Protein Phosphorylation as a Mechanism of Resistance Against Complement Damage

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INTRODUCTION

Complement inflicts cell damage by insertion of a membrane attack complex (MAC) comprised of the C5b, C6, C7, C8 and C9 proteins into the plasma membrane of target cells (Müller-Eberhard 1986). Nucleated cells vary in their sensitivity to complement-mediated lysis, however, generally, they are more resistant to immune damage than erythrocytes (Koski et al. 1983). It has been suggested that this resistance of nucleated cells is due to a postulated capacity of the cells to actively resist and repair complement damage (Ohanian and Schlager 1981). Recently, a Ca\(^{2+}\)-dependent process of rapid MAC removal from the surface of nucleated cells has been suggested to be involved (Carney et al. 1986; Morgan et al. 1987). Yet, the cellular regulatory mechanisms which support these and other postulated resistance/repair processes are poorly characterized.

Sub-lytic concentrations of MAC may elicit triggering events in nucleated cells, many of which are mediated by Ca\(^{2+}\) (Hänsch et al. 1988; Imagawa et al. 1983; Morgan 1989). MAC binding to platelets stimulates secretory responses and phosphorylation of platelet proteins (Wiedmer et al. 1987). Results presented herein demonstrate that sub-lytic concentrations of complement induce phosphorylation of membrane and cytosolic proteins in human leukemic cells. Furthermore, reagents which inhibit protein kinase activity are also shown to increase sensitivity of these leukemic cells to complement damage. Based on these results and others, we are proposing that protein kinases and protein phosphorylation play a major role in the resistance of nucleated cells to complement-mediated damage.

RESULTS AND DISCUSSION

Interference with protein, DNA and RNA synthesis can increase sensitivity of tumor cells to lysis by antibody and complement (Ohanian and Schlager 1981). That 3'5' cAMP can protect cells already damaged by complement from lysis (Boyle et al. 1976), further supported the notion that nucleated cells are equipped with a damage-repair mechanism. As shown in Fig. 1, in agreement with the latter finding, pretreatment (for 30 min at 37°C) of human leukemic cells with dibutyryl cAMP or reagents known to induce elevated intracellular cAMP level (3-isobutyryl 1-methyl xanthine (MIX) and forskolin) (Montague and Cook 1970; Daly 1984) reduced their sensitivity to lysis by antibody and human complement. Similar protection was induced in both K562 and U937 cells by phorbol 12-myristate 13-acetate (PMA), a known activator of protein kinase C (Nishizuka 1984). In contrast, four inhibitors of protein kinases, i.e. polymyxin B (PMB), N-(6-aminohexyl)-5-chloro-2- napthalene sulfonamide (W-7), 1-(5-isoquinolinyl sulfonyl)2-methylpipерazine

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(H-7) and tamoxifen (Nel et al. 1985; Hidaka et al. 1984; O’Brien et al. 1985) markedly enhanced complement-mediated cell lysis (Fig. 1). These results led us to propose (Fishelson et al. 1987) that processes of protein phosphorylation effected by cAMP-dependent protein kinase, protein kinase C and possibly other serine/threonine kinases (Edelman et al. 1987; Cohen 1988) promote cell resistance to complement damage.

![Fig. 1. Effect of chemical pretreatment on the sensitivity of K562 and U937 cells to lysis by antibody and complement. Human erythroleukemic K562 and histiocytic leukemic U937 cells (1 x 10⁵) were treated for 30 min at 37°C with various drugs (concentrations indicated in parenthesis) in 100 µl PBS. Rabbit antibodies were then added followed by NHS at concentrations which yielded 30-70% lysis (trypan blue inclusion after 60 min incubation at 37°C) of control cells preincubated without drugs. Results are expressed as the percent deviation from controls. Only the drug concentrations which produced the maximal effect are presented, i.e. higher and lower concentrations were less or as effective. None of the drugs was toxic to cells at these concentrations.](image)

To determine whether complement activation can indeed trigger protein phosphorylation in cells under attack, K562 cells were pre-equilibrated for 60 min at 37°C with [32P]-orthophosphoric acid (0.5 mCi, NEN, Boston) in phosphate-free medium, washed and exposed to normal human serum (NHS) or heat-inactivated (HI, 30 min at 56°C) NHS for 60 min at 37°C. The cells were pretreated or not with rabbit anti-K562 cells antibodies (Ab) during the last 30 min of the pre-equilibration with [32P]-orthophosphate. In all cultures, cell viability at the end of the experiment was above 85%. The cells were then washed, disrupted by sonication and sedimented at 100,000 x g for 30 min. The supernatant (cytoplasmic fraction) was removed and the membrane pellet was solubilized in 1% NP-40 and sedimented (as above) to remove insoluble material. All procedures were performed at 4°C in presence of protease and phosphatase inhibitors. Samples of the cytoplasmic and membrane preparations were subjected to TCA (10%) precipitation on ice. The radioactivity and protein concentration of the resulting protein pellets were determined; and the quantity of 32P incorporated per mg protein in each sample was then calculated. In absence of antibody, NHS caused a 46% increase in phosphorylation of membrane proteins when compared with HI-NHS (p < 0.01 in a t-test) (Fig. 2). A similar increase in membrane proteins phosphorylation (p < 0.05) was induced by NHS in antibody-pretreated cells. An increase in phosphorylation of cytoplasmic proteins also occurred upon NHS and Ab + NHS treatment as compared with HI-NHS AND Ab + HI-NHS, respectively, but the differences were not statisti-