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Induction and regulation of murine emphysema by elastin peptides

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1EA4683, SFR CAP-Santé, Université de Reims Champagne-Ardenne, Reims, France; 2Plateforme d’Imagerie Cellulaire et Tissulaire, SFR CAP-Santé, URCA, Reims, France; and 3Laboratoire d’Anatomie et de Cytologie Pathologiques, CHU R. Debré, Reims, France

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Sellami M, Meghraoui-Kheddar A, Terryn C, Fichel C, Boulard N, Diebold M, Guenounou M, Héry-Huynh S, Le Naour R. Induction and regulation of murine emphysema by elastin peptides. Am J Physiol Lung Cell Mol Physiol 310: L8–L23, 2016. First published October 30, 2015; doi:10.1152/ajplung.00068.2015.—Emphysema is the major component of chronic obstructive pulmonary disease (COPD). During emphysema, elastin breakdown in the lung tissue originates from the release of large amounts of elastase by inflammatory cells. Elevated levels of elastin-derived peptides (EP) reflect massive pulmonary elastin breakdown in COPD patients. Only the EP containing the GXPG conformational motif with a type VIII β-turn are elastin receptor ligands inducing biological activities. In addition, the COOH-terminal glycine residue of the GXPG motif seems to be a prerequisite to the biological activity. In this study, we endotracheally instilled C57BL/6J mice with GXPG EP and/or COOH-terminal glycine deleted-EP whose sequences were designed by molecular dynamics and docking simulations. We investigated their effect on all criteria associated with the progression of murine emphysema. Bronchoalveolar lavages were recovered to analyze cell profiles by flow cytometry and lungs were prepared to allow morphological and histological analysis by immunostaining and confocal microscopy. We observed that exposure of mice to EP elicited hallmark features of emphysema with inflammatory cell accumulation associated with increased matrix metalloproteinases and desmosine expression and of remodeling of parenchymal tissue. We also identified an inactive COOH-terminal glycine deleted-EP that retains its binding-activity to EBP and that is able to inhibit the in vitro and in vivo activities of emphysema-inducing EP. This study demonstrates that EP are key actors in the development of emphysema and that they represent pharmacological targets for an alternative treatment of emphysema based on the identification of EP analogous antagonists by molecular modeling studies.

murine model; elastin peptides; parenchyma inflammation; molecular modeling

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) is a major worldwide respiratory health problem (58) resulting in chronic airway inflammation and pulmonary emphysema. Emphysema is characterized by air space enlargement and alveolar extracellular matrix destruction (19). The COPD-associated inflammatory process is characterized by the secretion of large amounts of proteases by lung-infiltrating inflammatory cells (44). Some proteases such as neutrophil elastase, matrix metalloproteinases (MMP)-2, and MMP-9 promote breakdown of elastin. An increased secretion of desmosine, a specific marker for elastin degradation (20, 37), and elevated levels of elastin peptides (EP) in various biological fluids, reflect massive pulmonary elastin breakdown in COPD patients (5, 14, 49).

EP display a wide range of biological activities (3, 7, 13, 18, 51). These biological activities can be attributed to the VGVAPG hexapeptide, which is the major ligand for the high-affinity binding site of the elastin receptor (21, 48). The elastin-receptor complex is a heterodimer consisting of the 67-kDa elastin-binding protein (EBP), which is a spliced variant of human β-galactosidase designed as spliced galactosidase (S-gal) (23, 24, 46). Among EP, only those containing the GXPG conformational motif such as VGVAPG (3, 51), PGAIPG (7, 22), GVAPGV (7), and GLVPG (6) are EBP ligands inducing biological activities. Using molecular dynamic stimulations, we and others demonstrated that biologically active EP have a high propensity to form a type VIII β-turn in the GXXP motif, which modulates anchoring to EBP (16, 17, 39, 40). The presence of a COOH-terminal glycine residue following the GXXP motif seems to be a prerequisite to the biological activity (7).

In rodents, pulmonary exposure to porcine pancreatic elastase (PPE) induces acute lung inflammatory response and parenchyma destruction, impairing lung function as observed in human emphysema (25, 53, 55). PPE-induced emphysema is associated with elastin breakdown in vivo (31). Macrophage accumulation and development of air space pathology are suppressed in PPE-recipient mice treated with monoclonal antibodies directed against elastin fragments (25). In addition, intratracheal injection of the VGVAPG peptide induces a migration of peripheral blood monocytes to murine lungs (25). Collagen synthesis is further altered during elastase-induced emphysema (30).

In this study, we postulated that pulmonary exposure of wild-type (WT) mice to biologically active XGXXPG EP could induce the typical features of emphysema and that this emphysema could be modulated with use of EP analogous antagonists. We carried out molecular modeling calculations to design analogous EP obtained by deletion of the COOH-terminal glycine while keeping the high propensity of the GXXP sequence to fold as a type VIII β-turn. We observed that exposure of mice to active EP elicited hallmark features of...
emphysema, and we identified an analogous EP susceptible to antagonize the elastin peptide/elastin receptor interactions.

**MATERIALS AND METHODS**

**Animal procedure.** Six-week-old C57BL/6j mice purchased from Harlan Sprague Dawley were housed in an animal facility that was maintained at 22–26°C with 40–69% humidity and a 12-h light-dark cycle. Animals were fed with a commercial diet and received water ad libitum. Animals were humanely cared for, and all animal experiments were approved by the Committee for Animal Research at University of Reims Champagne-Ardenne. All protocols involving mice were approved by the University of Reims Champagne-Ardenne Institutional Animal Care and Use Committee and were carried out in accordance with institutional guidelines and regulations. Mice were anesthetized intraperitoneally with ketamine (50 mg/kg) and xylazine (5 mg/kg). After appropriately sedation, mouse lungs were endotracheally instilled with either PBS, one unit (7.4 μg) PPE (Sigma-Aldrich, Saint-Quentin Fallavier, France), 10 μg VGVAPG (Genepep, Saint Jean de Védas, France), 10 μg VVGPGA (random permutation of the VGVAPG peptide, Genepep), 10 μg PGAIPG (Genepep), 10 μg PGAIP (Genepep), or 10 μg GPPIA (random permutation of the PGAIP peptide, Genepep) via a 22-gauge intravenous catheter in a total volume of 50 μl PBS. PPE was chosen as a positive inducer in the study because previous data have shown that it induced pulmonary emphysema in mice (35, 52). Animals were euthanized by CO2 inhalation on days 1, 3, 7, 14, and 21. In some experiments, we coadministered PGAIP to VGVAPG- or to PPE-reipient mice to evaluate the capacity of PGAIP to neutralize the VGVAPG- or PPE-induced pulmonary damage. In these experiments, animals received either a single mixture of VGVAPG (10 μg) and PGAIP (10 μg) or PPE (10 μg) and PGAIP (10 μg) at time 0 and were euthanized on the 21st day. Control mice received a mixture of either VGVAPG (10 μg) and GPPIA (10 μg) or PPE (7.4 μg) and GPPIA (10 μg). In these different experimental approaches, bronchoalveolar lavage (BAL) fluids were recovered to analyze cell profiles and to determine MMP activities and desmosine concentrations. After BAL fluid collection, the lungs were inflated, removed, and either embedded in paraffin to allow histological and morphological analysis or frozen to analyze cell infiltration in parenchyma. In some experiments, peripheral blood of mice was also collected by cardiac puncture to perform circulating neutrophil isolation.

