

**CERAMIDE GENERATED IN THE OUTER LEAFLET
OF THE PLASMA MEMBRANE CONTROLS SIGNALING
OF FCIIA IMMUNORECEPTOR**

ABO BAKR ABDEL SHAKOR, KATARZYNA KWIATKOWSKA,
AGNIESZKA STRZELECKA-KILISZEK and ANDRZEJ SOBOTA*
Nencki Institute of Experimental Biology, Department of Cell Biology,
02-093 Warsaw, Poland

Raft microdomains have been demonstrated to serve as signaling platforms for numerous immunoreceptors. Upon activation, the receptors are accumulated in rafts where signaling tyrosine residues of the receptors are phosphorylated by Src-family kinases.

The question is what is the driving force promoting association of activated immunoreceptors with rafts and concomitant raft coalescence. We found that robust generation of externally oriented ceramide in U937 monocytes preceded association of activated Fc gamma receptor IIA (FcRIIA) with rafts and the receptor phosphorylation. Ceramide was released from sphingomyelin located in the outer leaflet of the plasma membrane. Ceramide production was correlated with activation of acid sphingomyelinase (ASMase). The enzyme was extruded from intracellular compartment to the cell surface evoking ceramide generation. ASMase translocation and ceramide generation in intact cells was demonstrated by immunoelectron microscopy studies performed on plasma membrane sheets prepared by mechanical cleavage of cells. FcRIIA clustering and association and the receptor phosphorylation were diminished under the influence of ASMase inhibitors. The inhibitory effects were reversed by incorporation of exogenous C16-ceramide to the plasma membrane of intact cells.

The presented data demonstrated that FcRIIA activation induces cell surface ceramide production. The lipid, due to its strong tendency to self-aggregation evokes fusion of activated FcRIIA with rafts and enables raft coalescence. This circumstance triggers the receptor phosphorylation by Src kinases located in rafts and downstream signaling is started.

Parts of the data were presented in *J. Biol. Chem.* 279:36778-36787 (2004).

* E-mail: a.sobota@nencki.gov.pl