Protein myristoylation in human mononuclear phagocytes: modulation by interferon-γ and tumor necrosis factor-α

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Summary

Labelling of cells with [^{3}H]myristic acid and analysis of labelled proteins by SDS–PAGE and fluorography, enabled the identification of a limited number of myristoylated proteins in human monocytes and monocyte-derived macrophages. In human monocytes, cultivated for one to three days, major myristoylated proteins observed were of 18 kDa, 44 kDa, 60–62 kDa, 90 kDa, and a doublet of 38–40 kDa. Differentiation of monocytes to macrophages by *in vitro* cultivation was accompanied by a selective decrease in the 60–62 kDa protein. Cultivation of the cells in the presence of the macrophage-activating cytokines interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α), prevented the decrease in the expression of the 60–62 kDa myristoylated protein. The effect of cytokines was observed when monocytes were treated with IFN-γ or TNF-α for 24 or 48 h and protein myristoylation analyzed at day four of culture. Maintenance of monocytes in culture for up to nine days in the presence of cytokines prevented the decrease in the expression of the 60–62 kDa myristoylated protein. IFN-γ had additional effects on myristoylation of macrophage proteins. Treatment of monocytes with IFN-γ for a few hours caused the induction of a 66 kDa protein. Induction of this myristoylated protein by IFN-γ was time-dependent and peaked at six hours. Analysis of the subcellular distribution of the 66 kDa protein induced by IFN-γ showed that, analogously to other myristoylated proteins, most of it was associated with cell membranes. Initial attempts to identify the myristoylated proteins in human mononuclear phagocytyes showed that p60^{src} is one of the myristoylated proteins which is down-modulated during differentiation of monocytes to macrophages, and that cultivation of monocytes in the presence of IFN-γ and TNF-α prevents this phenomenon.

Key words: mononuclear phagocytes, protein myristoylation, p60^{src}, interferon-γ, tumor necrosis factor-α.

Introduction

Macrophages are cells distributed in all tissues (Gordon *et al.* 1988) and derive from circulating monocytes, which, once emigrated into the extravascular space, undergo a complex process of differentiation. Studies on macrophages isolated from the peritoneal cavity of mice (Cohn, 1978) or human alveoli (Murray *et al.* 1985; Fels *et al.* 1987; Cassatella *et al.* 1989), and on monocytes differentiated to macrophages by *in vitro* cultivation (Nakagawara *et al.* 1981; Murray and Cartelli, 1983; Nathan *et al.* 1983; Cassatella *et al.* 1985; Dusi *et al.* 1990) have established the notion that differentiation to tissue macrophages is accompanied by profound phenotypic changes.

The functional state of mononuclear phagocytes can also vary considerably, depending on exposure to both microbial-derived molecules and cytokines. It has been recognised that both exogenous and endogenous factors are capable of modulating mononuclear phagocyte functions, and are responsible for the process of 'macrophage activation', that accompanies the acquisition of resistance against facultative intracellular parasites and tumor cell growth (reviewed by Adams and Hamilton, 1984; Petit and Lemaire, 1986).

Studies with both microbial-derived molecules and cytokines have clearly indicated that modulation of mononuclear phagocyte functions requires *de novo* RNA and protein synthesis. Several proteins whose enhanced synthesis accompanies modulation of mononuclear phagocyte functions have been identified, including surface molecules such as class I and II MHC antigens, proinflammatory mediators such as IL-1, TNF/cachectin, colony-stimulating factors, and molecules implicated in selective functions such as components of the NADPH oxidase, indoleamine 2,3-dioxygenase and others.

Attempts to identify other proteins whose synthesis is enhanced in the course of modulation of mononuclear phagocyte functions have been based on analysis of *de novo* synthesized polypeptides after cell labelling with radioactive amino acids (Largen and Tannenbaum, 1986; MacKay and Russell, 1986, 1987; Johnston *et al.* 1987). More recently, interesting observations have been made with mouse macrophages by restricting the analysis to a limited number of proteins containing covalently attached myristic acid (Aderem *et al.* 1986, 1988*a*,*b*).

Myristoylation of protein is a co-translational event catalyzed by the enzyme myristoyl-CoA:protein *N*-myristoyl transferase, that attaches myristic acid to amino-terminal glycine residues *via* an amide linkage (reviewed by Towler *et al.* 1988). Several proteins which undergo myristoylation in eukaryotic cells have already been identified, including the catalytic subunit of protein

kinase A (Carr *et al.* 1982), the B subunit of calcineurin (Aitken *et al.* 1982), NADH cytochrome b_5 reductase (Ozols *et al.* 1984), the G_o and G_i α subunits of GTP-binding proteins (Schultz *et al.* 1987; Buss *et al.* 1987; Jones *et al.* 1990), the alanine-rich protein kinase C substrate (Aderem *et al.* 1988; Stumpo *et al.* 1989), IL-1 α and β precursors (Bursten *et al.* 1988) and members of the src family of intracellular protein tyrosine kinases (reviewed by Perlmutter *et al.* 1988).

