



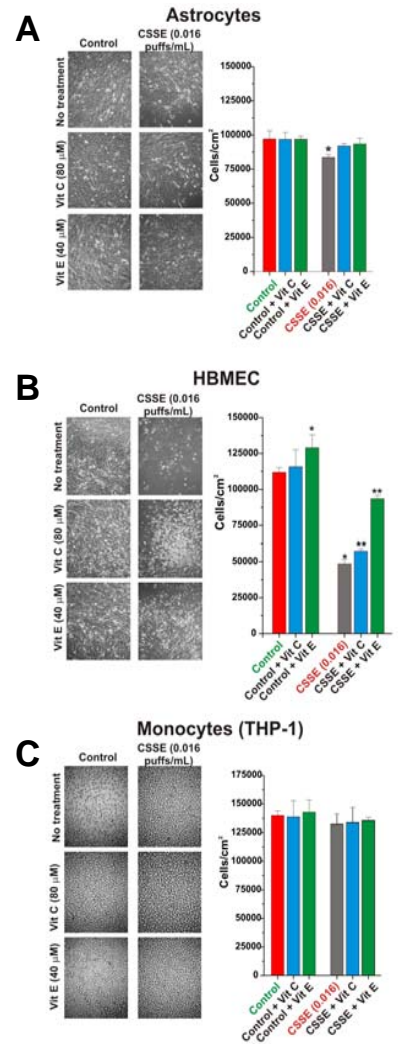
# Assessment of tobacco smoke toxicity at the human blood-brain barrier

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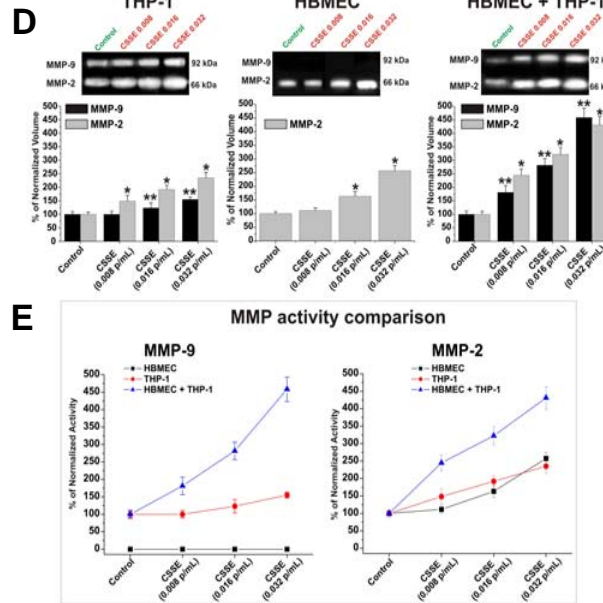
Cigarette smoke is known to contain high concentrations of free radicals and oxidants. However, nothing is known about the oxidative damage associated with cigarette smoke on the human brain microvasculature and more specifically on the cellular components of the blood-brain barrier (BBB). In this study we assessed whether exposure to cigarette side smoke extract (CSSE) affects the BBB integrity and the specific effect on BBB endothelial cells. Our results clearly show that chronic exposure to CSSE induced the pro-inflammatory activation of the microvascular endothelium demonstrated by the increased level of locally secreted pro-inflammatory cytokines (interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β)). In addition, the expression level of relevant vascular adhesion molecules such as VCAM-1, P-selectin and E-selectin were also increased. Exposure to CSSE facilitated the differentiation of a well know human acute monocytic leukemia cell line (THP-1) into mature and activated macrophages. This differentiation process was accompanied by a CSSE dose-dependent MMP-2 and MMP-9 activation. MMP activity was also detected by zymography in the culture medium of endothelial cells following the exposure to CSSE. These data strongly suggest that cigarette smoke synergistically modulates WBC differentiation as well as WBC and endothelial cells pro-inflammatory activation thus, ultimately hampering BBB integrity. BBB integrity was monitored by real time measurements of trans-endothelial electrical resistance (TEER) while the levels of adenylate kinase released in the culture medium were used to assess for cell viability. CSSE exposure also caused a cellular shift toward a more anaerobic and therefore less efficient metabolism. This was determined by an unparalleled increase in lactate production. Interestingly, antioxidant supplementation with vitamin C and E reduced or fully prevented the oxidation and the inflammatory damage induced by cigarette smoke.

## Cigarette smoke exposure affect viability of human blood-brain barrier endothelial cells



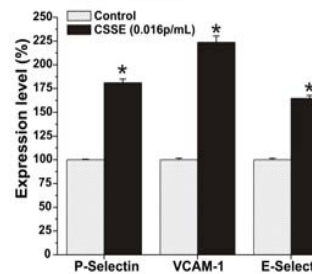
**Panel A:** TS significantly decreases cell viability of BBB endothelial cells. Astrocytes are negligible affected and only at the highest TS dose (**Panel B**). TS did not affect monocyte viability (**Panel C**).

