The role of matrix metalloproteinase (MMP)-9 in cigarette smoke-induced emphysema.

Jeffrey J Atkinson, Barbara A Lutey, Yoko Suzuki, Holly M Toennies, Diane G Kelley, Dale K Kobayashi, Whitney G Ijem, Gaetan Deslee, Carla H Moore, M. Eileen Jacobs, Susan H. Conradi, David S Gierada, Richard A Pierce, Tomoko Betsuyaku, Robert M Senior

ONLINE DATA SUPPLEMENT

Supplemental Methods:

Mouse MMP-12 Western. Based on protein concentration, equal amounts of protein from mouse left lung homogenates were separated under reducing conditions on a 10% SDS polyacrylamide gel and transferred onto Immobilon-P PVDF transfer membranes (Millipore Corp). After blocking overnight (5% nonfat dried milk, TBS, 0.1% Tween 20) at 4 °C, the membranes were incubated for 1 h with a rabbit monospecific polyclonal anti-MME antibody (1:1000), followed by 1 h of incubation with horseradish peroxidase-conjugated donkey anti-rabbit secondary antibody (1:25,000; Jackson ImmunoResearch). After washing, immunoreactive proteins were detected by chemiluminescence using the ECL Plus Western Blotting Detection System (Amersham Pharmacia) and subsequent autoradiography.

Zymography. Lung lavage fluid gelatinase activity was determined by gelatin zymography. Unconcentrated lung lavage fluid was electrophoresed in a 7.5% acrylamide gel containing 1 mg/ml casein, and gels were washed in 2.5% Triton X-100 and incubated for 24 hours in buffer containing 50 mM Tris (pH 8.0), 10 mM $CaCl_2$, and 1 mM $ZnCl_2$. Following incubation, gels were stained with Coomassie blue, destained, and proteolytic activity was detected as clear bands on a blue background. Gels were scanned and inverted to improve visualization of bands.

Alveolar macrophage MMP-9 expression. Specimens described previously were utilized to determine the alveolar macrophage production of non-smokers without emphysema and former smokers with lesser severity of COPD. Specimens obtained at the time of resection surgery were processed as described and evaluated by qPCR without determining local lung CT densisty. All values expressed are normalized to GAPDH. Mean macrophage MMP-9 from all 10 lung cores sampled of the 5 GOLD stage 4 subjects described in table 1 were utilized for comparisons.

Supplemental Figure Legends:

Figure E1. Gelatin zymography and Western blot for MMP-12 in lung homogenates of wildtype and MMP-9 KO mice. Gelatinolytic bands at 110 and 92 kD representing pro- and active MMP-9 are present only in wild-type (WT) animals. An additional band is also seen at 72 kD is pro-MMP-2. This is seen in both WT and MMP-9 KO (9KO) mice after smoke exposure. There are no gelatinolytic bands in the MMP-9 KO mice that are of greater intensity than in the WT controls. Western Blotting of the same lung homogenates reveals equal quantities of pro-MMP-12 at 54 kD and lower molecular weight intermediates as well as active MMP-12 at 29 kD. Although the pro- and intermediate forms can be detected in non-smoking mice (9KO NS) the quantity of active form is greater in the smoke exposed animals but there is not difference in either the pro-MMP-12 or active MMP-12 intensity between the smoke exposed mice.

Figure E2. Macrophage MMP-9 expression in lungs of individuals with different GOLD severity. CD68+ alveolar macrophages were isolated from frozen lungs after resection by laser capture microdisection of stained slides and MMP-9 expression was determined by real-time PCR. Expression of MMP-9 in the CD68+ cells of 3 never smokers (blue), 3 former smokers with no emphysema (red), 3 former smokers with GOLD 1 disease (yellow), 2 former smokers with GOLD 2 disease (green) and the mean macrophage expression of the 5 subjects in Table 1 graphed on a $\log_{10}$ scale with all values of 0 converted to 1. Similar quantities of macrophage MMP-9 expression can be seen in the absence of smoking related lung disease as is seen in more severe GOLD classes.

