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A possible role for mono(ADP-ribosyl)transferase in the signalling pathway mediating neutrophil chemotaxis

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1 Mono(ADP-ribosyl)transferase activity has been identified on the external surface of human polymorphonuclear neutrophil leucocytes (PMNs). The enzyme is released from the plasma membrane by phosphoinositide-specific phospholipase C, suggesting a glycosylphosphatidylinositol (GPI) linkage of the enzyme to the plasma membrane. Partial sequence of cDNA encoding the enzyme suggests that it is identical to the GPI-linked mono(ADP-ribosyl)-transferase identified previously on human skeletal muscle.

2 A panel of inhibitors of mono(ADP-ribosyl)transferase (including vitamins K_1 and K_3, novobiocin and nicotinamide) showed a rank order of inhibitory potency similar to that described for other mono(ADP-ribosyl)transferases. Furthermore, the mono(ADP-ribosyl)ation of agmatine was inhibited also by diethylamino(benzylidineamino)guanidine (DEA-BAG), another substrate of the enzyme related structurally to arginine.

3 There was a close linear correlation between the IC_{50} values for inhibition of mono(ADP-ribosyl)ation of agmatine by DEA-BAG or the enzyme inhibitors and their IC_{50} values for inhibition of receptor-dependent polymerization of cytoskeletal actin and chemotaxis.

4 These results suggest a role for mono(ADP-ribosyl)transferase in the transduction pathway involved in receptor-dependent re-alignment of the cytoskeleton during neutrophil chemotaxis.

Keywords neutrophil leucocytes mono(ADP-ribosyl)transferase chemotaxis actin polymerization

Introduction

Chemotaxis

Human polymorphonuclear neutrophil leucocytes (PMNs) exhibit chemotaxis and are competent to migrate up a chemical gradient both *in vitro* and *in vivo*. An early event following exposure of the cell to chemotaxin is a shape change, with transformation to a torpedo-like morphology. The cell is aligned in its long axis in the direction of the gradient, with polarization of the receptors for the chemotaxin at the leading edge [1]. Movement of the cell through the gradient towards a region of greater concentration of the chemotaxin requires selective adhesion of the cell at its leading edge to the extracellular matrix or adjacent cells, and alignment of the microfilamentous elements of the cytoskeleton in the direction of the gradient. Thereafter, there is contraction of the microfilaments with simultaneous release at the trailing edge of the cell. In this way the body of the cell is pulled up through the gradient (Figure 1).

Continued movement up the gradient then requires the cell to push out an extension or lamellipodium from the leading edge of the cell [2]. There is further re-alignment of the cytoskeleton in the direction of the gradient, and re-attachment of the leading edge of the lamellipodium to the extracellular matrix. With release of the trailing edge, the cell is then set for further microfilament contraction and cell movement [1, 2].

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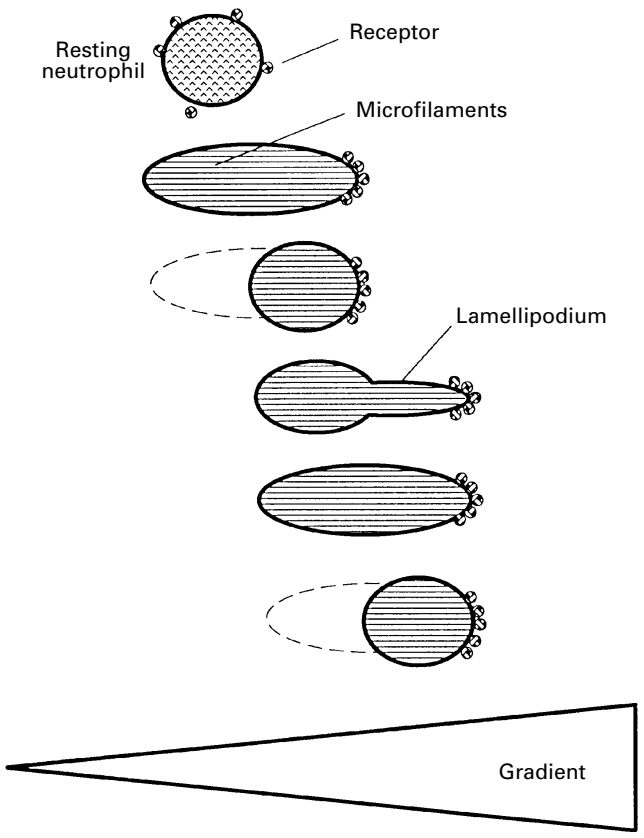