## Cigarette smoke exposure affect MMP activity in BBB endothelial cells and THP-1 culture media



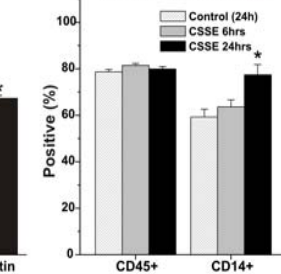
Activity of MMP-9 and, more significantly, MMP-2 are upregulated following exposure of THP-1 cells to TS. MMP-2 activity in HBMEC culture medium is also upregulated by smoke exposure (**Panel D**). When endothelial and THP-1 cells are both exposed to cigarette smoke and then co-cultured, a synergistic effect of MMP activity is observed (**Panel E**).

## Pro-inflammatory endothelial adhesion molecules



Cigarette smoke exposure upregulates the expression of pro-inflammatory EC adhesion molecules P-selectin, VCAM-1 and E-selectin (**Panel F**). The percentage of CD14 positive cells (CD14 is a monocyte marker) significantly increases after 24 hrs' exposure to TS indicating an increased level of differentiation along the monocytic lineage (**Panel G**).

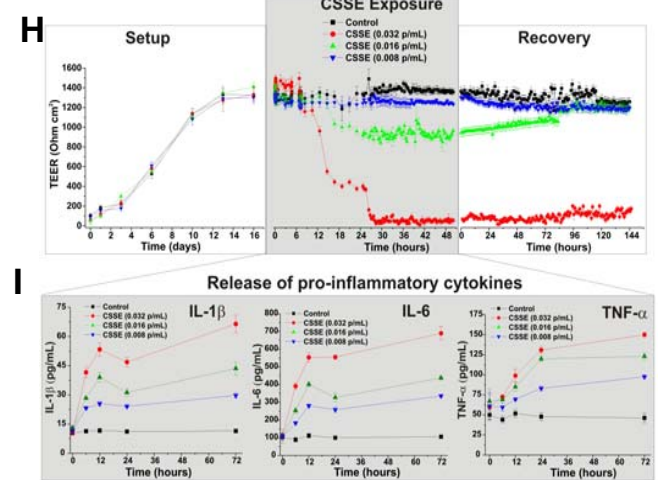
## THP-1 differentiation along the monocytic lineage



## Conclusions

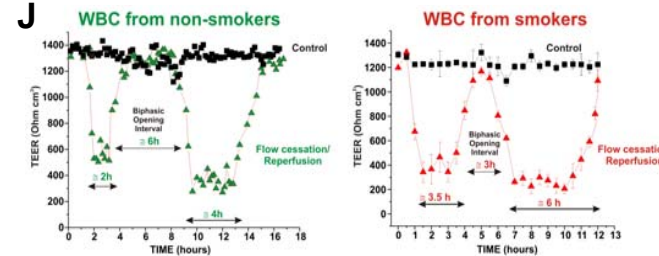
Cigarette smoke has previously been shown to contribute to a pro-atherosclerotic environment by triggering a complex pro-inflammatory response. This includes the recruitment of leukocytes through cytokine signaling, matrix metalloproteinase upregulation (e.g., MMP-2 and MMP-9), and promotion of monocyte adherence to the vascular endothelium. In this study we have shown that exposure to cigarette smoke determine a similar direct vascular damage imposed on brain microvascular EC where it causes rapid and complex loss of baseline BBB function and integrity.

## Cigarette smoke exposure affect BBB integrity and determine the release of pro inflammatory cytokines

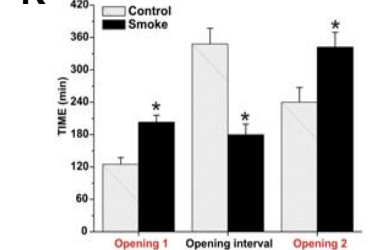


The exposure to cigarette smoke is associated with a rapid fall in TEER that is dose dependent and the effect on BBB integrity is reversible at the lower concentration if smoke-containing media is replaced with smoke-free one (**Panel H**). Loss of BBB integrity is paralleled by the release of TNF-α, IL-1β, and IL-6 which are also strongly smoke concentration dependent (**Panel I**).

## BBB Failure Associated with an Ischemic-Event Worsens in Presence of Monocytes Chronically Exposed to Smoke.



## BBB opening intervals



Monocytes from a smoker significantly increase Flow Cessation-Reperfusion dependent injury in the DIV-BBB in comparison to Monocytes from a non-smoker. **Panel J:** Flow cessation-reperfusion performed on viable DIV-BBB in the presence of circulating monocytes resulted in a biphasic BBB opening. **Panel K:** The time interval between the peak openings was significantly shorter and the peak opening time windows were drastically longer in the presence of monocytes isolated from smoker than non-smoker.