SIGNAL TRANSDUCTION IN ACTIVATED AND INACTIVATED HUMAN NATURAL KILLER CELLS: THE ROLE OF PHOSPHOINOSITIDE METABOLISM AND GUANINE NUCLEOTIDE-BINDING PROTEINS

by

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ABSTRACT

TITLE: SIGNAL TRANSDUCTION IN ACTIVATED AND INACTIVATED NK CELLS: THE ROLE OF PHOSPHOINOSITIDE METABOLISM AND GUANINE NUCLEOTIDE-BINDING PROTEINS

Human natural killer (NK) cells are a population of lymphocytes capable of lysing tumor cell lines without prior antigen stimulation. Prior investigations suggested that hydroxyl radical production may be important in NK cell activation. Hydroxyl radical scavengers, n-propyl gallate and catechin, and a spin trap specific for hydroxyl radicals, 5,5-dimethyl-1-pyrroline-N-oxide, inhibited NK cell-mediated cytotoxicity in a dose-dependent manner. We used electron spin resonance spectroscopy to measure hydroxyl radical production by stimulated neutrophils or NK cells. Neutrophils incubated with opsonized zymosan showed hydroxyl radical production that peaked in five minutes whereas target cell-stimulated NK cells showed no hydroxyl radical production in the first fifteen minutes of activation.

Prior investigations showed that phosphoinositide metabolism was important in target cell-stimulated NK cell activation and that NK cells can be functionally inactivated by incubation with NK-sensitive target cells for 6 hr. We linked NK cell phosphoinositide metabolism to cytotoxicity by showing that inactivation of NK cells abrogated target cell-stimulated phosphoinositide metabolism and that IL 2-reactivation restored cytotoxicity and phosphoinositide turnover.

In many cell systems, guanine nucleotide binding proteins (G-proteins) couple cell surface receptors to second messenger pathways such as adenylate cyclase and phospholipase C. Cholera toxin but not pertussis toxin inhibited NK cell-mediated cytotoxicity and antibody-dependent cellular cytotoxicity in a dose-dependent manner. Cholera toxin induced cAMP accumulation, and the kinetics of inhibition of target cell lysis correlated with the kinetics of cAMP production. Cholera toxin and cAMP inhibited effector cell-target cell conjugation. Inhibition of cytotoxicity by cholera toxin and by a protein kinase C (PKC) inhibitor, H-7, were additive suggesting that the effects of cholera toxin were PKC-independent. Cholera toxin and pertussis toxin specifically ADP-ribosylated an NK cell membrane protein of 42 kd and 40 kd, respectively. Therefore, NK cytotoxic activities seem to be regulated by cAMP induction through a cholera toxin-sensitive G-protein.

Finally, we examined the direct role of G-proteins in NK cell activation. GTP and GDP analogues introduced into permeabilized NK cells did not alter NK cell-mediated cytotoxicity. However, mastoparan, a peptide that binds G-proteins and increases GTP binding, stimulated phosphoinositide metabolism in NK cells, thus indicating that a G-protein couples an unrecognized NK receptor to phospholipase C in NK cells.

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