Factor XII Independent Pathways of Bradykinin Generation

Activation of human plasma is typically dependent on activation of Factor XII and any exogenous initiators of bradykinin formation (e.g endotoxin, sulfated mucopolysaccharides, salicates, uric acid crystals) are in fact initiators of Factor XII autoactivation. The subsequent steps are converting prekallikrein to kallikrein and cleavage of high molecular weight kininogen (HK) to release bradykinin. It should be noted that about 75-80% of plasma prekallikrein circulates bound to HK as a bimolecular complex.

Historically, the first evidence that bradykinin formation could occur in the absence of factor XII was studies of endothelial cells which observed that when the complex of prekallikrein and HK was bound, prekallikrein was converted to kallikrein. The “activator” was found to be heat shock protein-90 (HSP-90) liberated from the cell (or expressed along its surface) to form a trimolecular complex. Prekallikrein was converted to kallikrein in direct proportion to the HSP-90 present, thus the reaction is stoichiometric and if prekallikrein is not bound to HK, (in contrast to activated Factor XII) there is no conversion to kallikrein. This was reproduced by adding purified HSP-90 to prekallikrein-HK in the absence of cells and zinc ion is required. Thus activation of endothelial cells could result in bradykinin formation (yet to be proven) and the presence of Factor XII (which would normally be here) results in a marked increase in the reaction rate (i.e. become non-stoichiometric, as factor XII is activated to factor XIIa) due either to binding of Factor XII to endothelial cell gC1qR, which acts as a “surface” or by feedback enzymatic activation of factor XII by kallikrein. All these reactions, including the HSP-90 alternative, are inhibited by C1 inhibitor (C1 INH).
A needed laboratory “control” for the HSP-90-prekallirekin-HK reaction, employing purified proteins, is to leave out the HSP-90 or leave out the zinc. Although prekallikrein is not converted to kallikrein, the HK is cleaved to release bradykinin. Thus prekallikrein turns out to have enzymatic activity. The reaction with HK is inhibited by a synthetic peptide which prevents their stoichiometric binding or can be inhibited by corn trypsin inhibitor, which has no effect on kallikrein. The prekallikrein activation of HK is inhibited by C1 INH, thus it is not apparent in normal plasma. Since prekallikrein, thus far, has no demonstrable active enzymatic site, it appears that the active site is induced upon binding of prekallikrein to HK. Another permutation of these reactions is the fact that doing them in chloride buffers or phosphate buffers gives a different result. In chloride, prekallikrein cleaves HK to liberate bradykinin. In phosphate, not only is bradykinin released but the prekallikrein slowly auto-digests and auto-activates to kallikrein as if some HSP-90 or factor XIIa were present. Prekallikrein does not auto-activate in phosphate buffer unless bound to HK again suggesting that the active site is induced upon binding.

Finally we wished to demonstrate these reactions in hereditary angioedema plasma since it lacks C1 INH so that the active site within prekallikrein should be expressed. However plasma contains sufficient phosphate so that kallikrein will be formed. There are two possibilities to explain that. Either minute amounts of Factor XIIa present could activate prekallikrein or the prekallikrein-HK complex autoactivates so that the mechanism for HK cleavage and bradykinin formation could not be discerned. We therefore obtained factor XII deficient plasma and demonstrated that it could not be activated either by initiators of factor XII activation or upon
prolonged incubation. However immunoadsorption to deplete C1 INH lead to kallikrein formation and HK cleavage on incubation at 37°C which is also demonstrable in plasma from patients with type I HAE (see abstract this meeting – Joseph K and Kaplan AP).