The results reported in this paper were obtained from studies initiated with the aim of identifing myristoylated proteins in human mononuclear phagocytes. Besides identifying the pattern of myristoylated proteins in human monocytes and monocyte-derived macrophages, we provide evidence that protein myristoylation is modulated during the differentiation of monocytes to macrophages, and treatment with interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α). We also show that one of the myristoylated proteins which can be modulated in human mononuclear phagocytes is $p60^{src}$.

Materials and methods

Monocyte isolation and cultivation

Monocytes were obtained from blood 'buffy coats' of healthy volunteers (Cassatella *et al.* 1985; Berton *et al.* 1991). After centrifugation over Ficoll-Hypaque gradients, mononuclear white cells were washed 3–4 times with phosphate buffered saline (PBS) containing 2% human serum (pooled from 10 donors and heat inactivated) and resuspended in RPMI 1640 medium containing 50 μg ml^{-1} streptomycin and 50 i.u. ml^{-1} penicillin, and supplemented with 5% human serum. Mononuclear cells were plated in 10 cm^2, 6-well trays at a density of 15×10^6 cells/well. After 2 h of cultivation in 5% CO_2 at 37 °C, the non-adherent cells were removed by three washings with PBS and the monocyte monolayers cultivated in the above medium. The medium was replaced every 2–3 days and the cultivation prolonged up to 8–9 days. For treatment of monocyte monolayers with cytokines, human recombinant IFN-γ, produced by Genentech Inc. and kindly provided by Boehringer Ingelheim, Wien, Austria, (specific activity 2×10^7 U mg^{-1}; final dilution in the culture medium 10^6 fold) and human recombinant TNF-α, kindly provided by Bachem Inc, Hannover, F.R.G. (specific activity 1.7×10^8 U mg^{-1}; final dilution in the culture medium 10^7-fold) were used.

Labelling of cells and analysis of myristoylated proteins

[9,10-^{3}H]myristic acid in ethanol (40–60 Ci mmol^{-1}, Amersham International plc) was dried under a stream of nitrogen and resuspended in culture medium. Labelling of mononuclear phagocyte monolayers was performed in culture medium using 60 μCi ml^{-1} [^{3}H]myristic acid. After 4 h of labelling, the medium was aspirated, monolayers washed twice with ice-cold PBS and the cells scraped with a rubber policeman in ice-cold PBS. After centrifugation in a microfuge (12 000 *g*) for 30 s the cell pellet was lysed in PBS containing 1% Nonidet P-40 (NP-40), 2 μM leupeptin, 2 μM pepstatin, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 5 mM EDTA (lysis buffer). The lysates were kept on ice for 30 min, and after centrifugation in a microfuge for 5 min, the protein content of the supernatants was determined according to the method of Bradford (1976). Samples containing the amounts of proteins specified in the legends to figures were subjected to SDS–PAGE on 8% or 12% gels using the buffer system of Laemmli (1970), as detailed by Bellavite *et al.* (1987). Fluorography was performed according to the method of Laskey and Mills (1975), using X-Omat AR film (Kodak) at −70 °C. To test for hydroxylamine resistance of the myristic acid–protein linkage, gels were treated for 60 min at room temperature with 1 M hydroxylamine-HCl, pH 7.5, before fluorography (Magee *et al.* 1984). For analysis of subcellular localization of myristoylated proteins, monocyte monolayers were labelled with [^{3}H]myristic acid, as described above. After two washings with ice-cold PBS, the cells were scraped in 10 mM Tris, pH 7.5, 0.25 M sucrose, 0.1 mM $MgCl_2$, 2 μM leupeptin, 2 μM pepstatin, 1 mM PMSF, and sonicated 3 times at 90 W for 5 s. After removal of nuclei and cell debris by centrifugation at 1000 *g* for 10 min at 4 °C, supernatants were centrifuged at 100 000 *g* for 45 min at 4 °C to obtain a cytosolic and a membrane fraction.

Analysis of protein-bound fatty acid

This was performed using the method described by Olson *et al.* (1985) with the modifications introduced by Towler and Glaser (1986). Monocyte monolayers in one 6-well plate were labelled with 0.7 mCi ml^{-1} [^{3}H]myristate for 4 h. After the labelling period, cells were scraped, centrifuged and lysed with the buffer described by Olson *et al.* After delipidation by extraction six times with chloroform–methanol (2:1), proteins were subjected to acid methanolysis for 24 h, and fatty acid methyl esters were extracted into petroleum ether and analyzed by reverse-phase high pressure liquid chromatography (Towler and Glaser, 1986).