Figure E3. Macrophage MMP-12 mRNA expression. Macrophage MMP-12 expression in CD68+ alveolar macrophages compared with emphysema severity of the lung core. Emphysema severity is expressed as the mean density of the region of the lung core on CT. MMP-12 mRNA quantity from alveolar macrophages plotted on a linear scale demonstrates the paucity of macrophage MMP-12 production in these samples. All subjects have quit smoking for at least 2 years prior to collection. Despite the severe emphysema in many of these cores very few cores demonstrate any macrophage expression of MMP-12.

Figure E4. Plasma CCSP and SP-D production relative to emphysema severity. A) Plasma CCSP in current smokers (black circles) and former smokers (gray circles) compared with emphysema severity (percent low

attenuation area, LAA). Current smoking causes a significant decrement in CCSP (t test vs former smokers, $p<0.05$) but no relationship with emphysema severity is seen. B) plasma SP-D in current smokers (black circles) and former smokers (gray circles) compared with emphysema severity (percent low attenuation area, LAA). Current smoking causes a significant increase in SP-D (t test vs former smokers, $p=0.02$) but no relationship with emphysema severity is seen. Plasma CCSP and SP-D of current smokers with emphysema was not examined.

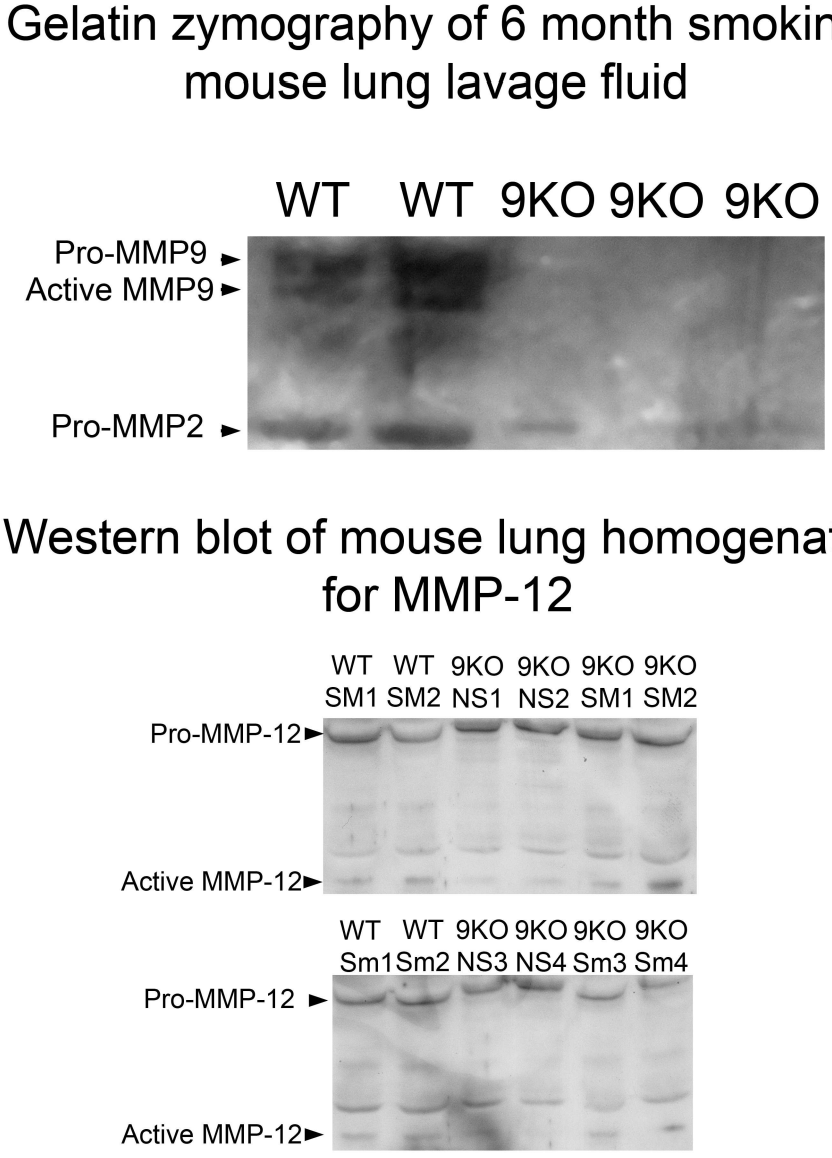

Figure E1
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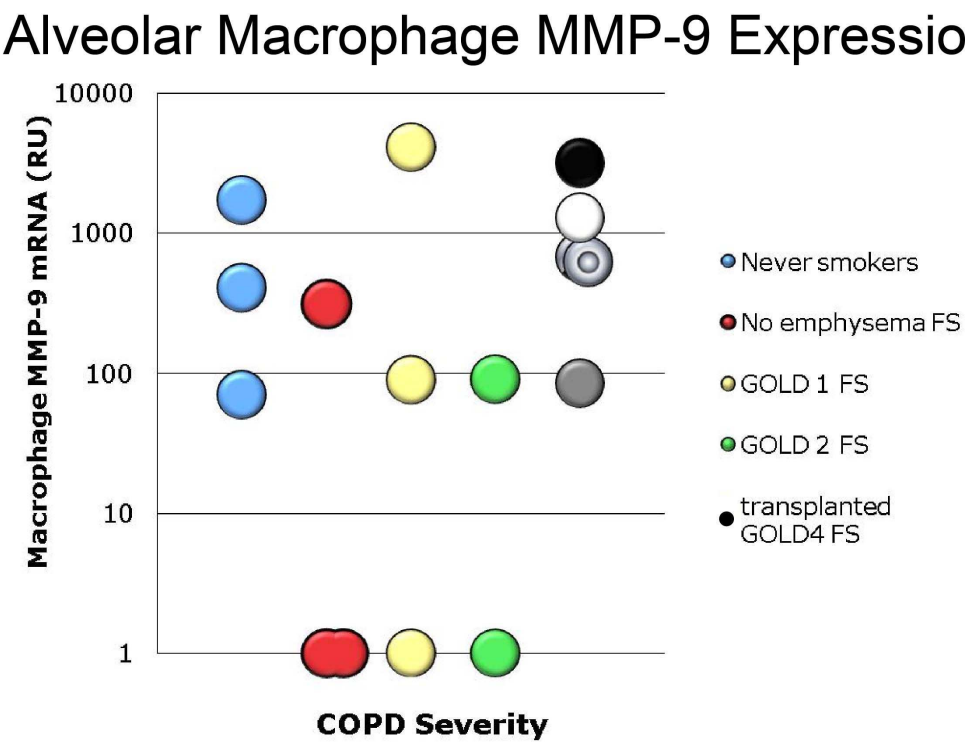

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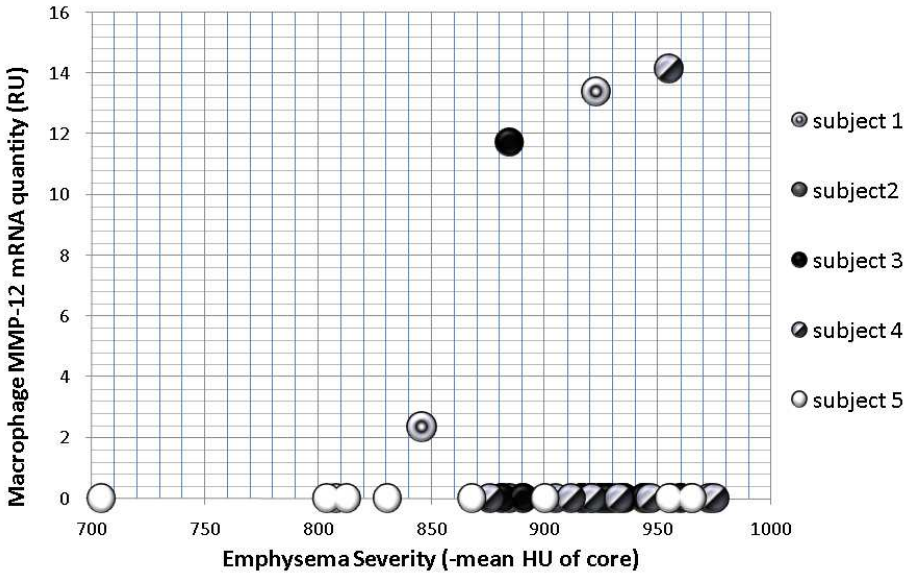

