SerpinB1 deficiency is not associated with increased susceptibility to pulmonary emphysema in mice

Tiziana P. Cremona,1,4 Stefan A. Tschanz,2 Christophe von Garnier,3 and Charaf Benarafa1

1Theodor Kocher Institute, University of Bern, Bern, Switzerland; 2Institute of Anatomy, University of Bern, Bern, Switzerland; 3Respiratory Medicine, Berne University Hospital and Department of Clinical Research, University of Bern, Bern, Switzerland; and 4Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland

Submitted 10 July 2013; accepted in final form 18 October 2013

THIS YEAR MARKS the 50th anniversary of the landmark study by Laurell and Eriksson (33) that describes the association between reduced levels of the plasma protein α1-antitrypsin (AAT) and early development of severe pulmonary emphysema. The characterization of AAT as a major inhibitor of neutrophil elastase and the replication of features of early-onset of severe emphysema in human (7). AAT is a prototypic member of the serpin superfamily, which in human includes 50 different proteins with varying regulatory functions. Serpins are proteins with a conserved three-dimensional fold including an exposed reactive center loop (RCL) that acts as a bait for proteases. Cleavage of the RCL by target proteases triggers a fast conformational change in the serpin structure that results in the formation of an irreversible covalent complex between the protease and the serpin, thereby inactivating both proteins (27, 47). Like AAT, SERPINB1 is a highly potent inhibitor of neutrophil serine proteases, and the two serpins have comparable kinetics of inhibition against NE, PR-3, and cathepsin G (CG) (14). SERPINB1 (also known as MNEI, monocyte neutrophil elastase inhibitor) is a member of the clade B serpins and was initially found in the cytosol and nucleus of monocytes and neutrophils, where it is expressed at high levels (4, 38, 40). In addition, SERPINB1 is expressed in lung epithelial cells and has been found in lung fluids of cystic fibrosis patients and in the lungs of premature infants (13, 15, 57). In such inflammatory conditions associated with neutrophil influx, it remains unclear whether SERPINB1 is actively secreted via an alternative caspase-1 dependent pathway (31) or whether it is released passively from dying cells. We have previously reported that mice deficient in serpinB1a, the mouse homolog of SERPINB1, have a severe defect in bacterial clearance and increased mortality following infection with mucoid and nonmucoid strains of Pseudomonas aeruginosa (5). This defective phenotype was associated with increased neutrophil protease activity in the lung fluids, increased neutrophil death, and severe inflammation. Furthermore, rats or serpinB1a-deficient mice treated with recombinant SERPINB1 showed increased Pseudomonas clearance and reduced associated inflammation (5, 55). In addition, serpinB1a-deficient mice showed increased lung injury, morbidity, and mortality following influenza A virus infection (20). These findings demonstrate that SERPINB1 is a critical regulator of neutrophil proteases during lung infection. On the basis of this evidence, we postulated that SERPINB1 may also play a physiological
role in the lungs in noninfectious conditions. To test the hypothesis that SERPINB1 protects against the development of emphysema, we investigated the pulmonary function and lung structural features of serpinB1a-deficient (KO) mice during aging and after chronic exposure to secondhand cigarette smoke.

**MATERIALS AND METHODS**

*Mice and cigarette smoke exposure model.* SerpinB1a-deficient (KO) mice were generated previously in 129S6/SvEv background. Cigarette smoke was generated by a microprocessor-controlled Teague TE10z smoking machine (Teague Enterprises, Davis, CA) connected to whole-body exposure chambers. 3R4F reference cigarettes (University of Kentucky, Lexington, KY) were stored at 4°C until 48 h prior to use and then placed into a closed container at 23°C containing a glycerin/water mixture to maintain a 60% relative humidity. WT and KO adult female mice (3 mo old) were randomized in two groups: exposed either to filtered room air (ctr) or to cigarette smoke (smk) 5 h/day, 5 days/wk for 6 mo. To gradually acclimatize the mice to cigarette smoke exposure, mice were exposed for 2 h/day for 2 days in the first week and then 2 h/day for 5 days in the second week of exposure. To model exposure to secondhand cigarette smoke, a mixture of mainstream (11%) and sidestream (89%) smoke was used and the total suspended particulate density was maintained at 100 ± 6 mg/m³. Carboxyhemoglobin (Co-Hb) levels were measured using a cobas b 123 POC system (Roche). Typically, blood CO-Hb levels were 4.9 ± 0.3% in smokers 1 h after exposure and returned to baseline (2.9 ± 0.2%) 24 h later. All mice were weighed at the start and every 14 days thereafter. At the indicated time points, separate subgroups of mice were used for lung morphometry, for biochemical assays, and for pulmonary function tests. All animal studies were approved by the Cantonal Veterinary Office of Bern and conducted in accordance with the Swiss federal legislation on animal welfare.