Figure 1 Chemotaxis of polymorphonuclear neutrophil leucocytes. The upper element shows a resting neutrophil with receptors for chemotaxin dispersed across the cell surface. In the presence of a chemotactic gradient, the receptors are polarised to the leading edge and the cell adopts a torpedo-like morphology with its long axis in the direction of the gradient. By a process of sequential adhesion and release of the leading and trailing edges from the extracellular matrix, contraction of cytoskeletal microfilaments draws the cell up through the gradient. The cells are competent also to extend outgrowths (lamellipodia) upfield into the gradient.

The polymerization of monomeric (globular, G) actin to filamentous (F) actin is a complex process, and is reviewed in [3]. Initiation of the polymerization occurs by a process of nucleation, in which short oligomeric assemblies of actin form the focus for the further addition of G actin monomers. The actin monomers are assymetric with a blunt end and sharp (or pointed) end. Extension occurs by addition of G actin monomers to the blunt end of the growing microfilament, with a sharp-to-blunt end alignment. The assembly requires ATP (or possibly ADP), which is coupled to the actin monomers prior to assembly. Additions to the growing microfilament are made in the region of plasma membrane, where the microfilament is tethered to specific transmembrane anchoring proteins. The anchoring points for cytoskeletal microfilaments are complex, comprising several classes of proteins with either high or low affinity binding interactions between each other and the actin-containing microfilament. A simplified version of the anchoring point, showing some of the proteins known to be involved with high affinity interactions, is shown in Figure 2. A more comprehensive account can be found in [4].

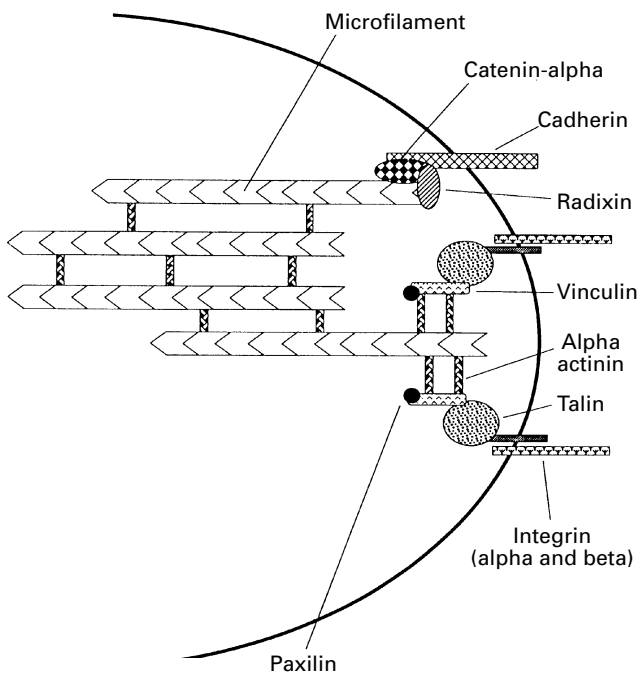

Figure 2 Anchoring points of cytoskeletal microfilaments at the plasma membrane. Actin-containing microfilaments are illustrated with a complex of proteins anchoring them to the plasma membrane. The cartoon shows only proteins with high affinity interactions with the cytoskeleton; other proteins (not shown) with lower affinity for the cytoskeleton appear to serve a role as regulators of microfilament assembly and extension.

Signalling in chemotaxis

Numerous receptors are expressed on the surface of PMNs which recognise chemotaxins. These include receptors for formyl-Met-Leu-Phe (FMLP) [5], the complement fragment C5a [6], interleukin 8 (IL-8) [7] and platelet activating factor (PAF) [8]. In each case, the receptors are coupled via G proteins to phospholipase C, and activation of the receptors by agonist facilitates hydrolysis of phosphatidylinositol 4,5-*bis*phosphate to yield inositol 1,4,5-*tris*phosphate (IP_3) and diacylglycerol (DAG). The functions of these second messengers are well understood, and are known to include IP_3-dependent release of Ca^{2+} ions from endoplasmic reticulum, and DAG-dependent activation of protein kinase C (PKC) [9].