**Synthesis, purification, and characterization of EP.** Synthesis of the elastin peptides used in this study (VGVAPG, PGAIPG, GGVPG, VVGPGA, PGAIP, VGVAP, and GPPIA) were performed by Genepep (Genepep). Crude peptides were purified by reverse-phase HPLC on a semiprepared C18-bonded silica column by use of a Shimadzu SPD 10A UV/VIS detector, with detection at 210 and 254 nm. The column was perfused at a flow rate of 3 ml/min with solvent A [10%, vol/vol, water in 0.1% aqueous trifluoroacetic acid (TFA)], and a linear gradient from 10 to 90% of solvent B (80%, vol/vol, acetonitrile in 0.1% aqueous TFA) over 40 min was adopted for peptide elution. Analytical purity and retention time of each peptide were determined under HPLC conditions in the above solvent system (solvents A and B). All analogous peptides showed >98% purity when monitored at 215 nm. Homogeneous fractions, as established by analytical HPLC, were pooled and lyophilized. Peptides molecular weights were determined by electrospray ionization mass spectrometry (ESI-MS). ESI-MS analysis was made in positive ion mode. The sample was dissolved in a mixture of water and methanol (50/50) and injected directly into the electrospray source, using a syringe pump, which maintains constant flow at 5 μl/min. The temperature of the capillary was set at 220°C. All peptides used in this study were solubilized or diluted either in PBS or RPMI.

**Bronchoalveolar lavage.** The trachea was inserted with a 22-gauge intravenous catheter, and the whole lungs were washed three times with 1 ml PBS. BAL fluid was centrifuged at 900 g for 10 min at +4°C, and the supernatant was collected and stored at −80°C until required. Cell pellet was resuspended in 1 ml of PBS, and cell count was performed. Cell viability was more than 95% as determined by Trypan blue exclusion. Aliquots of cells were then stained with fluorescent mAb specific for neutrophils (Ly-6G, BD Biosciences, Le pont de Claix, France) or macrophages (Mac-3, BD Biosciences). Flow cytometry analysis was performed and absolute number in cell populations was calculated.

**Lung tissue preparation.** The lungs were inflated for 10 min at a constant pressure of 25 cmH2O in the presence of either 10% neutral buffered formalin solution (Sigma-Aldrich) or PBS and then removed. Formalin-inflated lungs were fixed in 4% paraformaldehyde (PFA) for 24 h at room temperature before embedding in paraffin. Then transverse 3-μm or 20-μm sections of lung were cut for morphological or collagen and elastin network analysis, respectively. PBS-inflated lungs were frozen in liquid nitrogen to analyze cell infiltration by immunofluorescent staining from cryosections.

**Peripheral blood collection.** Peripheral blood was collected from the abdominal vena cava of mouse by use of a heparinized syringe and transferred into dextran (3 ml, 1.25% wt/vol in saline). Pooled blood from six to eight mice was used to perform neutrophil isolation. Tubes were then filled to a total of 10 ml dextran solution and inverted. Erythrocytes were allowed to sediment for 30 min at room temperature and the leukocyte-rich supernatant was collected. Peripheral blood neutrophils were isolated from leukocyte suspension by using a density gradient medium (OptiPrep, Sigma-Aldrich, Saint-Quentin Fallavier, France) modified to obtain a hypoomotic 1.077 g/ml density barrier. After centrifugation at 700 g for 20 min at room temperature, the liquid above the pellet was discarded and residual erythrocytes were lysed by ice-cold lysing buffer (1.5 mM NH4Cl and 0.1 mM KHCO3). Neutrophils were washed of erythrocyte debris and resuspended in PBS. Neutrophils were shown to be >98% by flow cytometry analysis (Ly-6G, BD Biosciences) and cell viability was over 95% as determined by Trypan blue exclusion (data not shown).

**Flow cytometry analysis.** Blood neutrophils and isolated BAL fluid cells were incubated with 1 μg/ml phycoerythrin (PE)-conjugated anti-mouse Ly-6G (clone 1A8, BD Biosciences, Le pont de Claix, France) or with a mixture of 1 μg/ml FITC-conjugated anti-mouse Mac-3 (clone M3/94, BD Biosciences) and 1 μg/ml PE-conjugated anti-mouse Ly-6G (clone 2IC, BD Biosciences), respectively. After incubation at room temperature for 20 min in the dark to prevent fluorescence quenching, cells were washed twice (400 g, 10 min) and then resuspended in 500 μl PBS containing 1% PFA. In parallel, isotype-matched controls were used to determine nonspecific labeling. Fluorescence emission was assessed by flow cytometric analysis using an LSRFortessa cell analyzer (BD Biosciences).

**Morphological analysis.** Serial midsagittal sections (3 μm thick) stained with hematoxylin phloxin saffron were used for morphological analysis. Ten randomly selected (×100) fields per slide were photographed by use of the ZEN imaging software (Carl Zeiss, Marly-Le-Roi, France). The images were analyzed with the Image J software (open source). From each fields 10 areas of interest, free of airways and muscular blood vessels, were picked for measurement of the number of intersections of virtual lines of known length, with alveolar septa. An increase in the average distance between intercepts (mean linear intercept) indicates enlarged air spaces.

