Human neutrophils (PMN) migrating across the gingival crevice during acute capsaicin challenge are fully activated.

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A novel model of acute neurogenic inflammation in the human oral cavity is reported in which a significant influx of PMN into saliva is observed at 120 min. following a single mouth rinse with 20ml of a 10% solution of capsaicin-containing Tabasco sauce (McIlhenny Co.,USA). 39 healthy, non-smoking subjects with no overt oral inflammatory disease were recruited with informed consent (21 M; 18F); average age 27.1 ± 1.4 yrs. Subjects showed a mean increase of 429% (±74.5) in total salivary PMN count measured by both haemocytometry and flow cytometry at 120 min. following Tabasco challenge (p < 0.001, Friedman test). The cell surface phenotype of the extravasating PMN indicated an activated status with increased CD11b (Integrin alpha M), reduced CD16 (Fc gamma receptor type III) and reduced CD62L (L-selectin) expression compared with blood-separated PMN quantified by fluorescence-labelled monoclonal antibody staining and flow cytometry. Functional confirmation of cell activation was achieved by measuring release of reactive oxygen species (ROS). PMN derived from blood by density gradient centrifugation, or from saliva pre- or post challenge, were incubated with 5 μM dichlorodihydrofluorescein diacetate (Invitrogen Ltd, UK) for 30 min. at 37°C. Mean fluorescence intensity readings for the FL-1 channel (green) were measured by flow cytometry with increased fluorescence proportionate to the level of superoxide anion generation. PMN elicited following capsaicin challenge (MFI 295±59 produced significantly more ROS than did PMN resident in the oral cavity prior to challenge MFI 166±34 or blood PMN from the same subject MFI 145±41 (n=8; p<0.01 (Wilcoxon test). PMN resident within the oral cavity or in blood could be further activated to release ROS in vitro by pre-incubation with 2μM fMLP (n=8; p=0.01 (Wilcoxon test) whilst ROS generation by PMN elicited by capsaicin stimulation could not be further enhanced. These data indicate a new, non-invasive model system for monitoring neurogenic inflammation within the human oral cavity. This could have future utility in testing orally-active anti-inflammatory modalities in vivo. Further we have demonstrated that the PMN migrating into the oral cavity in response to an acute neurogenic stimulus are maximally activated.