The relationship between the activation of PKC and either chemotaxis or cytoskeletal re-alignments is however very unclear. Furthermore, much of the relevant published work is contradictory [10,11]. Activation of PKC has been proposed as a critical step in actin polymerization or chemotaxis with responses mediated by phorbol ester or DAG [12], and inhibition by selected PKC inhibitors [11]. In contrast, other reports describe (i) the polymerization of actin following exposure to PKC inhibitors [13], and (ii) no capacity of phorbol ester to mimic FMLP-dependent assembly of F actin [14]. The relationship between receptor-dependent increases in $[Ca^{2+}]_i$ and chemotaxis or actin polymerization is similarly ambiguous. There are many reports implicating increases in $[Ca^{2+}]_i$ in chemotaxis [15], although others have observed (i) no changes in

$[Ca^{2+}]_i$ during chemotaxis following exposure of cells to FMLP [16], and (ii) dissociation of actin-containing microfilaments mediated by rises in $[Ca^{2+}]_i$ [17]. Clearly, there is much uncertainty about the primary signalling events mediating re-alignment of the cytoskeleton of PMNs during chemotaxis. Results are presented below which suggest that an Arg-specific mono(ADP-ribosyl)transferase may be involved in these processes.

Mono(ADP-ribosyl)transferase

Mono(ADP-ribosyl)transferases (EC 2.4.2.31) catalyse a two-step reaction, in which they (i) cleave NAD^+ to yield ADP-ribose and nicotinamide, and then (ii) facilitate the addition of ADP-ribose to a specific amino acid acceptor in a polypeptide [18]. Both Arg [19] and Cys-specific [20] eukaryotic mono(ADP-ribosyl)transferases have been reported, involving N- or S-glycosidic linkages to ADP-ribose respectively. The reactions they catalyse are thus similar to the ADP-ribosylation mediated by cholera or pertussis toxins, although the prokaryotic and eukaryotic enzymes are structurally quite unrelated.

There is still some uncertainty about the identity of Cys-specific eukaryotic mono(ADP-ribosyl)transferases since NAD^+ glycohydrolases yield ADP-ribose, and the covalent addition of free ADP-ribose to Cys residues is a relatively facile reaction that occurs non-enzymatically. Cys-specific isoforms of NAD^+ glycohydrolase with mono(ADP-ribosyl)transferase activity have however been reported [21]. Arg-specific mono(ADP-ribosyl)-ation occurs enzymatically by the addition of ADP-ribose to the guanidino nitrogen of a specific Arg residue. Several isoforms of the enzyme have been purified from turkey erythrocytes [19, 22–24] and rabbit skeletal muscle [25–27], and differences have emerged in their cellular distribution, regulatory, physical and kinetic properties. Arg-specific mono(ADP-ribosyl)transferases have more recently been cloned from rabbit [27] and human skeletal muscle [28] and unidentified cells in avian bone marrow [29]. The different enzymes show significant structural homology to each other, and about 40% homology with the RT6.2 NAD^+ glycohydrolase [29]. The deduced amino acid sequences of cloned mammalian mono(ADP-ribosyl)-transferases reveal that this enzyme is typical of a protein linked to membrane by a glycosylphosphatidylinositol (GPI) anchor. GPI-linked proteins are almost always located on the outer aspect of the plasma membrane, and this orientation of GPI-linked mono(ADP-ribosyl)transferase was later confirmed biochemically [28].

The protein substrates of eukaryotic Arg-specific mono(ADP-ribosyl)transferase have been explored [30], although much of this work has been performed with purified enzyme or in subcellular fractions. Measurement of mono(ADP-ribosyl)transferase activity in intact skeletal myocytes *in vitro* yielded a single 97 kDa substrate on the cell surface, which was identified as integrin α7 [31]. This is a muscle-specific and developmentally regulated matrix-binding integrin, which assembles with β1 integrin to form a receptor for laminin. There are as yet no reports of the consequences of the ADP-ribosylation of this particular laminin receptor, but clearly this modification might regulate various aspects of cell adhesion to matrix, or signalling responses mediated by the α7β1 dimer.

