(data not shown). Similarly, blockade of EPCR on HUVECs with this EPCR blocking mAb did not have a significant effect on FX or FXa binding (Figure 1B). FVIIa and APC binding studies performed in parallel with FX or FXa experiments clearly demonstrated that both FVIIa and APC bound to cells to a similar degree and in an EPCR-specific manner (Figure 1A-B). Analysis of the binding of biotinylated, active-site blocked FXa, FVIIa and APC to EPCR on CHO-EPCR and HUVECs revealed that little FXa was bound to EPCR on cell surfaces compared with FVIIa or APC (Figure 1C-D). In this assay, FVIIa and APC both bound to EPCR with an apparent Kd of ~15 to 25nM, whereas the Kd for FXa was >1µM. Consistent with these data that FX does not bind appreciably to EPCR, even a 100-fold molar excess of unlabeled FX (1µM) failed to compete effectively with the binding of 125I-FVIIa (10nM) to CHO-EPCR cells (Figure 1E). Analysis of FX binding to EPCR expressing cells by confocal fluorescence microscopy did not show any detectable fluorescence, either at the cell surface or intracellularly, in CHO-EPCR cells or HUVECs exposed to FX tagged with a fluorescent dye (AF488; Figure 1F).

In additional studies, we measured plasma levels of mouse factor X and protein C in EPCR overexpressing mice that received a high dose of active-site inhibited human APC. EPCR overexpression has been found to decrease circulating levels of protein C, while administration of human protein C has been shown to decrease circulating levels of protein C, while 

in hamsters administered intratracheal suspensions of diesel exhaust particles.2

Activation of platelets increases the tendency toward thrombosis in hamsters administered intratracheal suspensions of diesel exhaust particles.2

Linking air pollution exposure with thrombosis

To the editor:

We read with interest the review by Franchini and Mannucci examining the link between exposure to particulate matter air pollution (PM) and an increased tendency toward thrombosis.1 In their discussion of the potential mechanisms by which PM might induce thrombosis, the investigators highlight the excellent work by Nemmar and colleagues suggesting that PM enhances the release of histamine by mast cells and the resulting activation of platelets increases the tendency toward thrombosis in hamsters administered intratracheal suspensions of diesel exhaust particles.2

We were surprised that the authors did not discuss an additional mechanism. In mice, we reported that the intratracheal administration of fine urban particulates or the inhalation of concentrated ambient particulate matter air pollution from Chicago resulted in an increase in the plasma levels of thrombin-antithrombin (TAT) complexes and accelerated arterial thrombosis in the ferric chloride carotid injury model via a mechanism that required the release of IL-6 from alveolar macrophages.3 4 This mechanism is attractive as resident macrophages in the lung are likely the “first responders” to inhaled particles3 and the prothrombotic effects of IL-6 have been
Figure 1. The effect of loss of histamine signaling in particulate matter–induced thrombin generation. Fine urban particulate matter (National Institute of Standards and Technology Standard Reference Material, SRM 1649a 200 μg/mouse in 50 μL PBS) or vehicle was administered intratracheally to 20-25 g, 6-8 week old male C57BL/6 mice as previously described. A (A) After 24 hours BAL fluid was obtained and histamine levels were measured using a commercially available assay (EIA Histamine IM2015, Beckman Coulter). (B) Mice were treated with famotidine (10 mg/kg) and desloratadine (10 mg/kg) in 150 μL of PBS 4 hours before treatment with PM followed by an additional dose of famotidine 8 hours later. Twenty-four hours after PM administration, BAL fluid was obtained and IL-6 levels were measured as previously described (ELISA). C (C,D) H1R and H2R receptor double knockout mice (H1R−/−,H2R−/−) or littermate controls were treated with PM and 24 hours later IL-6 and TAT were measured in BAL fluid and citrated plasma as previously described. The protocol for the use of mice was approved by the Animal Care and Use Committee at Northwestern University. N = 4 or 5 animals for each group. *P < .05 compared with PBS control; and NS, not significant using ANOVA with Bonferroni posttest comparison.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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