*Lung fixation, sampling method, and processing.* Subgroups of 5–10 mice were used for stereological analysis of the lungs according to the ATS/ERS guidelines (26). Lungs were instilled with 1.5% paraformaldehyde-1.5% glutaraldehyde in 0.15 M HEPES pH 7.35 through a tracheal cannula at a constant pressure of 20 cm H2O. The trachea was ligated and lungs were placed in fresh fixative for at least 24 h to complete fixation. Total lung volume was determined by the water displacement method (42). Fixed lungs were embedded in toto in 2.5% agar and cut in 10–12 transversal slices of 1.2 mm. Systematic, uniform random sampling was used to pick six lung pieces representative of the whole organ. Briefly, lung slices were ordered from the largest to the smallest and each odd or even piece was picked by using a random start. Following paraffin embedding, 4-μm sections were cut and stained with hematoxylin and eosin. The acinar structures of the lung can be considered to be isotropic (26). Although all sections were taken from parallel slices, our sampling method is representative of the whole organ. Briefly, lung slices were ordered from the largest to the smallest and each odd or even piece was picked by using a random start. Following paraffin embedding, 4-μm sections were cut and stained with hematoxylin and eosin. The acinar structures of the lung can be considered to be isotropic (26). Although all sections were taken from parallel slices, our sampling method is representative of the whole organ. Briefly, lung slices were ordered from the largest to the smallest and each odd or even piece was picked by using a random start. Following paraffin embedding, 4-μm sections were cut and stained with hematoxylin and eosin. The acinar structures of the lung can be considered to be isotropic (26). Although all sections were taken from parallel slices, our sampling method is representative of the whole organ. Briefly, lung slices were ordered from the largest to the smallest and each odd or even piece was picked by using a random start. Following paraffin embedding, 4-μm sections were cut and stained with hematoxylin and eosin. The acinar structures of the lung can be considered to be isotropic (26). Although all sections were taken from parallel slices, our sampling method is representative of the whole organ. Briefly, lung slices were ordered from the largest to the smallest and each odd or even piece was picked by using a random start. Following paraffin embedding, 4-μm sections were cut and stained with hematoxylin and eosin. The acinar structures of the lung can be considered to be isotropic (26). Although all sections were taken from parallel slices, our sampling method is representative of the whole organ. Briefly, lung slices were ordered from the largest to the smallest and each odd or even piece was picked by using a random start. Following paraffin embedding, 4-μm sections were cut and stained with hematoxylin and eosin. The acinar structures of the lung can be considered to be isotropic (26). Although all sections were taken from parallel slices, our sampling method is representative of the whole organ. Briefly, lung slices were ordered from the largest to the smallest and each odd or even piece was picked by using a random start. Following paraffin embedding, 4-μm sections were cut and stained with hematoxylin and eosin. The acinar structures of the lung can be considered to be isotropic (26). Although all sections were taken from parallel slices, our sampling method is representative of the whole organ. Briefly, lung slices were ordered from the largest to the smallest and each odd or even piece was picked by using a random start. Following paraffin embedding, 4-μm sections were cut and stained with hematoxylin and eosin. The acinar structures of the lung can be considered to be isotropic (26). Although all sections were taken from parallel slices, our sampling method is representative of the whole organ. Briefly, lung slices were ordered from the largest to the smallest and each odd or even piece was picked by using a random start. Following paraffin embedding, 4-μm sections were cut and stained with hematoxylin and eosin. The acinar structures of the lung can be considered to be isotropic (26). Although all sections were taken from parallel slices, our sampling method is representa...
Protease activity assays. Enzymatic activity of NE, CG, and PR-3 in BAL and lung homogenates was measured in a standard colorimetric assay in 20 mM Tris, pH 7.4, 500 mM NaCl, 0.05% Tween-20, and 4 mM DTT by using MeO-Suc-Ala-Ala-Pro-Val-pNA, MeO-Suc-Ala-Ala-Pro-Phe-pNA, and N-Boc-Ala-ONp as substrates (500 μM final), respectively. Neutrophil lysates from WT and neutrophil protease-deficient mice (Cxcr4<sup>−/−</sup>) (1) were used as positive and negative controls, respectively.

