Macrophages play an important role in the pathogenesis of inflammation and other diseases. An early event in the activation of macrophages is the release of arachidonic acid (AA) and the subsequent formation of eicosanoids such as prostaglandins (PG), leukotrienes (LT), and thromboxanes (TX) (1). The metabolism of eicosanoids and their role in the inflammatory response have been described (2). The rate-limiting event in the biosynthesis of these inflammatory mediators is believed to be controlled by a phospholipase type enzyme, the simplest mechanism being the release of AA from the sn-2 position of phospholipids by a phospholipase A2 (PLA2) (3).

Even though several different PLA2’s have been isolated and characterized from a variety of macrophages and monocytic cell lines (3-9), the mechanism by which these enzymes are regulated in intact cells is still poorly understood. Since we have previously isolated and characterized (4-7) several phospholipases from the macrophage-like P388D1 cell line (10), we chose this cell line to study the mechanisms involved in cell activation and the intracellular signaling leading to the release of AA. The formation of prostaglandin E2 (PGE2), a major AA metabolite in this cell line (11), was used to measure cell activation following lipopolysaccharide (LPS) priming and stimulation with platelet-activating-factor (PAF) (12).

**PAF Stimulation of P388D1 Cells.**

Stimulation of P388D1 cells with PAF (20 nM) causes a receptor-mediated release of AA which is metabolized to PGE2 (see Figure). This release is increased significantly if the cells are pretreated with low doses of LPS (200 ng/ml) (12). Shortly after PAF stimulation, the inositol-trisphosphate (IP3) levels are markedly increased. This increase in IP3 is independent of LPS pretreatment and is not observed if the calcium ionophore A23187 (0.5 μM) is used as a stimulus (13). The increased IP3 production and the subsequent PGE2 formation are inhibited by pretreating the cells with pertussis toxin (13).

This implies that the PAF signal is transmitted via a PTX-sensitive G-protein to a phospholipase C that then liberates IP3 from phosphatidylinositol diphosphate. It has been shown in other systems that an increase in IP3 leads to an increase in cytosolic calcium concentration ([Ca2+]c) (14).

In peritoneal macrophages, PAF causes a biphasic rise in [Ca2+]c (15). While the initial increase is independent of extracellular Ca2+ (15), the second increase is probably due to the influx of exogenous Ca2+ (15,16). The release of AA in peritoneal macrophages is also dependent on the influx of...
extracellular Ca\(^{2+}\) (17). We, therefore, studied the role of intra- and extracellular Ca\(^{2+}\) in PAF stimulation of P388D\(_1\) cells. We have found that the PAF-stimulated PGE\(_2\) formation in P388D\(_1\) cells is dependent on an increase in [Ca\(^{2+}\)]\(_i\). If the augmentation of [Ca\(^{2+}\)]\(_i\) is buffered by BAPTA (18), the PGE\(_2\) production is reduced to basal levels. La\(^{3+}\) and EDTA experiments have shown that the PAF induced rise in [Ca\(^{2+}\)]\(_i\) requires neither the influx nor the presence of exogenous Ca\(^{2+}\) (13). The PAF induced increase in [Ca\(^{2+}\)]\(_i\) in P388D\(_1\) cells, therefore, seems to be the result of the release of Ca\(^{2+}\) solely from intracellular stores.

We have shown that protein kinase C most likely does not play a significant role in the P388D\(_1\) signaling system (12). However, genistein, a reportedly selective inhibitor of tyrosine-specific protein kinase (19), was a potent inhibitor of PAFstimulation of previous primed cells with an apparent IC\(_{50}\) of 7 \(\mu\)M, but only partially inhibited LPS priming. Furthermore, primed PAF stimulation of PGE\(_2\) formation is blocked by cyclohexamide (10 \(\mu\)M) (12). These results suggest that the primed PAF stimulated production of PGE\(_2\) in P388D\(_1\) cells is dependent on protein synthesis and possibly protein phosphorylation mediated by protein kinases other than protein kinase C. Others have also found that receptor-mediated stimulation of AA metabolism in macrophages is dependent on protein synthesis (20,21). How translation is induced by PAF stimulation, however, remains unclear.

LPS Priming of P388D\(_1\) Cells.

LPS is a potent modulator of macrophage function (22). Even though LPS is a poor trigger of AA release and eicosanoid production in P388D\(_1\) cells (11), LPS primes these cells for an enhanced PGE\(_2\).