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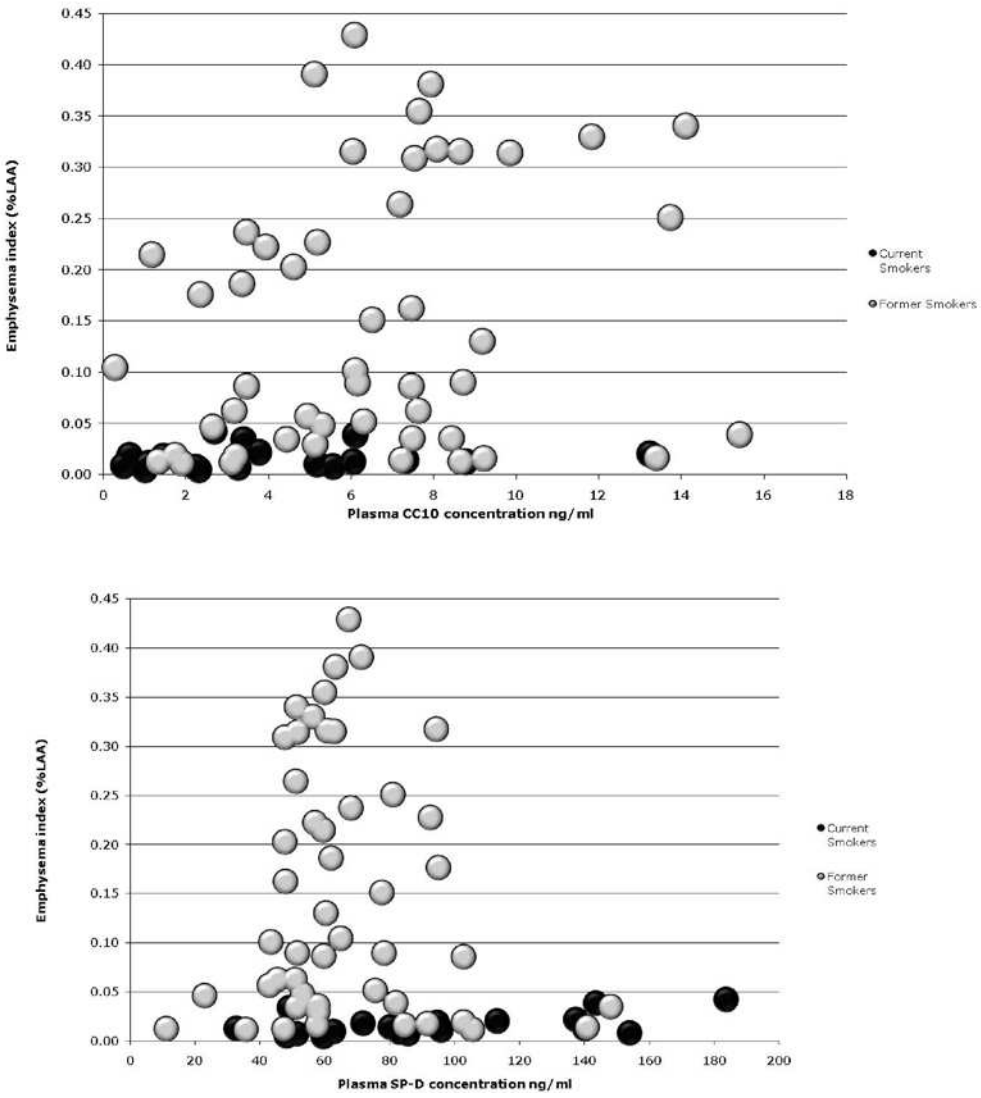

Figure E4
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