Statistical analysis. All analyses were performed with GraphPad Prism 6.0c by unpaired Student’s t-test or two-way ANOVA and Bonferroni posttest as indicated. A value of P < 0.05 was considered statistically significant.

RESULTS

Lung volume and pulmonary function in aging serpinB1a-deficient mice. To determine the role of serpinB1a in preventing the development of early-onset spontaneous emphysema, we measured lung volume and pulmonary function in young adult (3 mo) and older mice (15 mo). Mean body weight of groups of KO and WT mice gradually increased as a function of age in mice exposed to filtered room air up to 15 mo (Table 2). Lung volume also increased in 15-mo-old mice compared with younger mice (Fig. 1A). No difference in lung volume was observed between KO mice and age-matched WT mice at 5, 7, and 15 mo, indicating normal kinetics of age-related lung enlargement in serpinB1a-deficient mice (Fig. 1A). Pulmonary function test revealed decreased airway resistance, tissue damping, and elastance in 15-mo-old mice compared with 3-mo-old mice, but no significant difference was observed between WT and KO mice (Fig. 1, B–D).

Chronic cigarette smoke exposure in serpinB1a-deficient mice. We then examined the role of serpinB1a in cigarette smoke-induced emphysema. Groups of 3-mo-old WT and KO mice were exposed to secondhand cigarette smoke for 6 mo, while control mice were exposed to filtered room. Cigarette smoke exposure had a significant effect on body weight, as mice of both genotypes failed to gain weight compared with their corresponding control groups (Table 2). Absolute lung volume was significantly increased in cigarette smoke-exposed animals, suggesting structural changes in the lungs, but no difference was observed between the genotypes (Fig. 2A). Of note, the lung volumes of control mice (9-mo-old) were similar in WT and KO mice and consistent with their age (compare with data in Fig. 1A). Pulmonary function tests showed no significant changes in mice exposed to cigarette smoke for 6 mo compared with air-exposed mice and no difference was observed between genotypes (Fig. 2B–D). Values obtained for each parameter were in line with age-related decline shown in Fig. 1, B–D.

Lung stereology. Because pulmonary function tests in various strains of mice do not always correlate with morphological changes in lung structure after 6 mo of cigarette smoke injury (17, 22), we performed detailed stereological analysis of the lungs of WT and KO mice exposed to cigarette smoke for 6 mo and compared them with lungs of age-matched (9-mo-old) air-exposed mice. To measure the effects of age on lung structure, we also analyzed the lungs of 15-mo-old mice. Typically emphysema is structurally characterized by air space enlargement that is defined by increased alveolar volume and increased distance of interalveolar walls. These parameters are measured by reduced surface area density and increased Lm, respectively. Furthermore, emphysema generates a reduction of the diffusion surface area of the air-blood interface. Alveolar space enlargement was readily visible in lung histological sections of cigarette smoke exposed mice compared with controls (Fig. 3). Stereological analysis of lung sections of WT and KO mice exposed to cigarette smoke revealed statistically

---

Table 2. Effects of cigarette smoke exposure on body weight

<table>
<thead>
<tr>
<th>Age, mo</th>
<th>WT ctrl</th>
<th>WT smk</th>
<th>KO ctrl</th>
<th>KO smk</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>19.3 ± 0.4</td>
<td>19.7 ± 0.3</td>
<td>20.2 ± 0.3</td>
<td>19.3 ± 0.4</td>
</tr>
<tr>
<td>5</td>
<td>22.8 ± 0.6</td>
<td>20.2 ± 0.3*</td>
<td>20.4 ± 0.4</td>
<td>20.6 ± 0.5</td>
</tr>
<tr>
<td>7</td>
<td>24.2 ± 0.7</td>
<td>20.0 ± 0.3‡</td>
<td>22.2 ± 0.4</td>
<td>21.1 ± 0.4</td>
</tr>
<tr>
<td>9</td>
<td>24.6 ± 0.7</td>
<td>18.8 ± 0.3†</td>
<td>23.8 ± 0.6</td>
<td>20.9 ± 0.4*</td>
</tr>
<tr>
<td>15</td>
<td>27.5 ± 0.9</td>
<td>NA</td>
<td>27.3 ± 1.2</td>
<td>NA</td>
</tr>
</tbody>
</table>

Average body weight in grams of wild-type (WT) and SerpinB1a-deficient (KO) mice exposed to cigarette smoke (smk) or room air (ctrl) as function of age. At the age of 3 mo, mice of each genotype were randomly divided into 2 groups to be exposed or not to cigarette smoke. Cigarette smoke exposure ended 6 mo later (9 mo old). Data is shown as mean ± SE, N = 10–18 mice per group. "P < 0.01, †P < 0.001, ‡P < 0.0001 ctrl vs. smk within a genotype.