Results

Mono(ADP-ribosyl)transferase of human PMNs

In preliminary studies, we demonstrated the capacity of intact PMNs to ADP-ribosylate agmatine. The method used was a modification of previously published protocols [32, 33]. Inspection of Figure 3 shows that agmatine has an α-amino group as well as a guanidino nitrogen, and purely structural considerations led us to conclude that free ADP-ribose (if it were present) might form an adduct non-enzymatically with the α-amino moiety. We therefore undertook a study to exclude the possibility that the enzymatic event on the surface of PMNs was limited to NAD^+ glycohydrolase activity (with release of free ADP-ribose, and subsequent non-enzymatic addition of ADP-ribose to the α-amino group of agmatine). We synthesised diethylamino(benzylidineamino)guanidine (DEA-BAG) by the method of Soman *et al.* [34] and confirmed its purity and structure (Figure 3) by t.l.c. and desorption electron impact ionization mass spectrometry. DEA-BAG was then used as substrate in a labelling reaction with $[\alpha^{32}P]$-NAD^+ in the absence or presence of intact PMNs. The product, (ADP-ribosyl)-DEA-BAG, was purified using two separate high pressure liquid chromatography (h.p.l.c.) systems, and its chromatographic profile was shown to be identical with authentic (ADP-ribosyl)-DEA-BAG (prepared by the ADP-ribosylation of DEA-BAG in the presence of cholera toxin). The structure of the (ADP-

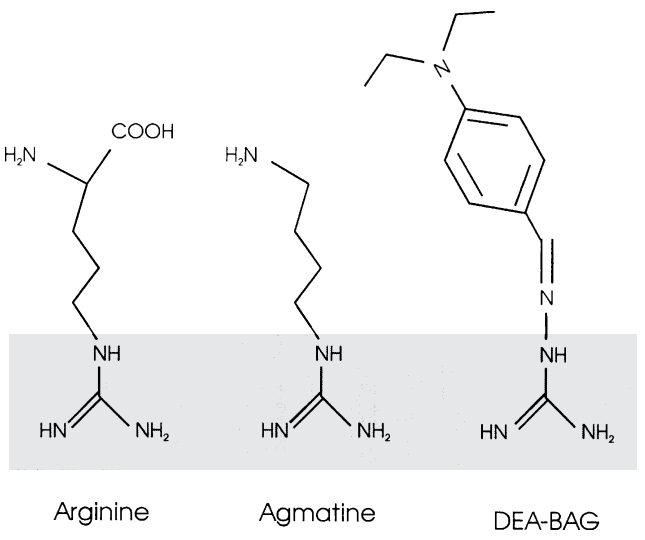

Figure 3 The structures of arginine, agmatine and diethylamino(benzylidineamino)guanidine (DEA-BAG). The shaded area shows the guanidino group available for enzymatic mono(ADP-ribosyl)ation.

ribosyl)-DEA-BAG was confirmed by electrospray mass spectrometry. The ADP-ribosylation of DEA-BAG was dependent on the presence of intact PMNs, and incubation of DEA-BAG with free ADP-ribose did not yield (ADP-ribosyl)-DEA-BAG. From these results we concluded that Arg-specific mono(ADP-ribosyl)-transferase activity is found on the surface of these cells.

Exposure of PMNs to phosphoinositide-specific phospholipase C released approximately 98% of mono(ADP-ribosyl)transferase catalytic activity from the cell surface, which suggests strongly that the PMN enzyme has a GPI side chain anchoring it to the plasma membrane. More recently, we have cloned the cDNA from human PMNs encoding mono(ADP-ribosyl)-transferase. At the time of writing, partial sequence of the cDNA suggests a typical GPI-linked protein, which is identical to the sequence of cDNA encoding mono(ADP-ribosyl)transferase of human skeletal muscle [28].

Measurement of mono(ADP-ribosyl)transferase activity in PMNs using agmatine as the substrate revealed that the enzyme is expressed at a level of no more than 1/50th of that found in human skeletal muscle. At saturating NAD^+ concentrations, we obtained V_{max} values of 1.4 ± 0.2 pmol (ADP-ribosyl)agmatine/h/10^6 cells, and the K_m value for NAD^+ was 100.1 ± 30.4 μM (mean ± s.e. mean; $n = 4$). The K_m value obtained was similar to that found in rat cardiac muscle [33].

