extracellular matrix components (*7*). One of these enzymes, which is designated macrophage elastase (MME) (*8, 9*), is a metalloproteinase that solubilizes many extracellular matrix proteins, including elastin.

To determine the contribution of macrophage elastase to the pathogenesis of emphysema due to cigarette smoke, we compared mice made genetically deficient in MME by gene targeting ($MME^{-/-}$ mice) (*10*) with wild-type littermates ($MME^{+/+}$) for the development of emphysema in response to chronic exposure to cigarette smoke. We exposed adult mice to smoke from two nonfiltered cigarettes for 6 days per week for up to 6 months, using a smoking apparatus (*11*) adapted for mice (*12*). Carbon monoxide (peak carboxyhemoglobin blood concentrations were 10 to 14%) did not reach toxic levels, and the mice appeared grossly normal during the entire experimental period. On completion of the smoking protocol, the mice were killed and the lungs were inflated and fixed by intratracheal instillation of 10% formalin at 25-cm H_2O pressure and sectioned for microscopic analysis.

To investigate the spectrum of elastolytic enzymes present in lung tissue in response to cigarette smoke, we made extracts of whole lung and analyzed them for elastase activity by zymography using κ-elastin as the substrate (Fig. 1A) (*13*). No elastase activity was found in extracts prepared from $MME^{+/+}$ mice that had not been exposed to cigarette smoke or from any $MME^{-/-}$ mice. Extracts from smoke-exposed $MME^{+/+}$ animals, however, exhibited two zones of elastolytic activity that comigrated at the same molecular size as the active 45-kD macrophage elastase and the 22-kD fully processed active form of macrophage elastase (*9*). The activity in both bands was inhibited by EDTA, which establishes them as due to metalloproteinases, and the identity of the enzymes responsible for the activity in these bands was shown to be macrophage elastase by protein immunoblot analysis. Immunohistochemistry of lung sections with affinity-purified antiserum directed against mouse macrophage elastase confirmed the presence of this enzyme specifically in macrophages within the lungs of $MME^{+/+}$ mice exposed to cigarette smoke, whereas the enzyme was only weakly detected in a few macrophages of nonsmoking $MME^{+/+}$ mice (Fig. 1B). As expected, no cells were immunoreactive in lungs of $MME^{-/-}$ mice regardless of whether the animals had been exposed to cigarette smoke. Thus, the expression of macrophage elastase was induced by cigarette smoke and appeared to be the only detectable elastase present in lung tissue of mice exposed to cigarette smoke.

$MME^{+/+}$ mice exposed to cigarette smoke for 6 months had enlarged air spaces compared with age-matched littermate controls that were not exposed to smoke. Calculation of mean linear intercepts (*Lm*), an estimate of the average distance between the opposing walls of a single alveolus (*14*), showed a 32% increase (Table 1), a degree of enlargement comparable with that seen after intrapulmonary instillation of neutrophil elastase (*5*). In contrast, $MME^{-/-}$ mice did not show changes in alveolar dimensions after 6 months of exposure to cigarette smoke (Table 1). Cigarette smoke exposure also led to enlargement of alveolar ducts (*15*) in $MME^{+/+}$ mice (Table 1), whereas in smoke-exposed $MME^{-/-}$ mice, there was only a modest, insignificant increase in the size of these structures.

Like human smokers, $MME^{+/+}$ mice exposed to cigarette smoke showed increased macrophages in their lung tissue (Fig. 2).

Table 1. Alveolar duct area and mean linear intercepts (*Lm*) of $MME^{+/+}$ and $MME^{-/-}$ mice after long-term exposure to cigarette smoke. Values shown are the mean and standard deviation (values in parentheses) for groups of 10 mice each. *P* values were derived from a two-tailed Student's *t* test.