---

Fig. 1. Lung volume and pulmonary function of wild-type (WT) and SerpinB1a-deficient (KO) mice as a function of age. A: absolute lung volume at a constant instillation pressure of 20 cmH2O. Data were analyzed by 2-way ANOVA and Bonferroni posttest. "P < 0.001 indicates differences between old (15 mo old) mice compared with young mice (5 or 7 mo old) of the corresponding genotype. B–D: lung function analysis of young adult (3 mo) and old (15 mo) WT and KO mice. Each symbol represents value for 1 mouse (WT, circles; KO, triangles). Data are shown as means ± SE (N = 5–6 mice per group) and analyzed by 2-way ANOVA and Bonferroni posttest. **P < 0.01 for young vs. old mice within each genotype.
we observed a significant increase in air space volume compared with 9-mo-old mice (ctrl) (Fig. 4A). Of note, septal surface area had an upward trend in older mice and the surface area density was reduced but did not reach statistical significance (Fig. 4, B and C). Lm was increased in old WT but not KO mice compared with their respective control (9-mo-old) mice (Fig. 4D).

Taken together, these results indicate that cigarette smoke exposure and age were both associated with significantly increased air space volume, suggestive of reduced resistance to constant inflation pressure and/or destruction of the lung parenchyma. Of note, septal surface area in smoke-exposed and old mice varied in opposite directions compared with ctrl mice (Fig. 4B). Importantly, deficiency in serpinB1a was not associated with increased severity in the development of emphysema following cigarette smoke exposure. In fact and in contrast to our hypothesis, the structural changes in KO mice induced by cigarette smoke or age were generally subtly less severe than in WT mice.

**Pulmonary Inflammation.** To investigate differences in the inflammatory cell profile in lungs of WT and KO mice after chronic exposure to cigarette smoke, BAL cells were counted and analyzed on cytospins. Total cell numbers were not significantly altered in mice exposed to cigarette smoke compared with age-matched control groups but the percentages of lung neutrophils were increased in mice of both genotypes exposed to cigarette smoke. We found no difference between WT and KO mice (Fig. 5, A–C). In mice of both genotypes, we observed a profound change in the phenotypic appearance of macrophages of mice exposed to cigarette smoke with increased size, abundant vacuolization of the cytoplasm, and a brown coloration that was readily noticeable in the cell pellet after centrifugation. Multinucleated macrophages in the lungs have been reported as a measure of cumulative toxicity of inhaled compounds and cigarette smoke (6), and the percentage of multinucleated cells was proposed as a biological marker for semiquantitative evaluation of cigarette smoke exposure (39). We observed a significant increase in the percentage of multinucleated alveolar macrophages in mice exposed to cigarette smoke but no difference was observed between

![Fig. 2. Effects of cigarette smoke exposure for 6 mo on lung volume and pulmonary function. A: absolute lung volume at a constant instillation pressure of 20 cmH2O. B–D: lung function analysis of 9-mo-old WT and KO mice exposed to room air (ctrl) or to cigarette smoke for 6 mo (smk).](image)

**Fig. 3. Lung histopathology.** Representative micrographs of lung sections from 9-mo-old and 15-mo-old (old) WT and KO mice exposed to air or from 9-mo-old mice exposed to cigarette smoke for 6 mo. Note air space enlargement in mice exposed to smoke compared with control mice. Scale bars are 50 μm.
WT and KO mice (Fig. 5D). Reporting multinuclear cells may thus be used as an independent biological measurement of cigarette smoke exposure across laboratories where smoking regimen, exposure systems, and cigarette source may vary.