Inhibitors of mono(ADP-ribosyl)transferase activity

There are no specific inhibitors of this enzyme, and so the assignment of a functional role for mono(ADP-ribosyl)ation in any particular biological process must be based on a tight correlation between K_i values for the inhibition of mono(ADP-ribosyl)transferase activity in that cell or tissue and inhibition of the particular biological event. Nicotinamide is a product of NAD^+ cleavage by mono(ADP-ribosyl)transferase, and it serves as an effective inhibitor of the enzyme activity. There are also numerous structural analogues of nicotinamide which inhibit mono(ADP-ribosyl)transferase activity, in addition to molecules unrelated structurally to nicotinamide such as the antibiotic novobiocin and vitamins K_1 and K_3 which also inhibit the enzyme [35, 36]. Finally in this context, Arg analogues which act as efficient substrates of mono(ADP-ribosyl)transferase can also be used to inhibit the mono(ADP-ribosyl)ation of endogenous substrate(s) in a particular cell or tissue. We have exploited DEA-BAG which inhibits the ADP-ribosylation of agmatine (and indeed the endogenous substrates-see below) by mono(ADP-ribosyl)transferase of human PMNs.

Mono(ADP-ribosyl)transferase, chemotaxis and receptor-dependent actin polymerization

A panel of inhibitors of mono(ADP-ribosyl)transferase activity and DEA-BAG were compared for their capacity to inhibit the ADP-ribosylation of agmatine in the presence of intact PMNs. Enzyme activity was measured in the absence or presence of selected concentrations of these compounds, and the concentration required for 50% inhibition of enzyme activity (IC_{50}) determined. Measurements were also made of PMN chemotaxis in response to 100 nM FMLP. The protocol involved migration of cells through cellulose nitrate filters in a modified Boyden chamber, with quantification of cell movement by the leading front method. Chemotaxis was then measured in the absence or presence of selected concentrations of the various inhibitors or DEA-BAG, and IC_{50} values determined in each case. A correlation was then made between the IC_{50} values for inhibition of chemotaxis and inhibition of the mono(ADP-ribosyl)-ation of agmatine ($r^2 = 0.873$, $P < 0.0001$). The results are shown in Figure 4a.

A similar approach was adopted to examine the possibility of a link between mono(ADP-ribosyl)-transferase activity and receptor-dependent actin polymerization. The assembly of G actin into cytoskeletal microfilaments may be quantified by the binding of 7-nitrobenz-2-oxa-1,3-diazole (NBD)-phallacidin to F actin in permeabilized cells. The abundance of the fluorescent probe is quantified in a fluorescence activated cell sorter, with an excitation wavelength of 488 nm, and emission recorded at 525 nm [37]. Actin polymerizes rapidly after exposure of PMNs to chemotaxin, with peak fluorescence intensity at about 40 s. The polymerization of actin was inhibited by the panel of mono(ADP-ribosyl)transferase inhibitors and DEA-BAG, and IC_{50} values for each compound were determined in a series of experiments examining the inhibition of actin polymerization at selected concentrations of the mono(ADP-ribosyl)transferase inhibitors.

A comparison was then made of the IC_{50} values for inhibition of mono(ADP-ribosyl)transferase activity and inhibition of receptor-dependent actin polymerization (Figure 4b; $r^2 = 0.905$; $P < 0.0001$). Inspection of the results in Figure 4 reveals a close correlation between the IC_{50} values for inhibition of mono(ADP-ribosyl)-transferase activity and inhibition of both receptor-dependent chemotaxis and actin polymerization. Furthermore, DEA-BAG, which is an efficient substrate for mono(ADP-ribosyl)transferase, also showed similar IC_{50} values for inhibition of the mono(ADP-ribosyl)-ation of agmatine (187 ± 28 μM; $n = 3$) and inhibition of receptor-dependent chemotaxis (97.3 ± 17.2 μM; $n = 4$) or actin polymerization (213 ± 62 μM; $n = 3$).

