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Identification of potential PAR-regulating proteinases in agricultural dust using an activity-based serine proteinase probe

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Abstract

In previous work from our laboratories (PMIDs 26092994, 24152160, 21270400) we found that environmental allergens, including cockroach- and barn-derived particulates, contain proteolytic enzymes that contribute to lung inflammation by cleaving and activating proteinase-activated receptors (PARs). Preliminary work using in-gel zymography demonstrated the presence of multiple serine proteinase inhibitor-sensitive enzymes in hog barn dust extract (HDE; PMID 26092994). Because these HDE enzymes were blocked by alkylation of the active serine in many of the proteinases with PMSF and related agents, we reasoned that an activity-based serine proteinase probe (ABP; PMID16554154) would also be able to alkylate the enzymes in the HDE. Using this novel approach (PMID 21270400), we ABP-biotinylated the enzymes in HDE with ABPs selective for tryptic, chymotryptic, and elastase enzymes, respectively. Our data show differential reactivity of these ABP probes with enzymes in the HDE, identifying several enzymes in the mass range from 10 to 36 kDa, overlapping with the mass of ABP-labeled porcine trypsin (28 kDa). Porcine trypsin itself is a major PAR-regulating proteinase that is present in HDE, and this enzyme may play a prominent role in triggering lung inflammation in agricultural workers. Thus, HDE trypsin

and other proteinases represent novel therapeutic targets both for preventing dust-induced disease and for monitoring as environmental risk factors in many agricultural settings.

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