**Proteases and anti-proteases in the lungs.** To start investigate potential compensatory mechanisms in regulating neutrophil proteases in serpinB1a KO mice, we measured changes in mRNA expression levels of serine protease inhibitors in the lungs of mice exposed to cigarette smoke and age-matched controls. We found that cigarette smoke exposure led to a strong upregulation of serpinB1a as well as the mouse homologs of AAT (serpinA1a and serpinA1b) and SLPI in WT mice. In KO mice, cigarette smoke did not induce the upregulation of the homologs of AAT and SLPI (Fig. 6). In contrast, cigarette smoke induced higher expression of intracellular serpinB6a and serpinB6b in serpinB1a KO mice but not in WT mice (Fig. 6). We found no detectable expression of serpinB1b and serpinB6c in the lungs of mice of both genotypes (not shown). Because AAT is mainly expressed in liver and...
secreted in plasma, we next determined AAT levels in serum and BAL and found no significant difference between genotypes and no changes following chronic cigarette smoke exposure (Fig. 7, A and B). As expected, levels in serum were several orders of magnitude higher than in BAL. Our findings suggest that although AAT mRNA expression was induced severalfold by cigarette smoke in WT mice this increase in local mRNA transcription was not reflected in higher levels of AAT protein in BAL. Finally, we measured the activity of the neutrophil serine proteases inhibited by serpinB1 in lungs. Although no protease activity was detected in BAL, cell-free NE activity was slightly but not significantly increased in the lungs of WT and KO mice after chronic smoke exposure (Fig. 7C). CG activity was not altered by cigarette smoke exposure (Fig. 7D), and PR-3 was not detected in lung tissue homogenates. Protease activity was not statistically different between WT and KO mice, although NE activity appeared slightly lower in KO mice. These results indicate that cigarette smoke exposure differentially upregulates the expression of both secreted and intracellular protease inhibitors in the lungs of WT and KO mice. However, these changes did not result in significant changes in extracellular protease activity in the lungs.

DISCUSSION

Since SERPINB1 and AAT have a uniquely shared feature of inhibiting the three neutrophil serine proteases NE, CG, and PR-3 with high efficiency, we postulated that serpinB1a deficiency may be an alternative approach to generate a protease-inhibitor imbalance model in the lungs leading to early-onset emphysema. Indeed, we previously showed increased proteolysis of surfactant protein-D in lungs of serpinB1a-deficient mice during Pseudomonas infection (5). Furthermore, expression of serpinB1a mRNA was found to be downregulated in alveolar macrophages of mice overexpressing interleukin-13 in the lung and of integrin-β6-deficient mice, which both develop emphysema spontaneously, implying a potential causative link (54). However, although we observed an age-dependent decline in lung function associated with increased lung volume, we did not measure significant changes in pulmonary function and structure in young adult and old serpinB1a-KO mice compared with age-matched control mice. In addition, we did not observe any significant difference in serine protease activity and in protease inhibitor expression levels in lungs and in BAL resident cell numbers between WT and KO mice in the absence of cigarette smoke exposure. Overall, our findings indicate that serpinB1 is not required for normal lung development and aging in noninflammatory state.

Chronic obstructive pulmonary disease (COPD) is a complex disease entity and many attempts have been made to model it in vivo with variable success using different triggering...
stimuli, animal species and genetic approaches (35, 56). Despite its known shortcomings in faithfully reproducing all features of COPD and despite the practical difficulties associated with the time to develop the disease, chronic exposure to cigarette smoke in mice for 6 mo has become a prevalent model (11, 19). In our exposure model, cigarette smoke induced a significant increase in total lung volume measured by fluid displacement, and stereological analysis revealed concurrent statistically significant increase in air space volume and Lm and, importantly, a significant decrease in surface area density of the lungs. We also measured a decrease in surface area but the reduction failed to reach statistical significance. In old mice, total lung volume was highly increased but the reduction in surface septal density was only marginal and, most surprisingly, the absolute surface area showed an increased trend. Mechanisms leading to increased airway volume are therefore different in chronic smoke injury and aging. Our results indicate that our model of chronic smoke exposure induced a mild form of emphysema and that the nature of structural changes observed in old mice may not be associated with overwhelming emphysematous changes.

Following chronic cigarette smoke exposure, we failed to observe significantly worsened smoke-induced emphysema in serpinB1a KO mice compared with WT mice. The most plausible interpretation for the absence of a more severe disease would be the relatively lower importance of intracellular protease inhibitors compared with AAT and other secreted protease inhibitors in protecting the lung matrix proteins from proteolytic degradation in this model. Indeed, low AAT levels in certain mouse strains have been correlated with increased susceptibility to develop emphysema (10, 48). Similarly, treatment with purified human AAT partly protected against increased Lm and matrix protein degradation induced by cigarette smoke exposure (12, 16). However, reproducing AAT deficiency in mice by reverse genetic approaches proved to be challenging because multiple functional homologs were found in mice (8, 21), and deletion of serpinA1a was associated with embryonic lethality (32, 53). We found no difference in AAT levels in BAL and serum of WT and KO mice and no changes were induced by cigarette smoke exposure. These results should, however, be interpreted with caution since ELISA measurements may not differentiate between active and inactive oxidized forms of AAT that are generated by cigarette smoke (9). Future attempts at targeting AAT in mice should include a better characterization of the mouse AAT genes and proteins and, importantly, conditional knockout strategies should be designed for deletion after birth and/or in a tissue-specific manner.