	Alveolar duct area (mm^2)*	*Lm* (μm)†
	$MME^{+/+}$	
Control	7.6 (1.2)	50.4 (1.5)
Smoke-exposed mice	12.2 (2.0)	66.6 (4.8)
P	0.0002	<0.0001
Increase with smoke exposure (%)	60	32
	$MME^{-/-}$	
Control	6.7 (0.9)	47.9 (2.2)
Smoke-exposed mice	7.8 (1.2)	49.6 (3.5)
P	0.12	0.26
Increase with smoke exposure (%)	16	3

*The alveolar duct area was calculated as described (*15*). †*Lm* was calculated as described (*14*).

Smoke-exposed $MME^{-/-}$ mice had no increase in the number of lung macrophages compared with $MME^{-/-}$ control mice. Also, the small accumulation of neutrophils in alveolar walls seen in $MME^{+/+}$ animals exposed to smoke was not significantly altered in $MME^{-/-}$ mice throughout the 6-month study.

Because the lack of macrophages in the lung, and not the lack of elastase per se, could be the reason for the absence of emphysema development in $MME^{-/-}$ mice, we intratracheally instilled monocyte chemoattractant protein–1 (MCP-1), a monocyte chemokine, which resulted in substantial macrophage accumulation in lung interstitium and alveolar spaces in both $MME^{+/+}$ and $MME^{-/-}$ mice (half-life for both ~18 days). Thus, $MME^{-/-}$ monocytes could egress from the pulmonary vasculature if there was a stimulus. Monthly MCP-1 instillation in $MME^{-/-}$ smoke-exposed mice caused an increase in macrophage recruitment compared with wild-type $MME^{+/+}$ mice exposed to cigarette smoke alone (Fig. 3, inset). However, despite the presence of $MME^{-/-}$ macrophages in lungs of mice exposed to cigarette smoke, the mean linear intercept (Fig. 3) and alveolar duct areas remained unchanged (7.0 mm^2 or 4% greater than mice that were not exposed to smoke). In contrast, MCP-1 treatment in $MME^{+/+}$ smoke-exposed mice enhanced emphysema (*Lm* = 76.2 μm, *n* = 4 or 15% greater than $MME^{+/+}$ smoke-exposed mice without MCP-1).

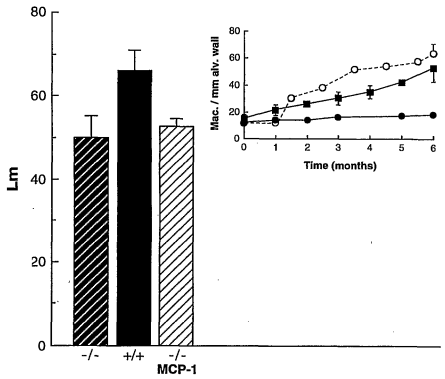

Fig. 3. Instillation of MCP-1 in the lungs of $MME^{-/-}$ mice exposed to cigarette smoke results in macrophage accumulation but not emphysema. MCP-1 (50 μg in 50 μl of saline) was given to $MME^{-/-}$ mice ($^{-/-}$ MCP-1) by monthly intratracheal administration, starting at 30 days after the initiation of cigarette smoke exposure. (**Inset**) Macrophage recruitment to the interstitium and alveolar surface (macrophages per millimeter of alveolar wall, Mac./mm alv. wall). Despite significant macrophage recruitment, the *Lm* of $MME^{-/-}$ mice exposed to cigarette smoke in the presence of MCP-1 ($MME^{-/-}$ + MCP-1) was not different from that of $MME^{-/-}$ animals. *n* = 4 to 6 mice per intermediate time point (for MCP-1, the numbers are averages of counts before and after instillation). *n* = 10 for 6-month data. Error bars represent the standard deviation. ●, $^{-/-}$; ■, +/+; and ○, −/− and MCP-1.