**Collagen and elastin network acquisition method.** Serial unstained midsagittal sections (20-μm thick) were used for the simultaneous visualization of collagen [second-harmonic generation (SHG)] and elastin [two photons excited fluorescence (2PEF)] in lung tissue section based on collection of SHG and 2PEF signals. We used a laser scanning Carl Zeiss microscope LSM 710 NLO (Carl Zeiss) merged with the ZEN imaging software (Carl Zeiss). Excitation was provided by a CHAMELEON femtosecond Titanium-Sapphire laser (Coherent, Courtaboeuf, France) set at 860 nm with 15% power. Samples were imaged with a ×63, 1.4-NA oil immersion objective lens. The emitted SHG and 2PEF signals generated from the sample were collected in

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the backward direction with two separate detectors by using a bandpass filter 420–440 nm and a bandpass filter 450–751 nm, respectively.

Collagen and elastin network quantification. Volume ratios of elastin and collagen structures were computed in term of the voxel volumes as previously described (1, 34). The volume fraction estimation of elastin and collagen structures was performed after 3D reconstruction images from 2PEF/SHG data sets processing with the Imaris software (Bitplane, Zurich, Switzerland). The volume fraction estimation was calculated from three different pulmonary tissue areas of six mice from each of the following groups: (1) control mice (PBS, VVGPGa); (2) mice that received PGE, VVGAPG, PgaIA, or gpPIa; and (3) mice coinfused with VGVAPG and PAGP or PGE and PGAIP. The depth of the tissues subjected to the analysis was 20-μm thickness. The ratio index, calculated to evaluate the elastin destruction in lung tissue, is defined as follows (34): ratio index = (Ev − Cv)/(Ev + Cv), where Ev and Cv represent elastin and collagen voxel volumes, respectively. This ratio index approaches maximum value of +1 when only elastin is present, and it approaches minimum value of −1 when only collagen is present. This ratio index therefore provides an estimation of the extent of ECM remodeling in terms of collagen and elastin voxel volumes in the lung tissues undergoing emphysema destruction.

Immunofluorescent staining. Lung-infiltrating macrophages and neutrophils were stained for 1 h at room temperature with rat antimouse macrophage marker (clone ER-MP58, Santa Cruz Biotechnology, Heidelberg, Germany) and rat anti-mouse LY-6G (clone M-66, Santa Cruz Biotechnology) primary antibodies, respectively. Slices were then rinsed twice with PBS and incubated with biotin-conjugated goat anti-rat secondary antibodies (Jackson ImmunoResearch Laboratories, Suffolk, UK). One hour after incubation at room temperature, slices were rinsed again before incubation with Alexa Fluor 488-conjugated streptavidin (1:100 dilution; Molecular Probes). Nuclei were stained with Hoechst and lung sections were counterstained with Harris hematoxylin and mounted with antifading solution (Biovaley, Marne-La-Vallée, France) onto glass slides. Slices were observed with an Axioptot fluorescence microscope (Carl Zeiss) and counted from 10 different high-powered ×40 fields per slide.

MMP analysis. The gelatinolytic activity of MMP-2 and MMP-9 and the MMP-12 expression in BAL fluids were, respectively, measured by gelatin zymography and Western blot as previously described (4, 43). For gelatin zymography, BAL fluids (10 μl) were eluted in nonreducing sample buffer (Bio-Rad, Marne-La-Coquette, France) and subjected to electrophoresis in 10% SDS-polyacrylamide gels containing 1 mg/ml of gelatin as substrate. Following electrophoresis, the gels were washed twice in 2.5% Triton X-100 for 1 h at room temperature and further incubated at 37°C overnight in 50 mM Tris-HCl containing 5 mM CaCl2 and 200 mM NaCl (pH 7.6). Proteolytic activity was visualized by staining the gels with 0.1% Coomassie blue containing 30% propanol-2 and 10% acetic acid, followed by destaining in 7.5% acetic acid and methanol. MMPs gelatinolytic activities were evidenced as white zones of lysis. Gelatinase activities were quantified by an automated image analyzer (Vulibier-Lourmat, Collégien, France). Linear range of enzyme activity was assessed by using recombinant MMP-2 and MMP-9 as standard. For Western blot analysis, equal amounts of proteins contained in BAL fluids were heated for 5 min at 100°C in Laemmli sample buffer, separated by 10% SDS-PAGE under reducing conditions (dithiothreitol), and transferred onto nitrocellulose membrane (Immobilon P, Millipore, Molsheim, France) with blotting apparatus (100 V, 110 min, Bio-Rad). Even transfer was verified by Ponceau staining. Membranes were blocked for 1 h at room temperature with buffer containing 5% nonfat milk in Tween-Tris-buffered saline (50 mM Tris, pH 7.5, 150 mM NaCl, and 0.1% Tween 20). The blots were incubated overnight at +4°C with antibodies raised against MMP-12 (1:250, Santa Cruz). After washing with Tween-Tris-buffered saline, the blots were incubated with horseradish peroxidase-coupled anti-goat antibodies (1:1,000, Santa Cruz Biotechnology) for 1 h at room temperature and then washed again with Tween-Tris-buffered saline. Blots were developed by chemiluminescence (Kit ECL/RPN 2069, Amersham Pharmacia Biotech, South Shield, UK).

Desmosine quantification. Determination of desmosine concentrations in BAL fluids was performed in triplicate by using a commercially available ELISA kit (Cusabio Biotech, Interchim, Montluçon, France) according to manufacturer’s instructions. The sensitivity of ELISA kit was 0.78 pg/ml.

Neutrophil chemotaxis assay. Neutrophil chemotactic activity was determined by using a Boyden chamber method as previously described (14). Briefly, 27 μl of RANTES (Regulated on Activation, Normal T Cell Expressed and Secreted) (0.1 μg/ml), VGVAPG (0.1 and 1 μg/ml), or PGAIP (0.1 μg/ml) were added to the lower wells of a 48-well Boyden chamber (Neuro Probe, Warwick, UK) and covered with a 5-μm polycarbonate filter (VWR International, Fontenay-sous-Bois, France). Then 50 μl of neutrophil suspension (5 × 105 cells/ml) were added to the upper wells of the chamber and incubated for 45 min at 37°C in 5% CO2. Migrating cells, fixed and stained with eosin and bromophenol blue at the lower part of the filter, were counted by microscopy in five randomly selected high-power (×400) fields per well. In some experiments, pretreatment of neutrophils for 1 h with 10 mM lactose (Sigma-Aldrich), a galactosugar known to induce S-Gal shedding from cell surface (38), was used to assess the specificity of VGVAPG effects. In some others, various concentrations of PGAIP (10 to 0.001 μg/ml) were used either for pretreatment of neutrophils 1 h before chemotaxis assay toward VGVAPG (0.1 μg/ml) or for coincubation with VGVAPG (0.1 μg/ml) during neutrophil chemotaxis assay. All experiments were performed in triplicate and results are reported as the mean number of cells per field.

