REGULATION OF INDUCIBLE CYCLOOXYGENASE EXPRESSION DURING MONOCYTE DIFFERENTIATION INTO MACROPHAGES

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Background. The two isoforms of the enzyme cyclooxygenase (Cox-1 and Cox-2) convert arachidonic acid into prostaglandins that control the inflammatory response and the homeostatic balance between blood cells and vessels (Marnett et al., 1999).
Cox-2 expression is increased in human atherosclerotic plaques and correlates with the degree of inflammation and subsequent risk of plaque rupture and acute ischemic events (Schonbeck et al., 1999; Stemme et al., 2000). Within atheromata Cox-2 colocalizes ainly with macrophages/foam cells (Baker et al., 1999). Plaque macrophages derive from blood monocytes, which once inflitrated into the intimal space, undergo differentiation (Gerrity et la., 1981).

Aim of this study.
1. To investigate Cox-2 expression during differentiation into macrophages of human monocytes and monocytic cell line U937;
2. To explore the molecular mechanisms involved;
3. To develop a pharmacologic approach;

Results. Phorbol ester (PMA)-induced differentiation resulted in Cox-2 expression, assessed by Western blot analysis in human monocytes and U937 cells. Increased Cox-2 protein levels were consequent to increased mRNA levels, determined by RT-PCR analysis. The newly synthesized Cox-2 enzyme is functionally active, as determined by the evaluation of thromboxane $_{B2}$ and prostaglandin E_2 levels in cell supernatants. The degree of monocytic differentiation into macrophages which goes in parallel with Cox-2 induction, was assessed through the evaluation of the expression of the surface macrophage marker CD14 by flow cytometry.
The transcription factors NF-kB, the peroxisome proliferator activated receptor g (PPARg) and MAP-kinase ERK1/2, are all involved in Cox-2 expression observed during monocyte differentiation induced by PMA. Moreover, we show that reactive oxygen species (ROS), generated in the intracellular compartment, represent a pivotal signaling pathway involved in Cox-2 expression during monocyte transition into macrophages. In particular, we have characterized a role of the NADPH oxidase enzyme and of its subunits $p47^{phox}$, $gp91^{phox}$ and Rac2 in Cox-2 induction during differentiation. The involvement of the small G protein Rac2 is suggested by results obtained with both fluvastatin, that prevents its essential geranylgeranylation by interfering with the mevalonate pathway, and lethal toxin isolated from Clostridium sordellii which selectively inactivates Rac by monoglucosylation.

Conclusion. This study provides evidence that monocyte differentiation into macrophages is accompanied by Cox-2 expression. This event involves the activation of several trascription factors and is mediated by multiple signal trasduction pathways, among which the intracellular generation ROS plays a prominent role. This latter finding indicates that

the redox state of monocyte/macrophage is essential for the mounting of the inflammatory response within the atheroma.

References

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