Immunoprecipitation of $p60^{src}$

Lysates were prepared from [^{3}H]myristic acid-labelled monocytes as described above and incubated with 3 μg of the monoclonal antibody 327 (Lipsich *et al.* 1983), kindly donated by Dr S. Courtneidge, EMBL, Heidelberg, or 3 μg purified mouse IgG. After one hour of incubation at 4 °C in a rotating mixer, 10 μl of 10% *S. aureus* (Pansorbin, Calbiochem), pre-coated with rabbit anti-mouse IgG, were added and lysates incubated as above for an additional hour. The immunoprecipitates were washed 5 times with RIPA buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS) and twice with TBS (20 mM Tris, pH 7.5, 150 mM NaCl). Immunoprecipitates were analyzed as described above for myristoylated proteins.

In vitro *kinase assays on anti-$p60^{src}$ immunoprecipitates*

Monocytes and macrophage monolayers were lysed in 20 mM Hepes, pH 7.5, 150 mM NaCl, 10 mM EDTA, 10 mM dithiothreitol (DTT), 100 μM sodium orthovanadate, 1 mM PMSF and 2% NP-40. Lysates were kept for 30 min in ice and then centrifuged for 5 min in a microfuge.

Immunoprecipitation of $p60^{src}$ was performed as described above. Immunoprecipitates were washed 3 times with RIPA buffer containing 1 mM EDTA, 1 mM DTT, and 100 μM sodium orthovanadate, and twice with TBS containing 1 mM DTT and 100 μM sodium orthovanadate. The final pellet was resuspended in 20 μl of kinase buffer whose composition was: 20 mM Hepes, pH 7.0, 5 mM $MnCl_2$, 1 mM DTT, 1 μM ATP, 2 μCi [γ-^{32}P]ATP (3 000 Ci mmol^{-1}, Amersham). After 15 min of incubation at room T, the reaction was stopped with 20 μl of SDS–PAGE sample buffer and the samples analyzed by SDS–PAGE and autoradiography.

Results

Myristoylated proteins in human monocytes and monocyte-derived macrophages

When human monocytes, maintained in culture for different times, are labelled for 4 h with [^{3}H]myristic acid and then the cell lysates analysed by SDS–PAGE and fluorography, several acylated proteins can be observed. Fig. 1 shows that in monocytes cultivated for 1–3 days, prominent bands of about 18 kDa and 60–62 kDa, and a doublet of about 38–40 kDa are observed. Other minor acylated proteins which were observed reproducibly are of about 44 and 90 kDa.

Evidence that these proteins were labelled by the covalent attachment of myristic acid was obtained by experiments showing that the protein–fatty acid linkage was hydroxylamine-resistant and occurred co-trans-

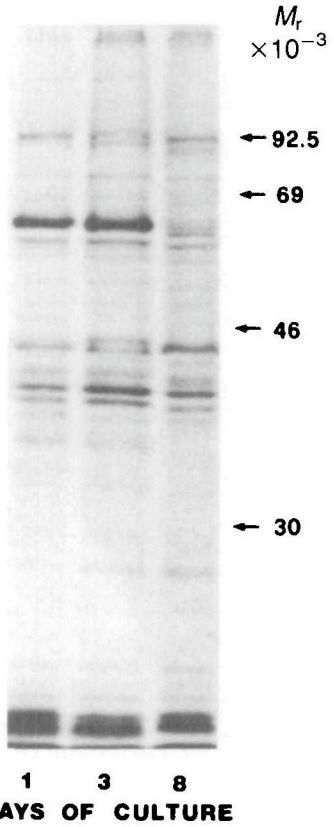

Fig. 1. 1, 3 or 8 day old monocytes were isolated and plated as described in Material and methods. After labelling for 4 h with [^{3}H]myristic acid, monolayers were washed twice with ice-cold PBS, and the cells scraped into ice-cold PBS. After centrifugation in a microfuge, the cells were lysed in 0.3 ml lysis buffer (see Materials and methods), lysates centrifuged for 5 min in a microfuge and supernatants analyzed on 12% SDS–acrylamide gels. The fluorograph shown was exposed for 12 days. In each case, 25 μg of protein were subjected to electrophoresis. Numbers at the right of the figure show migration of molecular weight markers.

lationally. In fact, treatment of the gels with 1 M hydroxylamine, before the autoradiographic analysis (see Materials and methods), did not release the label (data not shown), and the protein synthesis inhibitor cycloheximide completely blocked the incorporation of [^{3}H]myristic acid in the 4 h labelling period (see Fig. 4). Furthermore, analysis of protein-bound fatty acids (see Matherial and methods) revealed that, after acid methanolysis, 70% of the labelled fatty acid was eluted as methyl myristate, 20% as myristate and 2% as methyl palmitate (data not shown).