Molecular dynamic simulations. All the molecular dynamic (MD) simulations were performed by using the c37b1 version of the CHARMM program (8, 9), with the potential function parameter set 22 (36). MD simulations were performed at 300 K by the Verlet algorithm with an integration time step of 2 fs, as bonds involving hydrogen atoms were constrained by the SHAKE algorithm (47). All atoms were explicitly included in the calculation. The peptide was embedded in a 40-Å equilibrated TIP3P water box with a dielectric constant set to 1.0, and periodic boundary conditions were applied. The chosen starting structure for each peptide was a fully extended conformation (i.e., dihedral angles ϕ, ψ = 180°, except for proline, for which ϕ = −76.1° and ψ = 180°) in a zwitterionic form. This starting structure was chosen so as not to influence the peptide folding. For the nonbonded terms, we used a protocol that has been shown to be efficient and accurate in cutoff-based simulations using a force switching function for electrostatics and a potential shifting function for van der Waals interactions, both applied between 10 and 12 Å (56). The system was subjected to two cycles of energy minimization in each of which two series of 100 steps of Steepest Descent and 500 steps of Adaptive- Basis Newton Rapshon were performed, first with the fixed peptide and then with the fixed solvent. A final minimization, with all constraints removed, was performed in the full conformational space. The system was heated to 300 K in 100 picoseconds (ps) and then equilibrated in two 100-ps stages; in the early equilibration, velocities were assigned by using a Gaussian distribution, and in the late equilibration the velocities were scaled every 50 steps to keep the temperature at 300 ± 10 K. The production stage lasted 15 or 25 ns for each peptide. The MD simulations were systematically analyzed in terms of β-turn (26) spanning the GXXP motif. β-Turns are defined as secondary structures in which four consecutive amino acid residues (i, i + 1, i + 2, i + 3) fold the polypeptide chain back upon itself by nearly 180°, with a Cα(i) − Cα(i + 3) distance lower than 7 Å. Several β-turn types have been characterized and grouped into seven structural groups (I, I', II, II', IVa, Vb, and VIII), according to the backbone ϕ, ψ dihedral angle values of their two central residues (i + 1 and i + 2). From our MD trajectories, we extracted the Cα(i) − Cα(i + 3) distance and the central residues ϕ, ψ dihedral angles. A β-turn type
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was detected when its conformation followed the exact criteria given by Hutchinson and Thornton (26): a $C_C(i) - C_C(i + 3)$ distance $\leq 7$ Å together with an allowed tolerance for the central residue dihedral angles of $\pm 30^\circ$ for three of them and $\pm 40^\circ$ for the fourth one. $\beta$-Turns whose $C_C(i) - C_C(i + 3)$ distance fulfills the criterion but that cannot be classified in any structural type, according to their dihedral angle values, are grouped in class IV. Usually this category regroups distorted turns common in proteins. The studied dihedral angle values, are grouped in class IV. Usually this cannot be classified in any structural type, according to their

AutoDock 3.5 program (41) was used to perform the computational docking computations then have been carried out with several binding motif. This hypothesis was supported by the observation that fore proposed that these proteins might share a common ligand-

RESULTS

Exposure of mice to the VGVAPG peptide induces typical features of pulmonary emphysema. We delivered one single dose of the VGVAPG peptide (10 µg) into the trachea of WT C57BL/6J mice and we performed differential cell counts on BAL fluids and pulmonary tissues obtained on days 1, 3, 7, 14, and 21 following injection. Exposure to the VGVAPG peptide induced acute inflammation of the airways, characterized by transitory increased macrophage and neutrophil content in the BAL fluids (Fig. 1A and B). Inflammatory cell infiltration was maximal at day 1 after injection, then declined but remained significant until day 7. This kinetic profile was comparable to that obtained by administration of a single dose of PPE (7.4 µg). However, macrophage cellularity in BAL fluids at days 1

![Graph](https://via.placeholder.com/150)

**Fig. 1.** Exposure of C57BL/6J mice to the VGVAPG peptide induces acute and chronic inflammation of the airways. Macrophage and neutrophil numbers were measured in the bronchoalveolar lavage (BAL) fluids (A and B) and in the pulmonary tissue (C and D) of mice exposed to a single injection of PBS, porcine pancreatic elastase (PPE, 7.4 µg), VGVAPG (10 µg), or VVGPGA (random permutation of the VGVAPG sequence) on day 0. Inflammation of the airways was monitored between day 1 and day 21 following injection. In all experiments, $n = 6–8$ mice pooled per group and data represent mean of values from 4 separate experiments ± SE. *Significant difference comparing VGVAPG- and VVGPGA-treated mice ($P < 0.05$). **Significant difference comparing PPE- and VGVPGA-treated mice ($P < 0.05$).
and 3 was significantly higher in PPE-treated mice than in VGVAPG-treated mice, whereas neutrophil infiltration at days 1, 3, and 7 was significantly lower in the PPE group than in the VGVAPG group. PBS and the VVGPGA peptide, a random permutation of the VGVAPG peptide, used as a control of the specific VGVAPG effects (10 µg), had no effect on the cellular content in the BAL fluids. Exposure to the VGVAPG peptide also induced progressive chronic parenchymal inflammation with accumulation of macrophages and neutrophils as early as 1 day after injection, which is consistent with the cell counts obtained in BAL fluids. Cellular accumulation increased up to stable values around 7 or 14 days (Fig. 1, C and D). No significant differences were seen in the number of tissue-infiltrated macrophages between the group of VGVAPG-treated mice and the group exposed to PPE (Fig. 1C). However, VGVAPG was significantly more effective than PPE to induce neutrophil infiltration in pulmonary tissue at days 14 and 21 after instillation (Fig. 1D). VGVAPG-dependent parenchymal inflammation was associated with a breakdown of lung elastin and collagen, as determined by autofluorescent analysis of elastin and collagen fibers after 21 days (Fig. 2A). It was comparable to that obtained by administration of PPE. In contrast, the lungs of PBS or VVGPGA-treated mice showed a normal distribution of both elastin and collagen. The ratio index that provides a quantification of the extent of extracellular matrix remodeling in terms of elastin and collagen voxel volumes was significantly smaller in VGVAPG or PPE groups than in the control (PBS or VVGPGA groups) ones (Fig. 2B). Air space remodeling was accompanied by significant air space enlargement after 21 days (Fig. 2C). Mean linear intercept values, representative of the alveolar space size, were higher in both VGVAPG and PPE-treated mice, whereas no effect was seen in PBS or VVGPGA-treated groups (Fig. 2D). No significant differences were seen in the ratio index or mean linear intercept between the group of VGVAPG-treated mice and the group exposed to PPE.