Because only macrophages, and not blood monocytes, express MME, we hypothesize that MME expressed by constitutive alveolar macrophages after exposure to cigarette smoke generates monocyte chemotactic activity and that this activity is responsible for the monocyte recruitment in response to smoke. Thus, macrophage-mediated lung destruction after exposure to cigarette smoke is directly related to the presence of MME, which most likely is required for direct degradation of lung tissue. Additionally, macrophage elastase can inactivate α_1-antitrypsin (*16*), indirectly enhancing neutrophil elastase activity and potentially contributing to lung destruction.

In this study, wild-type mice exposed to cigarette smoke developed inflammatory cell recruitment and dilatation and destruction of alveolar walls and alveolar ducts. These changes mirror the pathology of smokers' lungs, although human lungs also have destruction and dilitation of respiratory bronchioles, a structure not present in mouse lungs. Unlike normal mice, mice without MME failed to recruit macrophages and did not develop lung destruction in response to cigarette smoke. These findings point to a primary role for macrophages and macrophage elastase in the development of emphysema induced by cigarette smoke. This study also sheds light on both the beneficial and harmful effects of macrophage elastase. On exposure to foreign material such as cigarette smoke, constitutive lung macrophages are activated to produce macrophage elastase, which modulates the host inflammatory response by recruiting more macrophages into the lung. However, production of macrophage elastase may overwhelm local inhibition and destroy the lung tissue it was designed to protect.

