EARLY CELLULAR EVENTS IN THE INDUCTION OF MURINE HEPATITIS VIRUS (MHV-3) INDUCED MACROPHAGE PROCOAGULANT ACTIVITY (PCA)

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INTRODUCTION

Interaction of a membrane receptor with its ligand results in the generation of second messengers which ultimately leads to alteration in cell function either by changes in nuclear transcription, or activation/inactivation of cellular enzymes by phosphorylation events. Although the cell-surface MHV receptor has been cloned and sequenced[1], the biochemical events leading to the expression of MHV-3 induced PCA after the binding of the virus to its receptor are unknown.

The mechanism of induction of tissue factor (TF), a distinct and separate procoagulant from the MHV induced prothrombinase has been explored by several investigators and is controversial. Lyberg and Prydz have demonstrated that induction of TF by human monocytes is a protein kinase C (PKC) dependent event, as TF could be induced by phorbol esters[2]. Furthermore, despite the findings that TF induction was calcium dependent and that the calcium ionophore A23187 was capable of inducing TF[3,4], no detectable changes in cytosolic calcium were associated with TF induction[3]. In contrast, Kucey et al. suggest that calcium ionophores and phorbol esters do not result in TF expression[5]. The differences in these results may be related to the cell populations studied, species of origin (human or rodent) and/or the incubation and culture conditions.

Experiments examining the early cellular events in the induction of the PCA by MHV-3 have recently been conducted, based upon the observation that 16,16 dimethyl prostaglandin E_2 (dmPGE) inhibits the induction of macrophage PCA[6,7,13]. As the actions of dmPGE are thought to de dependent upon an increase in intracellular cAMP[8,9], the effects of two agents which raise intracellular cAMP, forskolin, an adenylate cyclase agonist[10], and isobutylmethylxanthine, a phosphodiesterase inhibitor[11], have been examined. Both of these agents were able to attenuate the induction of macrophage PCA by MHV-3, consistent with the inhibitory action of dmPGE (Fig. 1.). Thus, cAMP would appear to have a downregulatory action on prothrombinase expression. Interestingly, although prothrombinase is functionally inhibited by dmPGE, by Western immunoblot and immunofluorescence analysis, antigenic expression is still present. This suggests that there are post-translational modifications to the protein which abrogate its activity as opposed to inhibition of transcription or translation. The contribution of other prostanoids in the regulation of PCA induction is less clear. Prostacyclin (PGI_2) has some inhibitory effects, but only at relatively high concentrations (100 uM), whereas PGF_{2a} has no effect on PCA induction, even at high concentrations[13] (Fig. 2).

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Although macrophages produce leukotriene B_4 (LTB_4) when stimulated with MHV-3[13], it does not appear that LTB_4 is a prerequisite for induction of PCA. At a time when induction of PCA is evident (2 hours), LTB_4 levels are minimal, and LTB_4 by itself cannot induce PCA, nor does it augment the activity in MHV-stimulated macrophages. PCA induction is not inhibited by the specific LTB_4 antagonist MK886. However, the 5-lipoxygenase inhibitor nordihydroguaretic acid (NDGA) does inhibit induction of PCA. This would suggest that leukotrienes may be involved in the induction of PCA, although as NDGA is not a specific agent, it is possible that its action may involve inhibition and/or scavenging of oxygen free radicals.

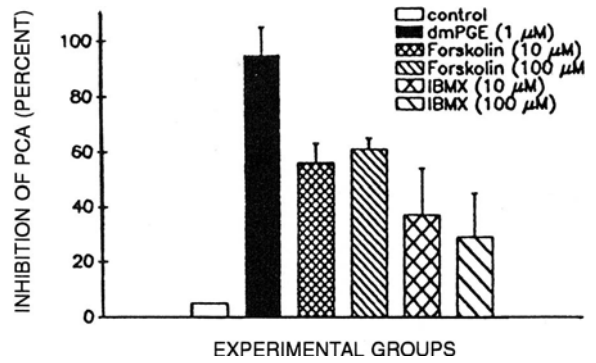

Figure 1. Inhibitory effects of agents that modify cAMP on the induction of procoagulant activity by MHV-3. 1 x 10^6 macrophages were treated with dmPGE (1 um), forskolin (10-100 uM) or IBMX (10-100 uM) 30 min. prior to infection with 1 x 10^6 PFU of MHV-3. Cells were incubated a further 6 hours, washed, frozen, and assayed for PCA in a one stage clotting assay.

The role of G-binding proteins, a heterotrimeric family of related membrane proteins that are involved in the coupling of cell surface receptors to effector enzymes[14], has been examined using pertussis toxin (PT). PT is a bacterial toxin that acts upon the *alpha* subunit of the G-binding protein resulting in ADP-ribosylation[14]. This covalent modification results in interference with the interaction between the G-binding protein and the membrane surface receptor. PT significantly inhibits induction of PCA by MHV in macrophages from susceptible Balb/cJ mice suggesting that G-binding proteins play an important role in prothrombinase activity (Fig. 3.). The recent availability of specific probes for specific G-binding proteins[15] will allow for the examination for constituitive differences in G-binding proteins in cells from resistant and susceptible strains of mice.

Calcium transients, however, do not appear to be involved in MHV-induced PCA. Following infection of macrophages by MHV, there are no detectable changes in the concentration of intracellular calcium, as measured fluorometrically using the dye Indo 1-AM. The calcium ionophores ionomycin and A23187 do not induce appreciable levels of PCA within the time period that PCA is induced by MHV (1-4 hours). However, MHV cannot induce an increase in PCA in macrophages cultured in calcium-free media, thus defining an absolute requirement for calcium. (Fig. 4).