The VGVAPG peptide contributes to MMP and desmosine accumulation in BAL fluids and induces neutrophil migration and MMP expression in vitro. To assess the role of the VGVAPG peptide in airway inflammation and matrix pro-

![Fig. 2. Exposure of C57BL/6J mice to the VGVAPG peptide induces parenchymal degradation. C57BL/6J mice were exposed to a single injection of PBS, PPE (7.4 µg), VGVAPG (10 µg), or VVGPGA (10 µg). In all experiments, n = 6–8 mice pooled per group. A: autofluorescent analysis of elastin and collagen fibers organization in the lungs of mice at day 21 (×63). Photomicrographs are representative of 3 separate experiments. B: ratio index of the elastin-to-collagen structures measured from a 3D mapping of lung extracellular matrix components performed between day 1 and day 21 following injection. Data represent mean of values from 3 separate experiments ± SE. C: morphological representation of hematoxylin phloxin saffron-stained lung sections obtained from mice at day 21 (×100). Photomicrographs are representative of 3 separate experiments. D: mean linear intercept measured from hematoxylin phloxin saffron-stained lung sections performed between day 1 and day 21 following injection. Data represent mean of values from 4 separate experiments ± SE. *Significant difference comparing VGVAPG- and VVGPGA-treated mice (P < 0.05). **Significant difference comparing PPE- and PBS-treated mice (P < 0.05).
tein degradation during pulmonary emphysema in mouse, we first evaluated, in BAL fluids, the effects of the VGVAPG peptide on the expression of MMP and desmosine, a biomarker of elastin breakdown. Zymography analysis, performed at days 3, 4, and 21 following peptide injection, showed a significant increase of the gelatinolytic activities of MMP-2 and MMP-9 compared with VVGPGA-treated mice (Fig. 3, A and B). Both activities increased from day 3 to a maximal value at day 21. Gelatinolytic activity of MMP-2 in BAL fluids of PPE-treated mice were delayed and consistently lower (Fig. 3A), whereas the MMP-9 protease activities in BAL fluids of PPE-treated mice did not significantly differ from that observed in the PBS control group (Fig. 3B). Western blot analysis showed that MMP-12 expression was increased after PPE or VGVAPG injection (Fig. 3C). MMP-12 expression was nearly the same in the two groups at days 3 and 14, whereas it was stronger after VGVAPG treatment at day 21. We then evaluated whether VGVAPG-dependent MMP expression could be related with elastin fiber degradation. Mice at 14 and 21 days after VGVAPG instillation exhibited significantly greater levels of desmosine in BAL fluids than VVGPGA-treated mice (Fig. 3D). Increase of desmosine levels was also found after instillation of PPE. However, the response appeared earlier as observed at 3 days postinstillation. Except for this time point, there were no obvious differences in the desmosine levels between VGVAPG and PPE groups (Fig. 3D). We next determined the capacity of the VGVAPG peptide to attract murine neutrophils and consequently to induce neutrophil MMP expression in vitro. The observed neutrophil chemotactic activity in response to the VGVAPG peptide (0.1 µg/ml) was similar to that of the RANTES chemoattractant, and it was totally suppressed when neutrophils were preincubated with lactose (Fig. 4A). Treatment of murine neutrophils with VGVAPG significantly increased MMP-2 and MMP-9 expression (Fig. 4B).

![Fig. 3. The VGVAPG peptide contributes to MMP and desmosine accumulation in BAL fluids. C57BL/6j mice were exposed to a single injection of PBS, PPE (7.4 µg), VGVAPG (10 µg), or VVGPGA (10 µg). Expression of MMP-2 (A), MMP-9 (B), and MMP-12 (C) and quantification of desmosine (D) were studied in BAL fluids collected at days 3, 14, and 21 following injection. In all experiments, n = 6–8 mice pooled per group and data represent mean of values from 4 separate experiments ± SE. *Significant difference comparing VGVAPG- and VVGPGA-treated mice (P < 0.05). **Significant difference comparing PPE- and PBS-treated mice (P < 0.05). ***Significant difference comparing VGVAPG- and PPE-treated mice (P < 0.05).](http://ajplung.physiology.org/Downloadedfrom)
The PGAIP peptide is a potential EBP-specific antagonist as suggested by molecular dynamic and docking simulations. We next tested the hypothesis that active XGXXPG sequences, still retaining a type VIII β-turn after deletion of the COOH-terminal glycine residue, could act as selective antagonists of EBP-dependent biological activities. We performed molecular dynamic simulations with the active VGVAPG and PGAIPG peptides. The frequency of type VIII β-turns observed with the two peptides was 28% or more (Fig. 5). For VGVAPG, efficient folding required several nanoseconds, resulting in 44% of type VIII β-turns in the last simulation period. For PGAIPG, efficient folding was reached immediately with 50% type VIII β-turns in the first 7 ns and an average percentage of 29.2% during the overall simulation. The inactive GGVPG peptide (10) had very low 2.7% propensity to fold as a β-turn. Deletion of the COOH-terminal glycine residue in the VGVAPG peptide significantly decreased the type VIII β-turns frequency down to 0.9%. On the contrary, glycine deletion in the VGVAP peptide significantly increased the type VIII β-turns up to 46.2%, compared with 29.2% for PGAIPG, and even up to 61.2% for the last 7 ns of the simulation (Fig. 5). The capacity of PGAIP to interact with EBP was further supported by molecular docking analysis. The AutoDock scoring function was used to compute the binding energy of each docked conformation and to rank these conformations into clusters of similar structures. As observed with the PGAIPG and VGVAPG peptides, the best structures calculated for PGAIP also correspond to a cluster of lower energy conformations (Table 1). This cluster contained 18 conformations, thus suggesting a high propensity for EBP binding. For the GGVPG peptide the docking program failed to identify an optimal interaction structure.