REFERENCES AND NOTES

1. U.S. Centers for Disease Control, *U.S. Natl. Cent. Health Stat. Mon. Vital Stat. Rep.* **44**, 1 (1996); M. Feinleib *et al.*, *Am. Rev. Respir. Dis.* **140**, S9 (1989); T. D. MacKenzie, C. E. Bartecchi, R. W. Schrier, *N. Engl. J. Med.* **330**, 975 (1994).
2. G. L. Snider, *Am. Rev. Respir. Dis.* **146**, 1334 (1992); *ibid.*, p. 1615; American Thoracic Society, *Am. J. Respir. Crit. Care Med.* **152**, S77 (1995).
3. A. Nagai, H. Inano, K. Matsuba, W. M. Thurlbeck, *Am. J. Respir. Crit. Care Med.* **150**, 1411 (1994).
4. D. E. Niewoehner, J. Kleinerman, D. B. Rice, *N. Engl. J. Med.* **291**, 755 (1974); R. K. Merchant, D. A. Schwartz, R. A. Helmers, C. S. Dayton, G. W. Hunninghake, *Am. Rev. Respir. Dis.* **146**, 448 (1992); R. Finkelstein, R. S. Fraser, H. Ghezzo, M. G. Cosio, *Am. J. Respir. Crit. Care Med.* **152**, 1666 (1995).
5. C. B. Laurell and S. E. Eriksson, *Scand. J. Clin. Lab. Invest.* **15**, 132 (1963).
6. A. Janoff *et al.*, *Am. Rev. Respir. Dis.* **115**, 461 (1977); R. M. Senior *et al.*, *ibid.* **116**, 469 (1977); G. L. Snider *et al.*, *ibid.* **129**, 155 (1984); R. C. Kao, N. G. Wehner, K. M. Skubitz, B. H. Gray, J. R. Hoidal, *J. Clin. Invest.* **82**, 1963 (1988).
7. H. G. Welgus *et al.*, *J. Clin. Invest.* **86**, 1496 (1990); H. A. Chapman Jr., J. S. Munger, G.-P. Shi, *Am. J. Respir. Crit. Care Med.* **150**, S155 (1994); S. D. Shapiro, *ibid.*, p. S160.
8. Z. Werb and S. Gordon, *J. Exp. Med.* **142**, 361 (1975); M. J. Banda and Z. Werb, *Biochem. J.* **193**, 589 (1981).
9. S. D. Shapiro *et al.*, *J. Biol. Chem.* **267**, 4664 (1992); S. D. Shapiro, D. K. Kobayashi, T. J. Ley, *ibid.* **268**, 23824 (1993); A. Belaaouaj *et al.*, *ibid.* **270**, 14568 (1995).
10. J. M. Shipley, R. L. Wesselschmidt, D. K. Kobayashi, T. J. Ley, S. D. Shapiro, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 3942 (1996).
11. J. L. Wright and A. Churg, *Am. Rev. Respir. Dis.* **142**, 1422 (1990).
12. The cigarette-smoking protocol was as follows: Groups of 10 $MME^{-/-}$ mice and 10 $MME^{+/+}$ littermates, 12 weeks of age, were subjected to smoke from two nonfiltered cigarettes per day (University of Kentucky research cigarettes), 6 days per week, for 6 months, with the use of a smoking apparatus with the chamber adapted for mice. Nonsmoking, age-matched littermates (10 $MME^{+/+}$ and 10 $MME^{-/-}$) were used as controls. Preliminary studies performed on pure 129/SvJ and C57Bl/6J mice revealed that both strains had similar inflammatory cell recruitment into the lungs followed by pathologic changes characteristic of emphysema in response to cigarette smoke. A preliminary time course study in $MME^{+/+}$ mice demonstrated progressive inflammatory cell recruitment beginning within the first month of smoking followed by air space enlargement after 3 to 4 months of cigarette exposure (R. D. Hautamaki, D. K. Kobayashi, R. M. Senior, S. D. Shapiro, data not shown). All animal procedures were approved by the institutional review committee of the Washington University School of Medicine.
13. Total lung tissue extracts were prepared by removing lungs en bloc, freezing them, and homogenizing them in 5 ml of 50 mM tris, 10 mM $CaCl_2$, and 150 mM NaCl buffer. After the extracts were centrifuged, the supernatants were applied to κ-elastin zymography as described [R. M. Senior *et al.*, *J. Biol. Chem.* **266**, 7870 (1991); (*9*)]. Elastolytic metalloproteinases and serine proteinases are capable of generating zones of lysis by means of this technique. κ-Elastin zymography of neutrophils confirmed that neutrophil elastase activity was equivalent in $MME^{+/+}$ and $MME^{-/-}$ mice.
14. M. S. Dunnill, *Thorax* **17**, 320 (1962). *Lm*, an indicator of air space size, was calculated for each mouse from 15 random fields at ×200 by means of a 50-line counting grid (10-mm total length). The results are the average of measurements done by two independent investigators.
15. To determine alveolar duct enlargement, we measured the proximal surface areas from the terminal bronchiole–alveolar duct transition extending 250 μm into the duct using Optimus 5.2 image analysis software (Optimus, Bothell, WA). An average of 12 ducts per mouse fit this criteria for the 40 mice (four groups of 10 mice).
16. P. E. Desrochers, J. J. Jeffrey, S. J. Weiss, *J. Clin. Invest.* **87**, 2258 (1991); U. I. Sires, G. Murphy, H. G. Welgus, R. M. Senior, *Biochem. Biophys. Res. Commun.* **204**, 613 (1994).
17. We quantified alveolar and interstitial macrophages by counting macrophages identified by murine Mac-3 [rat antibody to mouse (0.5 mg/ml), used at 1:4000 dilution; PharMingen, San Diego, CA] immunostaining using the avidin-biotin alkaline phosphatase technique with 3,3′-diaminobenzidine as the chromogenic substrate. Neutrophils were identified by hematoxylin and eosin. Cell counts were determined by two independent investigators counting 10 random fields each at ×400.
18. We thank T. J. Ley and H. G. Welgus for helpful advice, R. McCarthy and D. Young of our transgenic/gene disruption laboratory, and D. O'Neal and C. Sandford for performing the smoking protocol. Supported in part by NIH, an American Lung Association Career Investigator Award (S.D.S.), the Alan A. and Edith Wolff Charitable Trust, and the Marc and Marjorie Seldin Endowed Fund for Pulmonary Research.

18 June 1997; accepted 12 August 1997