Cultivation of monocytes for prolonged times (8–10 days), up to their differentiation to macrophages (see Nakagawara *et al.* 1981), caused only one clear alteration in the pattern of myristoylated proteins. In fact, as shown in Fig. 1, myristoylation of both the major 18 kDa protein and the doublet of about 38–40 kDa, and other proteins (for example those of 44 and 90 kDa) was the same in monocyte-derived macrophages as in monocytes cultivated for 1–3 days. However, the myristoylated 60–62 kDa protein became less evident when monocytes were maintained in culture and was almost undetectable in 8-day-old monocyte-derived macrophages. In several experiments, the detectability of the myristoylated 60–62 kDa protein did not change from day 1 to day 3 of culture but decreased substantially and reproducibly at day 4 (see Fig. 2).

Effect of IFN-γ and TNF-α on myristoylation of monocytes and monocyte-derived macrophages proteins

The observation that the detectability of the myristoylated 60–62 kDa protein decreased during *in vitro* differentiation of monocytes to macrophages prompted studies on a possible effect of cytokines, able to modulate mononuclear phagocyte functions, on protein myristoylation. Fig. 2 shows results obtained with IFN-γ and TNF-α. Monocytes that had been cultivated for 3 days were treated for a few hours with IFN-γ (lanes 1–3) or TNF-α (lanes 5,6) or maintained in culture up to day 4 with cytokines (lane 7: IFN-γ; lane 11: TNF-α). The same monocyte population was also treated with cytokines at day 2 of culture and myristoylated proteins analysed at day 4 (lane 8: IFN-γ; lane 10: TNF-α). As shown in Fig. 2, both IFN-γ and TNF-α prevented the decrease in detectability of the myristoylated 60–62 kDa protein which occurs at day 4 of culture (compare lane 9 with lanes 7,8,10,11). In fact, the myristoylation of the 60–62 kDa protein in monocytes cultivated for 4 days but previously exposed for 24 h (lanes 7,11) or 48 h (lanes 8,10) to IFN-γ or TNF-α, was comparable to that observed in monocytes cultivated for 3 days (lane 4).

Fig. 3 shows the results of experiments performed to extend the observation that cytokine treatment maintains the detectability of the myristoylated 60–62 kDa protein at elevated levels. As shown in the figure, maintenance of monocytes in culture for different times was accompanied by a progressive decrease in detectability of the myristoylated 60–62 kDa protein. However, when TNF-α was added at day 2 of monocyte culture and the cells were then cultivated in cytokine-containing medium, the apparent expression of the myristoylated 60–62 kDa protein was higher up to day 9 of cultivation. The same observation was obtained using IFN-γ instead of TNF-α (data not shown).

Induction of a myristoylated 66 kDa protein by IFN-γ

As shown in Fig. 2 (lanes 1–3), in monocytes cultivated for 3 days, IFN-γ induced the myristoylation of a protein of apparent molecular weight of 66 kDa which was undetectable in untreated monocyte cultures. The effect of IFN-γ was time-dependent and transient (lanes 1–3,7,8). Myristoylation of the 66 kDa protein increased for up to 6 h of treatment with IFN-γ (lanes 1–3) and returned to the undetectable levels of untreated monocyte cultures after 24–48 h (lanes 7,8).

Fig. 4 shows the results of experiments performed to characterize further the myristoylated 66 kDa protein which became detectable after a few hours of treatment of monocytes with IFN-γ. As shown in panel A, treatment of 3-day-cultivated monocytes with IFN-γ for six hours caused the induction of a 66 kDa myristoylated protein. Panel A also shows that, consistent with the established notion that myristoylation is a co-translational event (Wilcox *et al.* 1987), cycloheximide completely blocked the addition of myristic acid to the 66 kDa protein, as well as to other proteins.

Panel B of Fig. 4 shows the results of experiments performed to investigate the partition of monocyte myristoylated proteins in the cytosolic or membrane fraction. As previously established for other cell lineages, myrostoylated proteins were found in both cytosol and