Inhibitors of mono(ADP-ribosyl)transferase and changes in $[Ca^{2+}]_i$

The role of Ca^{2+} ions in cytoskeletal reorganization is complex and appears to be involved at several stages, most particularly those involved in dissociation of filamentous actin. Contractile responses mediated by several cytoskeletal proteins are also dependent on changes in $[Ca^{2+}]_i$ (see above). We addressed the possibility that the inhibition of chemotaxis or receptor-dependent actin polymerization by inhibitors of

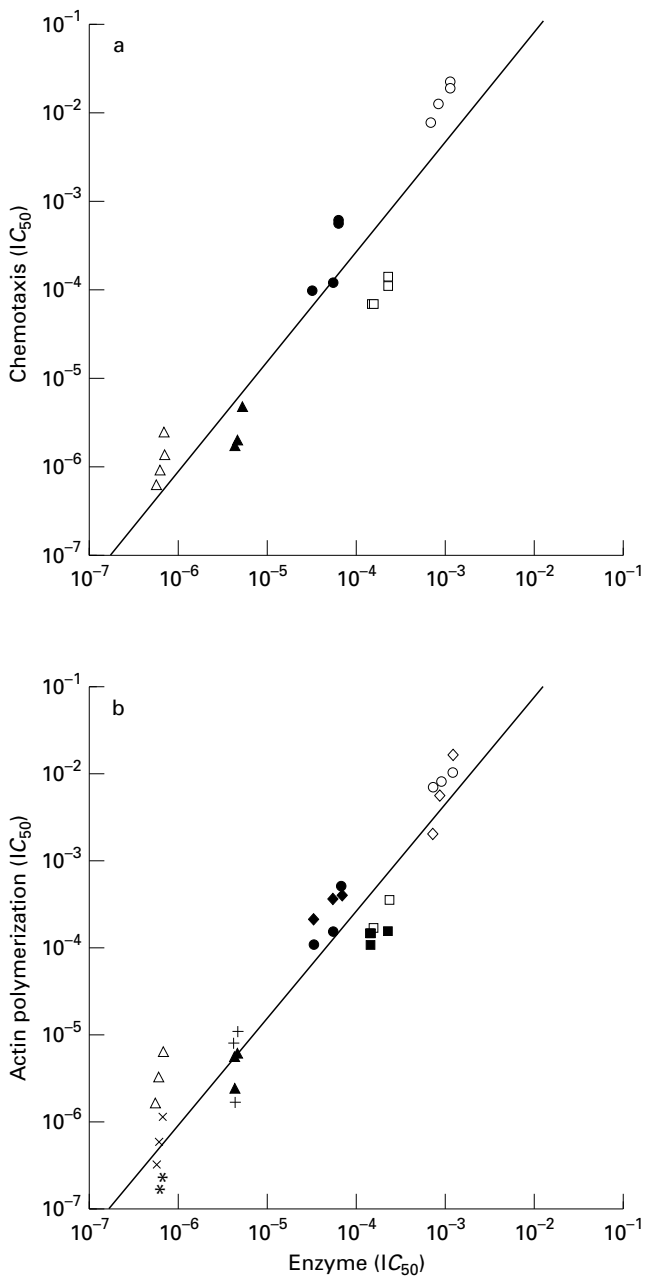

Figure 4 a) Inhibition of mono(ADP-ribosyl)transferase activity and receptor-dependent chemotaxis in human neutrophil polymorphs. Measurement were made of the IC_{50} values of nicotinamide (○), novobiocin (●), vitamin K_3 (▲), vitamin K_1 (△) and DEA-BAG (□) for inhibition of the mono(ADP-ribosyl)ation of agmatine and inhibition of chemotaxis mediated by 0.1 μM FMLP. The comparative inhibitory effects are correlated as a linear regression ($r^2 = 0.873$, $P < 0.0001$). b) Inhibition of mono(ADP-ribosyl)transferase activity and receptor-dependent polymerization of monomeric actin. Measurements were made of the IC_{50} values of nicotinamide (○,◇), novobiocin (●,◆), vitamin K_3 (▲,+), vitamin K_1 (△,x,*) and DEA-BAG (□,■) for inhibition of mono(ADP-ribosyl)ation of agmatine and inhibition of actin polymerization mediated by 1 μM FMLP (○,●,▲,△,□), 1 μM PAF (◇,◆,x) and 4 nM C5a (+*■). The comparative inhibitory effects are correlated as a linear regression ($r^2 = 0.905$; $P < 0.0001$).