An alternative explanation for the lack of increased severity of emphysema development in serpinB1a KO mice after cigarette smoke exposure may be linked to the reduced numbers of neutrophils in the bone marrow reserve of serpinB1a KO mice. Neutrophils are protected by serpinB1 from a cell-intrinsic death pathway mediated by CG, which induces neutrophil death both upstream and independently of caspases (2, 4). In highly acute neutrophil inflammation caused by bacterial and viral infections, we showed that reduced neutrophil survival led to an overwhelming inflammation, increased tissue injury, and, ultimately, increased mortality in serpinB1a KO mice (5, 20). In the emphysema model, neutrophil recruitment is considerably lower than in infectious settings, and the rapid demise of neutrophils may ultimately be less damaging, and thus, not associated with increased lung damage in KO mice after cigarette smoke exposure. Accordingly and in contrast with infection models characterized by massive neutrophil recruitment, we found no significant increase in cell-free protease activity in the lungs of KO mice. The finding that serpinB1 is cytoprotective in neutrophils may also be relevant because a role for AAT in inhibiting cell apoptosis via interaction with caspase-3 has been proposed as a contributing mechanism during emphysema in AAT deficiency (36, 37). However, molecular mechanisms of inhibition and pathways leading to localization of AAT in the cytoplasm, where caspase-3 is located, have not been entirely elucidated. Interestingly, we have shown here that expression of AAT homologs, as well as SLPI and serpinB1a, was strongly increased in the lungs of WT mice following smoke exposure. This upregulation was blunted or absent in serpinB1a KO mice and may be compensated in part by the increased expression of two serpinB6 homologs, which also inhibit CG. This is reminiscent of the absence of phenotype of serpinB6a KO mice, which have a strong compensatory increased expression of serpinB1a in neutrophils (41). Of note, we found no detectable expression of serpinB1b and serpinB6c in the lungs of mice of both genotypes. SerpinB1b inhibits CG but is cleaved by NE and PR-3 (3) and the activity of serpinB6c has yet to be determined. Further work is required to determine how intracellular and secreted serpins regulate death pathways in cells that do not express known target proteases. A striking example is the recent discovery that human subjects and mice lacking serpinB6 develop a gradual hearing loss associated with the degeneration of cells in the inner ear (46, 49). In conclusion, our study shows that serpinB1a alone is not essential in protecting the lungs in steady state or following chronic exposure to secondhand cigarette smoke. More work is required to understand the relative function of protease inhibitors in the lungs and to establish a model of protease-antiprotease imbalance leading to early-onset emphysema in mice.

ACKNOWLEDGMENTS

The authors acknowledge the excellent technical assistance of Patrizia Castiglioni, Beat Haenni, and Marianne Hofstetter. We are grateful to Petra Hirschi for CO-Hb measurements and to Paola Basilico for help in handling the mice. We thank Martin Zwahlen and Hansueli Zürcher for help in the maintenance of the smoking exposure system. Images were acquired on a device supported by the Microscopy Imaging Center of the University of Bern. We thank Eileen Remold-O’Donnell and Peter Gehr for critical reading of the manuscript and Ewald Weibel for insightful discussions.

GRANTS

This work was supported by grants to C. Benarafa from the Flight Attendant Medical Research Institute (FAMRI), the Swiss National Science Foundation (310030-127464), and the EU/FP7 Marie Curie International Reintegration grant.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


AJP-Lung Cell Mol Physiol • doi:10.1152/ajplung.00181.2013 • www.ajplung.org
REFERENCES


11. Caruthers AM, Raptis SZ, Kelley DG, Pham CTN.


47. **Stratikos E, Gettins PG.** Formation of the covalent serpin-proteinase complex involves translocation of the proteinase by more than 70 Å and full insertion of the reactive center loop into beta-sheet A. *Proc Natl Acad Sci USA* 96: 4808–4813, 1999.