**The PGAIPG peptide induces airway inflammation and remodeling whereas the PGAIP peptide is inactive.** To confirm the effect of the COOH-terminal glycine deletion of the PGAIPG peptide on its biological activities, we exposed mice to a single dose of either PGAIPG (10 µg) or PGAIP (10 µg) and monitored the typical features of emphysema between day 1 and day 21. The VGVAPG peptide (10 µg) and the VVGPAG peptide (10 µg) were used as a positive and negative control for lung inflammation and degradation, respectively. Exposure of mice to PGAIPG resulted in a parenchymal inflammation (inflammatory infiltrate in BAL fluids and tissue) (Fig. 6, A–D) in a similar way to that observed for VGVAPG. However, the cellular content in BAL fluids and tissue was significantly lower in the PGAIPG group compared with the VGVAPG group. In contrast, exposure to PGAIP did not significantly affect the number of macrophages and neutrophils in BAL fluids.

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**Fig. 4.** The VGVAPG peptide induces murine neutrophils migration and MMP expression in vitro. A: neutrophils pretreated or not with lactose (10 mM, 1 h) were studied for their chemotactic response toward RANTES (0.1 µg/ml; positive control) or VGVAPG. B: expressions of MMP-2 and MMP-9 by zymography were studied in the culture supernatants of murine neutrophils treated with formyl methionyl-leucyl-phenylalanine (fMLP) (100 nM; positive control) or VGVAPG. Data represent mean of values from 4 separate experiments ± SE. *Significant difference compared with RPMI or PBS (P < 0.05).
Table 1. Characteristics of the best PPE/peptides molecular docking models

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Activity Rank</th>
<th>Cluster</th>
<th>Cluster Size</th>
<th>Xα Residue</th>
<th>Xα Residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>VGVAPG</td>
<td>+</td>
<td>1</td>
<td>4</td>
<td>6.8</td>
<td>-102.7 -25.8 -139.2 121.8</td>
</tr>
<tr>
<td>PGAIPG</td>
<td>+</td>
<td>2</td>
<td>6</td>
<td>7.1</td>
<td>-77.6 -44.9 -145.2 124.0</td>
</tr>
<tr>
<td>GGVP</td>
<td>-</td>
<td>5</td>
<td>1</td>
<td>7.6</td>
<td>-153.7 -74.2 -126.5 137.6</td>
</tr>
<tr>
<td>VGVAP</td>
<td></td>
<td>1</td>
<td>7</td>
<td>6.6</td>
<td>-89.2 -148.7 -43.4 122.2</td>
</tr>
<tr>
<td>PGAIP</td>
<td></td>
<td>2</td>
<td>18</td>
<td>7.3</td>
<td>-89.2 -43.4 -148.7 122.2</td>
</tr>
</tbody>
</table>

For each peptide conformation retained, the specificity of its cluster (rank and size) is indicated. Except for the inactive GGVP peptide, the best complex structure is obtained for the first or second best energy conformation.

Structural characteristics of the GXXP motif are also detailed: the Cα – (Gly) – Cα – (Pro) distance and the central residues ϕ, ψ dihedral angles. According to Hutchinson and Thornton criteria (38), canonical type VIII β-turns are defined by a Cα – Cα distance ≤7Å, and ϕ(Xi) = -60.0°, ψ(Xi) = -30.0°, ϕ(Xi) = −120.0°, ψ(Xi) = 120.0° with an allowed tolerance of ±30° for three angles and ±40° for the fourth one. All the peptides except GGVP show a canonical type VIII β-turn spanning the GXXP motif with all the criteria fulfilled; a slight deviation is however observed for ϕ(Val 3) dihedral angle of VGVAPG.

For GGVP, a strong deviation for dihedral angles of Gly 2 is to be noticed. Presently, the dihedral angles of Gly 2 differ from −60.0° for three angles and −120.0° for the fourth one. The GGVP peptide inhibition of the effects of VGVAPG on chemotaxis in response to these peptide mixtures showed that the PGAIP peptide (0.001 to 10 µg/ml) had no effect, and no PGAIP–dependent chemotaxis of untreated neutrophils was observed. The VGVAPG peptide at a concentration of 0.1 µg/ml had no effect on neutrophil migration. To provide further evidence for the ability of the PGAIP peptide to impair neutrophil migration induced by the VGVAPG peptides, the PGAIP peptide (0.001 to 10 µg/ml) and the VGVAPG peptide (0.1 µg/ml) were coinoculated at various ratios and the effects of these mixtures on neutrophil migration were studied. Analysis of neutrophil chemotaxis in response to these peptide mixtures showed that the PGAIP peptide inhibition of the effects of VGVAPG on neutrophil migration was dose dependent (Fig. 8B). Pretreatment of neutrophils or coinoculation of the VGVAPG peptide with various concentrations of the PGAIP peptide (random permutation of the PGAIP peptide, 0.001 to 10 µg/ml) did not intercept values (Fig. 7D). Interestingly, there were no significant differences in the distribution of both elastin and collagen fibers and in the size of alveolar spaces between the group of PGAIP-treated mice and the control group exposed to VVGPGA (Fig. 7, A–D).

The PGAIP peptide antagonizes the VGVAPG peptide-induced neutrophil migration in vitro. We postulated that the VGVAPG peptide can trigger lung inflammation and degradation in mice by increasing neutrophil migration to the lung tissues. The VGVAPG peptide-induced neutrophil migration should therefore be downregulated by the antagonist activity of the PGAIP peptide. This hypothesis was tested in vitro by preincubation of neutrophils with increasing concentrations of the PGAIP peptide before a chemotaxis assay using the VGVAPG peptide at a concentration of 0.1 µg/ml. The VGVAPG peptide induced significant migration of untreated neutrophils whereas a 1-h preincubation with PGAIP concentrations ranging from 0.01 to 10 µg/ml significantly inhibited VGVAPG-dependent chemotaxis (Fig. 8A). A lower PGAIP concentration (0.001 µg/ml) had no effect, and no PGAIP-dependent chemotaxis of untreated neutrophils was observed. The VGVAPG peptide at a concentration of 0.1 µg/ml had no effect on neutrophil migration. To provide further evidence for the ability of the PGAIP peptide to impair neutrophil migration induced by the VGVAPG peptides, the PGAIP peptide (0.001 to 10 µg/ml) and the VGVAPG peptide (0.1 µg/ml) were coinoculated at various ratios and the effects of these mixtures on neutrophil migration were studied. Analysis of neutrophil chemotaxis in response to these peptide mixtures showed that the PGAIP peptide inhibition of the effects of VGVAPG on neutrophil migration was dose dependent (Fig. 8B). Pretreatment of neutrophils or coinoculation of the VGVAPG peptide with various concentrations of the PGAIP peptide (random permutation of the PGAIP peptide, 0.001 to 10 µg/ml) did not...
alter the VGVAPG-dependent neutrophil migration (data not shown).