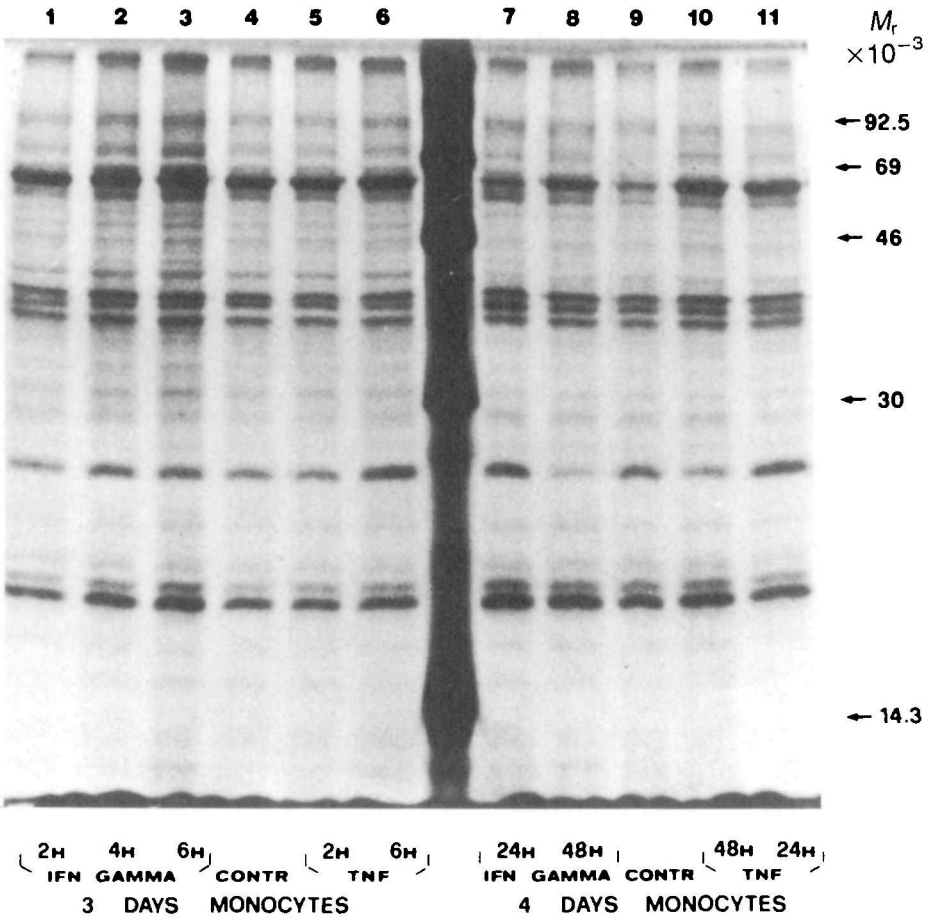

Fig. 2. Myristoylated proteins were analyzed as described in Materials and methods and the legend to Fig. 1. After culture for 3 days (lanes 1–6) or 4 days (lanes 7–11) Monocytes were exposed to 100 U ml^{-1} IFN-γ (lanes 1–3, 7, 8) or 5 ng ml^{-1} TNF-α (lanes 5, 6, 10, 11) for the time (in hours) indicated at the bottom of the figure, before cell lysis. 35 μg of protein obtained from cell lysates were subjected to electrophoresis in 12% SDS–acrylamide gels. The fluorograph was exposed for 15 days. Contr, control (lanes 4, 9).

membranes, although the majority of them were associated with the membrane fraction. Also the IFN-γ-induced 66 kDa protein appeared to have the same distribution.

Identification of p60^{src} as one of the myristoylated proteins modulated upon differentiation of monocytes to macrophages and upon treatment with cytokines

Among the best characterized myristoylated proteins of eukaryotic cells are members of the src family of intracellular tyrosine kinases (see Perlmutter *et al.* 1988). Since the myristoylated band whose detectability we observed to be modulated during monocyte differentiation and upon exposure to cytokines migrated in our SDS–PAGE gels to a position slightly above 60 kDa we asked whether this myristoylated protein could be p60^{src}.

As shown in Fig. 5, immunoprecipitation experiments with lysates of monocytes cultivated for 3 days and labelled for 4 h with [^{3}H]myristic acid showed that the anti-p60^{src} antibody 327 immunoprecipitated a protein that migrated in our gels to the same position as the myristoylated 60–62 kDa band. However, we could not exclude the possibility that the band migrating at about 60–62 kDa might represent other proteins of similar

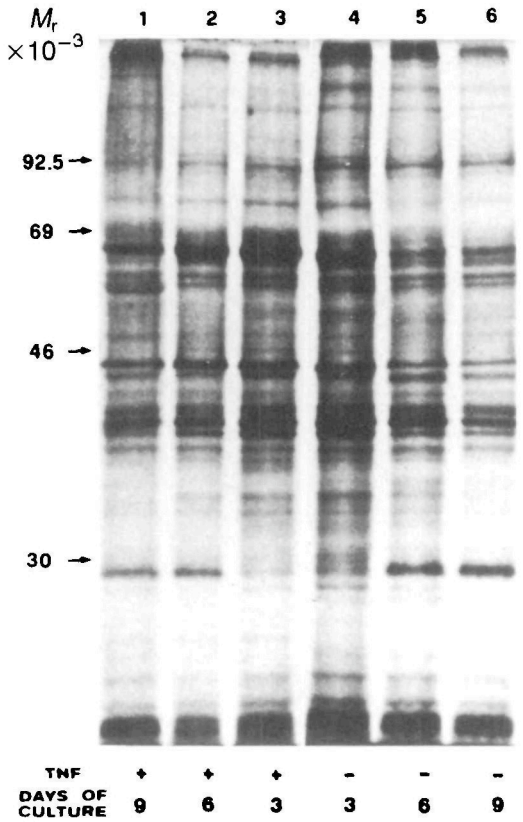

Fig. 3. Myristoylated proteins were analyzed as described in Materials and methods and the legend to Fig. 1. At day 2 of culture, monocytes were supplemented with 5 ng ml^{-1} TNF-α. Medium with (+) or without (−) TNF-α was replaced at days 4 and 6 of culture. 35 μg of proteins of cell lysates were subjected to electrophoresis in 12% SDS–acrylamide gels. The fluorograph was exposed for 18 days. The position of marker proteins is shown as the left of the figure.