Protein kinase C (PKC) is a calcium-dependent enzyme that is activated following the hydrolysis of cell membrane inositol phospholipids. The phorbol ester phorbol myristate acetate (PMA) had no effect on the induction of PCA in unstimulated macrophages from Balb/cJ mice following short incubation periods (1-4 hours). In contrast, inhibitors of PKC, staurosporine and H7, significantly inhibited MHV-induced PCA suggesting that PKC plays a role in its expression.

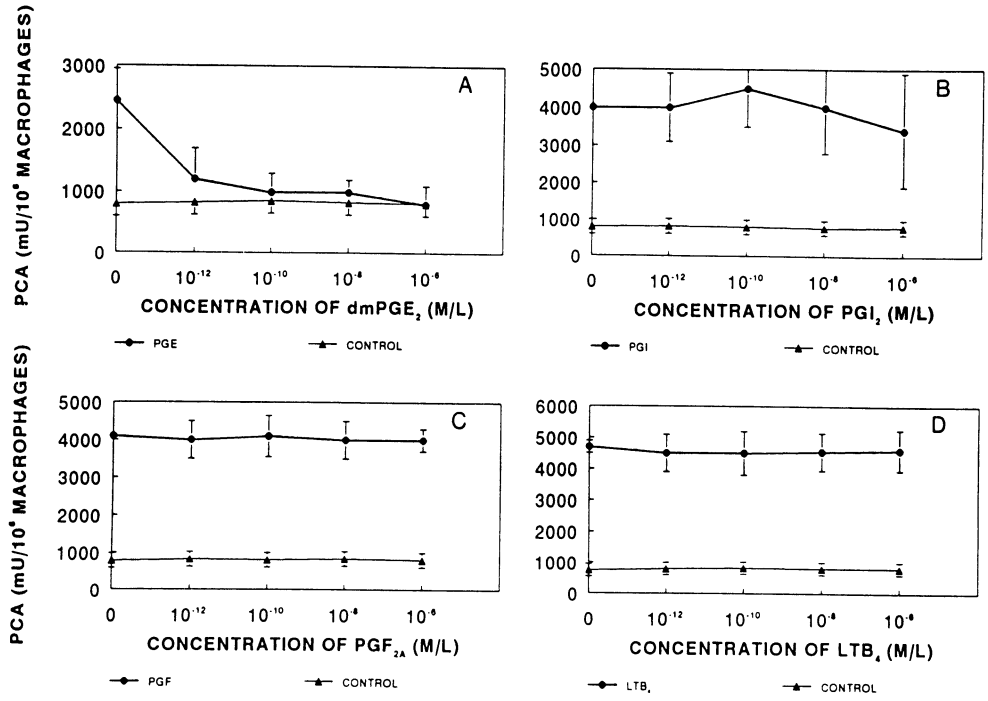

Figure 2. Ability of eicosanoids to inhibit MHV-3 induced procoagulant activity. 1 x 10^6 macrophages were pretreated with varying concentrations of dmPGE, PGI_2 or PGF_{2a} 1 hour prior to infection with 1 x 10^6 PFU MHV-3. The cells were incubated for 12 hours, then assayed for PCA in a one stage clotting assay. A=treatment with dmPGE; B=treatment with PGI_2; C=treatment with PGF_{2a}; D=treatment with leukotriene B_4.

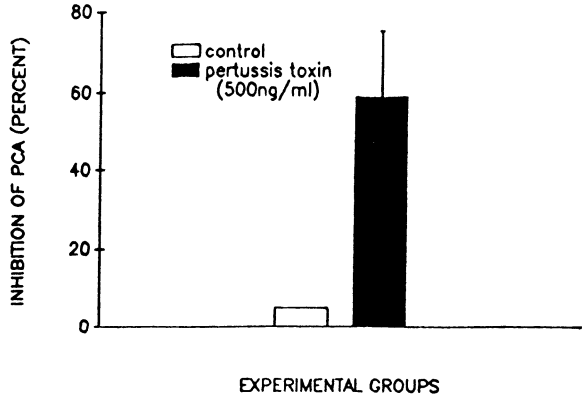

Figure 3. Inhibitory effect of pertussis toxin on the induction of procoagulant activity by MHV-3. 1 x 10^6 macrophages were treated with 500 ng/ml pertussis toxin for 1 hour prior to infection with MHV-3 (10^6). Cells were incubated a further 6 hours, then washed, frozen and assayed for PCA in a one stage clotting assay.

Clearly, much work remains in the elucidation of the biochemical events required for the induction of PCA in response not only to MHV, but also other stimuli. It is possible that differences in macrophage signal transduction pathways may determine whether the cell is capable of expressing procoagulant activity, and these differences may translate into determining whether an organism is resistant or susceptible to a particular stimulus.

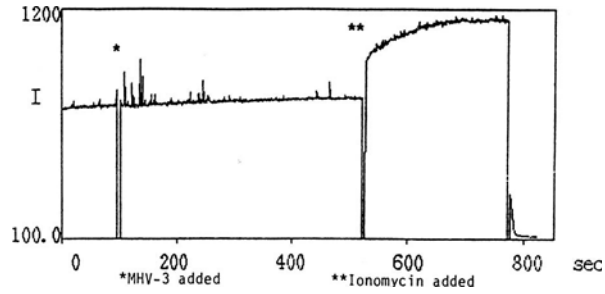

Figure 4. Cytosolic calcium $[Ca^{2+}]_i$ changes in response to stimulation by MHV-3 (M.O.I. of 1.0). $[Ca^{2+}]_i$ was determined as described.

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