The PGAIP peptide protects mice from the VGVAPG peptide- and the PPE-induced pulmonary emphysema. We next evaluated whether the PGAIP peptide could antagonize the effects of the VGVAPG peptide as well as those of PPE in vivo. We delivered one single instillation of a mixture containing either the VGVAPG and PGAIP peptides (10 μg each) or PPE (7.4 μg) and the PGAIP peptide (10 μg). As a negative control, the same protocol was done using the GPPIA peptide in combination with VGVAPG or PPE. At day 21 after injection, the mice were euthanized and inflammation was evaluated by both inflammatory cell counting in tissue and by protease analysis and desmosine quantification in BAL fluids. Parenchymal degradation was investigated by analyzing both the elastin and collagen fibers organization in the lungs of mice at day 21 (×63). Photomicrographs are representative of 3 separate experiments. B: ratio index of the elastin-to-collagen structures measured from a 3D mapping of lung extracellular matrix components performed at day 21 following injection. Data represent mean of values from 3 separate experiments ± SE. *Significant difference comparing VGVAPG- and PGAIPG-treated mice (P < 0.05). **Significant difference comparing PGAIPG- and PGAIP-treated mice (P < 0.05).

Fig. 7. The PGAIPG peptide induces parenchymal degradation, while the PGAIP peptide has no effect. C57BL/6J mice were exposed to a single injection of VVGPGA (10 μg), VGVAPG (10 μg), PGAIPG (10 μg), or PGAIP (10 μg). In all experiments, n = 6–8 mice pooled per group. A: autofluorescent analysis of elastin and collagen fibers organization in the lungs of mice at day 21 (×63). Photomicrographs are representative of 3 separate experiments. B: ratio index of the elastin-to-collagen structures measured from a 3D mapping of lung extracellular matrix components performed at day 21 following injection. Data represent mean of values from 3 separate experiments ± SE. *Significant difference comparing VGVAPG- and PGAIPG-treated mice (P < 0.05). **Significant difference comparing PGAIPG- and PGAIP-treated mice (P < 0.05).
breakdown matrix fibers was significantly decreased when the PGAIP peptide was coinjected to the mice (Figs. 11, A and B). Finally, as a consequence of PGAIP efficiency, airway remodeling was significantly lowered in PGAIP-cotreated mice as revealed by mean linear intercept values (Figs. 11, C and D, and 12, C and D). Nevertheless, a moderate degree of air space enlargement was still persistent in mice coinstilled with VGVAPG and PGAIP (Fig. 11D). Interestingly, mice coinfected with PPE and PGAIP were more sensitive to the PGAIP peptide effects with a complete inhibition of airway remodeling (Fig. 12D).

DISCUSSION

COPD is a major worldwide respiratory health problem resulting in chronic airway inflammation and pulmonary emphysema. Studies in humans with severe α-1 anti-trypsin deficiency (28, 33, 54) and in animal models exposed to proteases (25, 35, 55) suggest that emphysema partly results from proteolytic breakdown of extracellular matrix proteins by enzymes released from lung infiltrated inflammatory cells (15). EP produced in significant amounts during pulmonary elastin degradation (5, 14, 49) share miscellaneous biological activities, predominantly chemotactic activities and protease release (7, 51). Here, we report that in mice EP are potent inducers of the typical features of emphysema and that their deleterious effects on lungs can be antagonized by analogous peptides.

The first aim of this study was to characterize the effects of the administration of EP, i.e., the VGVAPG peptide, on the induction of proinflammatory response and emphysema in mice. We observed a positive correlation between the lung inflammatory response, the air space enlargement, the parenchymal tissue degradation, and the injection of a single endotracheal dose of the VGVAPG peptide. The VGVAPG peptide-treated mice exhibited inflammatory processes and emphysematous structural changes similar to those observed following endotracheal administration of PPE, a pulmonary inflammatory model whose traits closely resemble human emphysema (25, 35, 55). Controversy remains regarding the relative contribution of macrophage-derived vs. neutrophil-derived enzymes in initiating the emphysema process. It was previously suggested that neutrophil-derived MMP-2 and -9 are involved in the initial phase of PPE-induced pulmonary emphysema (between days 0 and 3), whereas macrophage-derived proteases are responsible for later destruction of the lungs (after day 8) (45).

Here we report that MMP-2 and -9 are present at no significant amounts in BAL fluids after a 3-day exposure of mice to PPE, suggesting that in our PPE experimental model the early phase of pulmonary inflammation is shortened (less than 3 days) as objectified by the decrease in the number of neutrophils in BAL fluids after a 3-day exposure of mice to PPE, or vari…
is related to the direct effect of PPE whereas the emphysema observed at 21 days after PPE instillation supported by a previous report that showed that only endogenous mechanisms to allow inflammatory cell activation is markedly reduced (29) and is cleared from the lung within 24 h (57), suggesting that PPE must trigger time-delayed endogenous mechanisms to allow inflammatory cell activation and consequently MMP expression. This analysis is supported by a previous report that showed that only ~20% of the emphysema observed at 21 days after PPE instillation is related to the direct effect of PPE whereas ~80% is related to the effects of inflammatory mediators (35). We can here hypothesize that these mediators are poorly involved in MMP-9 expression by tissue infiltrating inflammatory cells. In contrast, the VGVAPG peptide exerts direct chemotactic and activator effects on inflammatory cells (3, 13, 50, 51), leading to early MMP expression that trigger continuous degradation of elastin fibers. The genesis of such self-perpetuating process of inflammatory cell activation is probably more suitable for MMP-9 expression by these cells. In accordance with the VGVAPG-induced MMP elastinolytic activities, an increased secretion of desmosine, a specific marker for elastin degradation, was found in the BAL fluids at days 3, 14, and 21. Furthermore, we demonstrated in this study an increased neutrophil migration and MMP-2 and -9 expressions by murine neutrophils in response to VGVAPG activation in vitro. Since significant KC chemokine expression was not observed in BAL fluids of VGVAPG-treated mice (data not shown), neutrophil infiltration and MMP expression can be easily attributed to in vivo VGVAPG-mediated effects. Our data corroborate those of previous studies demonstrating that 1) EP activate proteases release (7) and exert potent chemotactic activities toward inflammatory cells (22, 42, 51), and 2) EP antagonism suppresses monocyte recruitment to the lung in PPE-recipient mice (25). A previous study has already shown that intratracheal administration of VGVAPG significantly increases the amount of lung macrophages (25). However, our report provides the first kinetic analysis of EP-induced emphysema in mice, which simultaneously analyzes and correlates lung inflammation and parenchymal destruction.