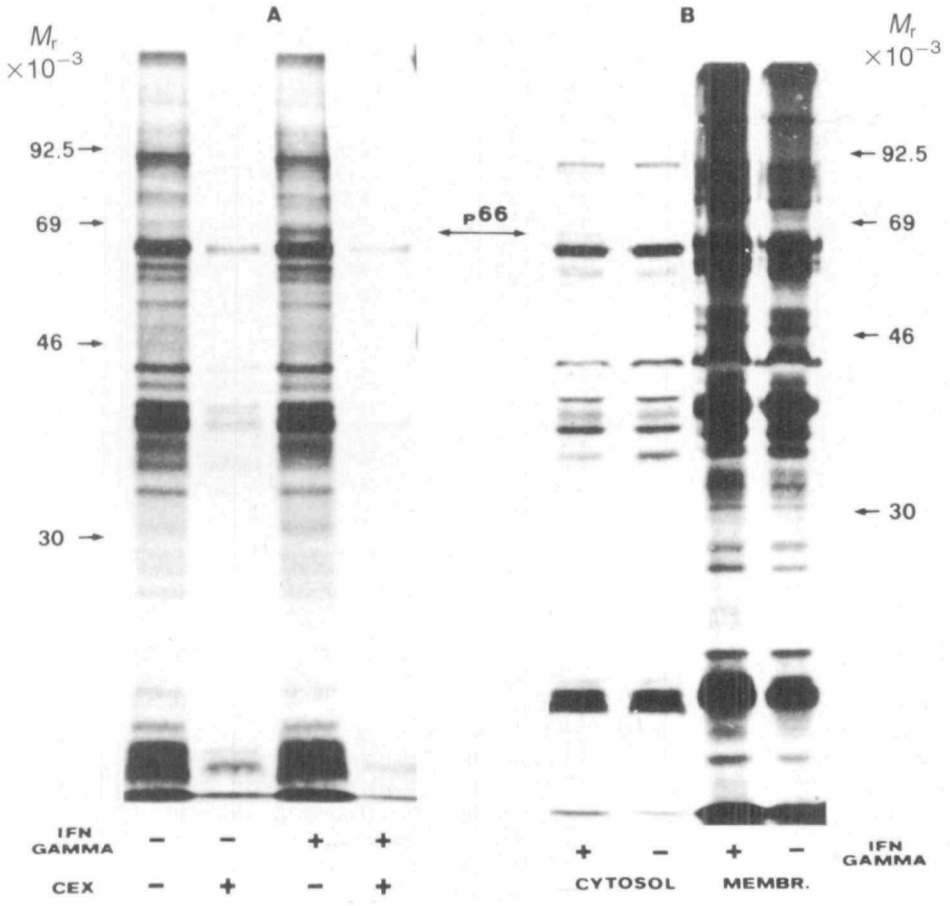

Fig. 4. Myristoylated proteins were analyzed as described in Material and methods and the legend to Fig. 1. (A) Monocytes cultivated for 1 day were treated with or without 10 $\mu g\,ml^{-1}$ cycloheximide (CEX) for 30 min and then incubated with [^{3}H]myristic acid for a further 4 h before lysis. IFN-γ (100 U ml^{-1}) was added 6 h before cell lysis. 23 μg of proteins from cell lysates were subjected to electrophoresis in 8 % SDS–acrylamide gels. The fluorograph was exposed for 15 days. (B) The cytosolic and membrane fractions were isolated from monocytes cultivated for 2 days as described in Materials and methods. 30 μg of cytosolic proteins and 55 μg of membrane proteins were subjected to electrophoresis in 8 % SDS–gels. The fluorograph was exposed for 15 days. The position of molecular weight marker proteins is shown.

molecular weight. In fact, initial attempts to deplete monocyte lysates of the 60–62 kDa myristoylated band by anti-p60^{src} antibodies were unsuccessful.

We then addressed the issue of a possible modulation of p60^{src} upon differentiation of monocytes to macrophages and treatment with cytokines by using an alternative approach, that is the assay of p60^{src} kinase acivity. It has been established that p60^{src} undergoes autophosphorylation in *in vitro* kinase assays performed on anti-p60^{src} immunoprecipitates (Lipsich *et al.* 1983; Courtneidge, 1985), and that the extent of autophosphorylation correlates with expression of the protein (Golden *et al.* 1986; Gee *et al.* 1986).