mono(ADP-ribosyl)transferase could reflect alterations in the magnitude of the agonist-induced calcium transient. PMNs were exposed to 10 nM FMLP or 100 nM PAF which induced rapid increases in $[Ca^{2+}]_i$ of 428 ± 86 nM ($n = 11$) and 716 ± 71 nM respectively ($n = 12$). The agonist-dependent calcium transient was also measured in the presence of 10 μM vitamin K_1, 20 μM vitamin K_3, 200 μM novobiocin, 50 mM nicotinamide or 2 mM DEA-BAG. There was however no attenuation of the response in the presence of any of these compounds.

Actin polymerization and inhibitors of enzymes implicated widely in signal transduction

Notwithstanding the contradictory reports of involvement of PKC and other enzymes in white cell chemotaxis, we addressed the possibility that selected inhibitors of enzymes with well-established involvement in signal transduction might show a similar pattern of correlation between a reduction in enzyme activity and reduced receptor-dependent polymerization of filamentous actin. Measurements were made therefore of actin polymerization in PMNs after exposure to 1 μM FMLP, and the responses were quantified in the absence or presence of a panel of putative inhibitors of protein kinase C (staurosporine, RO 31–8220, calphostin C), tyrosine kinase (genistein, tyrphostin A1, tyrphostin A25), protein kinase G (KT5823), protein kinase A (KT5720), myosin light chain kinase (KT5926) and phosphatases 1 and 2A (okadaic acid). Receptor-dependent actin polymerization was inhibited by calphostin C ($IC_{50} = 113 \pm 56$ nM) and tyrphostin A1 ($IC_{50} = 99 \pm 32$ μM), however the other compounds tested had no effect.

Preliminary experiments have been undertaken to identify the substrate of mono(ADP-ribosyl)transferase on the surface of PMNs. Exposure of intact PMNs to [α^{32}P]-NAD^+ revealed numerous [^{32}P]-labelled proteins that were separated by SDS-polyacrylamide gel electrophoresis. Products with mol wt values of 70, 67, 46, 36 and 25 kDa were identified, and phosphodiesterase 1 cleavage of the adducts yielded [^{32}P]-5′-AMP. These results are indicative of mono(ADP-ribosyl)ated proteins on the cell surface. Experiments are now under way to identify these individual protein substrates for the enzyme.

Discussion

The results presented here provide unequivocal evidence for expression of eukaryotic Arg-specific mono(ADP-ribosyl)transferase on the surface of human PMNs, and evidence of a rather more preliminary nature implicating the involement of mono(ADP-ribosyl)transferase activity in the re-alignment of cytoskeletal actin and chemotaxis. The enzyme is expressed at very low levels in this tissue, and particular care was taken to show that the mono(ADP-ribosyl)ation of agmatine was indeed dependent on mono(ADP-ribosyl)transferase activity and not simply on enzymatic hydrolysis of NAD^+ with subsequent non-enzymatic addition of ADP-ribose to the α-amino group of agmatine. The issue was resolved in a series of experiments that demonstrated mono(ADP-ribosyl)ation of DEA-BAG

by intact PMNs, and also by the cloning of cDNA encoding mono(ADP-ribosyl)transferase from these cells. Partial sequence of the cDNA cloned from PMNs (580 out of a total translated region of 981 base pairs) was identical to that encoding the human skeletal muscle enzyme, and is typical of a GPI-linked protein. In skeletal muscle cells, a higher mol wt pro-enzyme is translated, from which is cleaved both N- and C-terminal peptides of striking hydrophobicity. The modified protein is then translocated to the cell surface, where almost all the measurable mono(ADP-ribosyl)transferase activity is found. The GPI-linkage has been confirmed in the skeletal muscle enzyme, and was confirmed also in PMNs by hydrolysis with PI-specific phospholipase C. A more complete account of the enzymology of mono(ADP-ribosyl)transferase in PMNs can be found in [38].