The relationship between the inflammatory process development and the EP administration can be accounted for in our model. As the VGVAPG peptide is injected endotracheally it reaches the airways, and inflammatory cells are rapidly chemotactically migrating to the alveolar spaces. In addition, inflammatory cells infiltrating pulmonary tissue are activated by the VGVAPG peptide to release proteases contributing to parenchymal destruction, including elastin and collagen fiber degradation. Thus elastin breakdown generates peptides that further contribute to the development of pulmonary emphysema. Mice exposed to a single

Fig. 9. The PGAIP peptide protects mice from the VGVAPG peptide- and the PPE-dependent inflammatory cell infiltration in pulmonary tissue. C57BL/6J mice were exposed to either a single injection of PBS, VGVAPG (10 µg), VVGPGA (10 µg), PPE (7.4 µg), or PGAIP (10 µg) or to a mixture containing VGVAPG (10 µg) and PGAIP (10 µg), PPE (7.4 µg) and PGAIP (10 µg), VGVAPG and GPPIA (random permutation of the PGAIP peptide uses as a negative control, 10 µg), or PPE and GPPIA (10 µg). Macrophage (4) and neutrophil (8) numbers were measured in the pulmonary tissue of mice at day 21. In all experiments, n = 6–8 mice pooled per group and data represent mean of values from 3 to 4 separate experiments ± SE. *Significant difference compared with VVGPGA-treated mice (P < 0.05). ***Significant difference compared with PBS-treated mice (P < 0.05). **Significant difference compared with VGVAPG or PPE-treated mice (P < 0.05).
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Fig. 10. The PGAIP peptide protects mice from the VGVAPG peptide- and the PPE-dependent accumulation of MMP and desmosine in BAL fluids. C57BL/6J mice were exposed to either a single injection of PBS, VGVAPG (10 μg), VVGPGA (10 μg), PPE (7.4 μg), or PGAIP (10 μg), or to a mixture containing VGVAPG (10 μg) and PGAIP (10 μg), PPE (7.4 μg) and PGAIP (10 μg), VGVAPG and GPPIA (10 μg), PGAIP and GPPIA (10 μg). Expression of MMP-2, MMP-9, and MMP-12 (A) and quantification of desmosine (B) were studied in BAL fluids collected at day 21. In all experiments, n = 6–8 mice pooled per group and data represent mean of values from 3 to 4 separate experiments ± SE. *Significant difference compared with VVGPGA-treated mice (P < 0.05). **Significant difference compared with PBS-treated mice (P < 0.05). ***Significant difference compared with PBS-treated mice (P < 0.005). ****Significant difference compared with PBS-treated mice (P < 0.001).

dose of the PGAIPG elastin peptide also showed typical emphysematous tissue destruction. This strongly suggests that various peptides originated from elastin degradation can interact with the EBP subunit in the elastin-receptor complex to trigger signals responsible for deleterious pulmonary effects. We therefore envisaged EBP as a common factor in the development of EP-induced emphysema in mice and proposed a critical relationship between the occurrence of a COOH-terminal glycine residue in the EP with a type VIII β-turn conformation and the biological function of these peptides.

We performed molecular modeling studies of the VGVAPG and PGAIPG peptides and demonstrated the high propensity of the PGAIPG peptide to fold as a type VIII β-turn and to bind EBP. Moreover, in contrast to PGAIPG, administration of PGAIP to WT mice did not induce inflammatory response or alteration of lung tissue integrity. Moreover, no MMP expression was detected in the BAL fluids of PGAIP-treated mice (data not shown). The VGVAPG peptide and the well-documented inactive GGVPG (10) had a low probability of a type VIII β-turn, probably reflecting structures with a high flexibility. Our observation that the PGAIP peptide impaired the in vitro activity of the VGVAPG peptide on neutrophil migration in a dose-dependent way suggested that a similar effect might exist in vivo. A single dose of PGAIP injected into murine lungs simultaneously to the exposure to VGVAPG or PPE protects the animals from the VGVAPG- or PPE-dependent emphysema. However, PGAIP does not completely block VGVAPG effects on lungs, given that a low but significant infiltration of neutrophils associated with MMP-2 expression in BAL fluids and an increase of the size in alveolar spaces were observed. We cannot exclude the possibility that the PGAIP peptide contributes to some of these biological activities. The lack of effects observed on lung neutrophil migration, on MMP-2 expression in BAL fluids, and on pulmonary tissue integrity in mice exposed to the PGAIP peptide alone does not support this hypothesis. Moreover, the PGAIP incomplete protection of animals from the VGVAPG-dependent emphysema was not found when PGAIP was instilled into murine lungs simultaneously to the exposure to PPE. Competition between the two peptides is a dynamic event depending on the peptide affinity, avidity, and the dose ratio used. The amount of PGAIP selected here agrees both with the inhibition of the VGVAPG activity in vitro at a 1/1 ratio and with the minimal dose of VGVAPG required to trigger in vivo effects on murine lungs. Similar results were obtained by using two- to fivefold increased PGAIP amounts at a constant amount of VGVAPG (data not shown).
In summary, the present study showed that 1) various peptides originated from elastin degradation (EP) can interact with elastin receptor (EBP) to induce emphysema, and 2) molecular modeling studies are original approaches to screen analogous peptides susceptible to antagonize the EP/EBP interactions. More analogous peptides should be screened in the future to identify the best candidate to fully antagonize elastin peptides deleterious effects.

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**

M.S., S.H.-H., and R.L.N. conception and design of research; M.S., A.M.-K., C.F., and N.B. performed experiments; M.S., A.M.-K., C.T., M.-D.D.,
REFERENCES