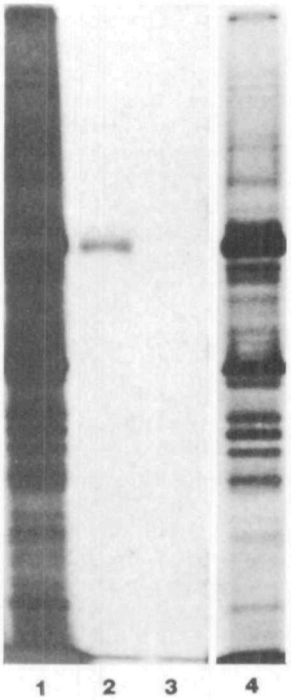

Fig. 5. Monocytes cultivated for three days, and labelled with [^{3}H]myristic acid, were lysed and p60^{src} immunoprecipitated as described in Materials and methods. Samples were subjected to electrophoresis in 8 % SDS–acrylamide gels. Lane 1 and 4: total myristoylated proteins. Lane 2: anti-p60^{src} antibody 327 immunoprecipitate. Lane 3: mouse IgG immunoprecipitate. The fluorographs were exposed for 1 month (lane 1–3) or 15 days (lane 4).

As shown in Fig. 6, the anti-p60^{src} antibody 327 precipitated from lysates of monocytes cultivated for 3 days a protein of about 60 kDa that was phosphorylated in the presence of [γ-^{32}P]ATP (lane 5). No phosphorylated band was present in immunoprecipitates done with control IgG, demonstrating the specificity of the assay (compare lanes 1–4 with lanes 5–8). In immunoprecipitates obtained from lysates of monocytes cultivated for 7 days, the amount of autophosphorylated p60^{src} was clearly lower (lane 6), but, when monocytes were cultivated in the presence of IFN-γ or TNF-α from day 3 to day 7 of culture, the kinase assay performed on anti-p60^{src} immunoprecipitates revealed the presence of higher amounts of the autophosphorylated p60^{src} (lanes 7 and 8).

Discussion

By incubation of human monocytes with [^{3}H]myristic acid, we have identified a limited number of acylated proteins. The fatty acid linkage is resistant to hydroxylamine and a protein synthesis inhibitor completely blocks the incorporation of the label into proteins, results that are consistent

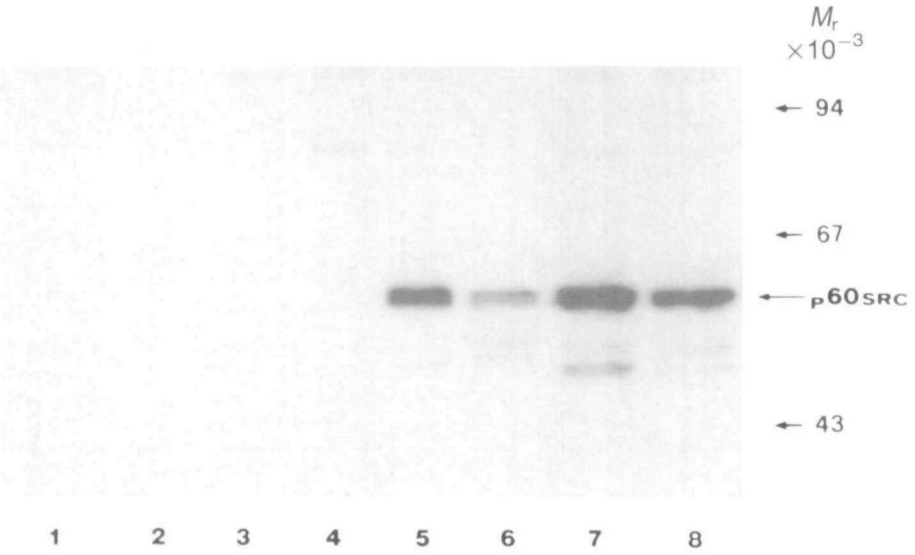

Fig. 6. $p60^{src}$ was immunoprecipitated from monocytes or monocyte-derived macrophages, and kinase assays on immunoprecipitates were performed as described in Materials and methods. Immunoprecipitations were done with lysates from monocytes cultivated for 3 days (lanes 1,5), monocytes cultivated for 7 days (lanes 2,6), monocytes cultivated for 7 days in the presence of 100 U ml^{-1} IFN-γ (lanes 3,7) and monocytes cultivated for 7 days in the presence of 5 ng ml^{-1} TNF-α (lanes 4,8). Lanes 1 to 4 show immunoprecipitations done with control mouse IgG, and lanes 5 to 8 with the anti-$p60^{src}$ antibody 327. One representative of three experiments is shown.

with properties which have been demonstrated as indicative of a true myristoylation process (see Towler *et al.* 1988). We have also obtained direct evidence that the protein-bound fatty acid is myristate. The prominent myristoylated proteins of human monocytes appear to be an 18 kDa protein, a doublet of 38–40 kDa and a 60–62 kDa protein. An 18 kDa protein has been demonstrated to be one of the major myristoylated proteins in animal kidney cell lines (Magee and Courtneidge, 1985) and mouse peritoneal macrophages (Aderem *et al.* 1986) and, to our knowledge, it remains to be identified.

