SEMINAIRE IBS

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Keynote Seminar

Friday March 26, 2010

11 a.m. - IBS seminar room

By Edgar Pick

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Fateful attraction - When p67^{phox} meets Nox2

The superoxide (O_2.-) generating NADPH oxidase complex of phagocytes consists of membrane-embedded flavocytochrome b$_{558}$, composed of 91 kDa (gp91^{phox} or Nox2) and 22 kDa (p22^{phox}) subunits, and four cytosolic components: p47^{phox}, p67^{phox}, p40^{phox}, and the small GTPase Rac. The catalytic element, responsible for O_2.- generation by NADPH-dependent reduction of O_2, is Nox2 and the process is initiated by the translocation of the cytosolic components to the plasma membrane and their interaction with flavocytochrome b$_{558}$. The "purpose" of this process is the induction of a conformational change in Nox2 but opinions diverge as to its mechanism. The "monotheistic" hypothesis proposes that the conformational change in Nox2 is the consequence of the interaction of Nox2 with p67^{phox}. p47^{phox} and Rac serve only as facilitators of the Nox2 – p67^{phox} interaction. Our principal findings are: 1) By using "peptide walking", in which the binding of p67^{phox} to overlapping 15-mer Nox2 peptides is measured, we identified three binding sites for p67^{phox} on the cytosolic tail of Nox2; 2) Replacing Nox2 peptides binding p67^{phox} with the Nox4 peptide equivalents eliminates binding, in agreement with the fact that Nox4 is not regulated by p67^{phox}; 3) Cys 369 in Nox2 is essential for binding. A Nox2 peptide in which Cys 369 was replaced by Arg does not bind p67^{phox}. A Cys369Arg mutation is found in a Nox2^{+} (X91^{+}) case of chronic granulomatous disease; 4) A recombinant protein corresponding to a cytosolic segment of Nox2 (357-570), fused to NusA, which comprises all three binding sites for p67^{phox}, binds full-length p67^{phox} (1-526) and truncated p67^{phox} down to residue 198 (but not 186); 5) Deletion of residues 187-193 reduces binding of full-length p67^{phox} to NusA-Nox2 (357-570); 6) In a parallel study, we found that p67^{phox} peptides covering residues 259-279 inhibit NADPH oxidase activation in a cell-free system. Residues 259-279 are located in the N-terminal SH3 domain (SH3-N), the role of which in NADPH oxidase function was never established and which is absent in NOXA1; 7) Deletion of the SH3-N domain or of residues 259-279 results in enhanced binding of p67^{phox} to NusA-Nox2 (357-570) and p67^{phox} with residues 259-279 deleted shows enhanced binding to a Nox2 peptide (369-383); 8) Recombinant SH3-N and peptides, within the 259-279 sequence, interfere with binding of p67^{phox} to NusA-Nox2 (357-570). These and other findings lead to the proposal that p67^{phox} exists in two conformations: closed, in which there are one or more intramolecular bonds, and open, in which these bonds are absent. The main intramolecular bond is between residues in the 259-279 sequence, within SH3-N, and yet unidentified upstream residues. The «opening» of this bond allows access of key residues in p67^{phox} to their binding partners in Nox2.

Host : F. Fieschi (IBS/LPM)

Biography: Edgar Pick is professor emeritus at Tel Aviv University. In the 70s, he greatly contributed to the concept of «cytokine» in inflammation. Since 1980 he has been specialized in understanding the mechanisms of production of oxygen radicals in phagocytes to eliminate pathogens. Over the past 30 years, he was at the origin of major advances in the NADPH oxidase complex in neutrophils. Edgar Pick has published over a hundred publications in peer review journals and has been editor of several journals in the domain of immunity.

Axe : Immunity and host-pathogen interactions