The biological roles of eukaryotic mono(ADP-ribosyl) transferases remain obscure, although the identification of numerous substrates in fractured cell systems (and in experiments employing purified enzyme) have suggested a number of possibilities. The topic is reviewed in [30], and the various mono(ADP-ribosyl)ated products include G protein α-subunits and several cytoskeletal and microtubular proteins. Whether any of these proteins are substrates for the enzyme in intact cells or tissues remains uncertain, furthermore in many instances the radiolabelled protein has not been submitted to any structural analysis. Thus the possibility remains that some of the putative substrates of mono(ADP-ribosyl)-transferase may in fact be poly(ADP-ribosyl)ated proteins, or products of the non-enzymatic addition of free ADP-ribose, or even intact NAD^+, to available Cys residues.

Previous research by this group has addressed the possibility that mono(ADP-ribosyl)transferase might have a role in heterologous desensitization mediated by activation of prostacyclin receptors [39]. Exposure of human platelets and several neuronal cell lines to prostacyclin has shown that the loss of prostacyclin receptors is accompanied by a coincidental loss of functional Gsα from the cell membrane [40]. The consequence of this is heterologous loss of responses mediated by adenosine (A_2) and other receptors on the cell surface which mediate activation of adenylate cyclase through Gsα. Previous work has suggested that inactivation or elimination of Gsα might be mediated by mono(ADP-ribosyl)ation of Gsα [41], although experiments in this laboratory did not support that hypothesis [40]. As part of further studies in this area, we are currently exploiting a cDNA library prepared from human platelets [42], and we are exploring the possibility of multiple prostacyclin receptor isotypes.

In the present report, we have shown that inhibition of PMN mono(ADP-ribosyl)transferase with any one of a panel of inhibitors yielded IC_{50} values with a similar rank order of potency as that described for other eukaryotic mono(ADP-ribosyl)transferases [35,36]. The same panel of enzyme inhibitors also inhibited receptor-dependent polymerization of monomeric actin, and there was a close correlation between the IC_{50} values for inhibition of mono(ADP-ribosyl)transferase and actin polymerization in intact human PMNs. A similar close correlation was also obtained in a comparison of the IC_{50} values of the various compounds as inhibitors of PMN chemotaxis and mono(ADP-ribosyl)-transferase activity. Further evidence in support of a role for mono(ADP-ribosyl)transferase in the re-alignment of actin-containing cytoskeletal microfilaments was provided by the demonstration that DEA-BAG, a substrate of mono(ADP-ribosyl)transferase, also inhibited receptor-dependent actin polymerization and chemotaxis, with similar IC_{50} values for each effect.

The possibility was considered that inhibition of mono(ADP-ribosyl)transferase might mediate its effects on chemotaxis and actin polymerization by a reduction in the magnitude of the Ca^{2+} transient in PMNs following exposure to chemotaxin. However, measurement of the changes in $[Ca^{2+}]_i$ following the addition of FMLP or PAF showed no evidence of attenuated responses in the presence of inhibitors of mono(ADP-ribosyl)transferase activity.

The signalling pathway mediating chemotaxis is unknown, but clearly includes events leading to cytoskeletal re-alignment, with microfilament assembly in the direction of the gradient. We examined the effects of inhibitors of several enzymes implicated widely in signal transduction (including PKC, PKA, PKG, myosin light chain kinase and phosphatases 1 and 2A), but there was no consistent pattern of inhibition. It seems unlikely therefore that there is a central role for any of these enzymes in agonist-dependent polymerization of cytoskeletal actin. In contrast, the results presented for the effects of a panel of inhibitors of mono(ADP-ribosyl)-transferase now prompt us to propose that this enzyme plays a central role in chemotaxis. Further work is required however to establish how the enzyme becomes activated (or is translocated to the cell surface) following exposure of PMNs to chemotaxin. The substrate is also unknown in this context, although we are currently exploring the possibility that it might be one of the many proteins which together comprise the membrane anchoring points of cytoskeletal microfilaments. These anchoring points appear to play a pivotal role in chemotaxis, providing (i) the ends from which the microfilaments extend, and also (ii) the domains on the cell surface which permit adhesion of the leading edge of the cell to extracellular matrix or adjacent cells.

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