The myristoylation of a doublet of about 40 kDa has been shown to be induced by lipopolysaccharide in mouse resident peritoneal macrophages (Aderem *et al.* 1986). Proteins of approximately the same molecular weight have been identified as substrates of myristoylation reactions and include the 39 kDa G_o (Schultz *et al.* 1987) and 42 kDa G_i (Buss *et al.* 1987; Jones *et al.* 1990), α subunits of GTP-binding proteins and the 39–42 kDa catalytic subunit of the cyclic AMP-dependent protein kinase (Carr *et al.* 1982).

Among the major myristoylated proteins we detected in human monocytes there is a protein with an apparent molecular weight of 60–62 kDa, which we demonstrated to be the major one modulated during *in vitro* differentiation to macrophages. The myristoylation of this protein does not change in the first few days of monocyte cultivation but drops substantially between day 3 and day 4 of monocyte cultivation, and further in fully differentiated macrophages (see Figs 1–3). Although any speculation is premature, it may be interesting to note that other reported studies on human monocyte-derived macrophages have shown that maintenance of monocytes in culture for times comparable to those used in our experiments is accompanied by down-modulation of monocyte effector funtions, such as the capability to produce toxic oxygen molecules (Nakagawara *et al.* 1981; Murray and Cartelli, 1983; Nathan *et al.* 1983; Cassatella *et al.* 1985; Dusi *et al.* 1990).

Cultivation of monocytes in the presence of IFN-γ or TNF-α prevented the down-modulation of the myristoylated 60–62 kDa protein. Upon differentiation to macrophages and as a result of cytokine treatment, the only myristoylated protein whose detectability changed was the 60–62 kDa one, which strongly suggests that we actually observed alterations in expression of a myristoylated protein and not of myristyl-CoA:protein myristoyl transferase (NMT) activity.

The results presented in this paper are the first to describe modulation of protein myristoylation by cytokines in human mononuclear phagocytes. The selective effect of both IFN-γ and TNF-α on the 60–62 kDa myristoylated protein suggests that this may be involved in macrophage functions modulated by cytokines.

In the attempt to identify this 60–62 kDa myristoylated protein, which may correspond to proteins of similar molecular weight, we obtained evidence that $p60^{src}$ is one of the monocyte myristoylated proteins which undergoes modulation upon differentiation to macrophages and as a consequence of exposure to cytokines. $p60^{src}$ kinase activity is induced upon differentiation of myelomonocytic cell lines to monocytes and granulocytes (Barnekow and Glaser, 1986; Gee *et al.* 1986). This observation, as well as the evidence that high levels of $p60^{src}$ kinase activity are detectable in post-mitotic neurons (Brugge *et al.* 1985) and platelets (Golden *et al.* 1986), allows one to conclude that this protein is implicated in the regulation of processes not related to cell proliferation. The role of $p60^{src}$ and its substrates in non-proliferating cells is largely unknown. However, evidence that $p60^{src}$ is enriched in chromaffin granules of adrenal medullary chromaffin tissues (Parsons and Cruetz, 1986), and that it phosphorylates tubulin in nerve growth cone membranes (Matten *et al.* 1990), points to a possible role for this protein in exocytosis and cytoskeleton dynamics, cellular functions which are of key importance in the role exerted by the macrophage in the inflammatory site. In this context, it is of interest that the targeted disruption of the c-*src* proto-oncogene leads to impairment of osteoclast functions in mice (Soriano *et al.* 1991).

As shown in Figs 2 and 4, besides its effects on the 60–62 kDa protein, IFN-γ selectively induces a new myristoylated protein of about 66 kDa in human monocytes. Myristoylation of this protein is observed within 2 h and increases up to 6 h, but it is undetectable after 24 h of treatment with IFN-γ. Studies with fully differentiated macrophages present in the peritoneal cavity of mice showed that this cytokine induces the myristoylation of a 48 kDa protein (Aderem *et al.* 1988*b*). More than a difference between species, it is likely that the use of cells in different states of differentiation is at the root of the discrepancy between our observation and that of Aderem

et al. (1988*b*). We do not know whether there is any analogy between the myristoylated 66 kDa protein induced in human monocytes by IFN-γ and the myristoylated 68 kDa protein induced in mouse peritoneal macrophages by lipopolysaccharide (Aderem *et al.* 1986), that has been identified as one of the major substrates of protein kinase C (Aderem *et al.* 1988*a*).

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