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Identification of a novel neutrophil membrane protein involved in modulation of oxidative burst

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Abstract

On the basis of selective recognition by antibodies directed against neutrophil membrane determinants, a new neutrophil protein (molecular mass 82 kDa) has been identified, and shown to be functionally correlated with the oxidative response evoked in these cells by agonist stimulation. The protein is present in neutrophil membrane fraction but only upon activation it becomes accessible to recognition by a specific monoclonal antibody. In these conditions a complete and selective inhibition of O_2^- production occurs. The presence of a new protein antigen in neutrophil membranes linked to the activation of the O_2^- producing multienzyme complex that becomes external to the cell surface in primed or activated cells, might be important for future approaches aiming at the control of neutrophil response and at the identification of the activated forms of these cells.

Key words: Surface antigen; Oxidative burst; Superoxide anion; Monoclonal antibody; Neutrophil

1. Introduction

The complex physiological function accomplished by neutrophils can be oversimplified as the result of two sequential and distinct phases: a first one characterized by the identification of the inflammation sites and a second one, involving activation of specific responses, directed to destroy microrganisms or inflammatory mediators [1–5]. Both neutrophil functions are mediated by the interaction of cell receptors with a variety of different and specific molecules present in the extracellular medium. Thus, adhesion of neutrophils to endothelium is mediated by receptors involved in cell–cell recognition [1,6], but not in the cell activation process. A different class of receptors seems to promote an agonist dependent, pre-activated or primed cell condition characterized by a considerably higher sensitivity to agonist stimulation [10–12]. For example, many cytokines [13–16], such as the colony stimulating factors, are efficient enhancers (priming factors) of neutrophil chemotaxis and of the respiratory burst in neutrophils stimulated with fMet-Leu-Phe. A third class of receptors, each specifically interacting with different types of stimuli, is responsible for the selective transduction of those signals, that are capable of evoking the neutrophil response [17,18]. This class of receptors is functionally reactive in resting neutrophils and is involved in the binding of N-formylated peptides, leukotriene B4, interleukine-8 and platelet activating factor (PAF) [19–23].

In inflammatory diseases, as well as in those pathological conditions characterized by tissue lesions, neutrophil activation generates the characteristic oxidative burst [4,24], promoted by the NADPH oxidase multienzyme system composed of four different proteins, some of which undergo a phosphorylation process in the course of activation [25,26].

In the present paper we report experiments performed with monoclonal antibodies (mAbs) obtained from mice injected with neutrophil cytoplasts, and selected for cross-reactivity with neutrophil surface anti-

Abbreviations: fMet-Leu-Phe, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine; PAF, platelet activating factor; Hepes, (*N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid]); DME, Dulbecco's modified Eagle medium; FCS, fetal calf serum; PMA, phorbol 12-myristate 13-acetate; SOD, superoxide dismutase; FITC, fluorescein isothiocyanate; PMSF, phenylmethylsulphonyl fluoride; EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid.

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gen proteins and for ability to modulate cell responses, particularly those involved in the activation of the oxigen burst.

The results obtained demonstrate the existence of a new protein antigen, involved in the regulation of NADPH activity, that becomes available for antibody recognition only on the surface of activated or 'primed' neutrophils. Binding of a specific monoclonal antibody to this surface protein results in the inhibition of the O_2^- production by neutrophils stimulated by a number of agonists.

2. Materials and methods

2.1. Isolation of human neutrophils

Neutrophils were isolated from heparinized peripheral blood of healthy subjects following the procedure described by Boyum [27] and modified as in Ref. [28]. The resulting cell preparation, containing 97% neutrophils, was washed in 10 mM Hepes buffer containing 140 mM sodium chloride, 5 mM potassium chloride, 5 mM glucose pH 7.4 (medium A) and resuspended in Dulbecco's modified Eagle medium (DME) containing 20% fetal calf serum (FCS) at a density of $20 \cdot 10^6$ cells/ml.

2.2. Preparation of cytoplasts

Release of intracellular organelles and separation of cytoplasts were accomplished by the technique described by Michael et al. [29]. The particles collected at the interface 12.5%/16% Ficoll were collected, washed and used as the source of cytoplasts. The integrity of the particles was tested by evaluating their ability to produce superoxide anion following treatment with PMA.

2.3. Preparation of monoclonal antibodies against neutrophil membrane proteins

Mice were immunized by intraperitoneal injections at 10 day-intervals with 50 μg of cytoplasts dispersed in complete Freund's adjuvant. Anti-cytoplast monoclonal antibodies (mAbs) were obtained following the procedure described by Zollinger et al. [30] and Corte et al. [31]. The mAbs were selected on the basis of their ability to bind neutrophil plasma membrane proteins, by a solid phase radioimmunoassay [32], revealed by a ^{125}I-labelled rabbit anti-mouse IgG.

2.4. Activation of human neutrophils and evaluation of their responses

Neutrophils at a density of 10^6 cells/ml were resuspended in 0.5 ml of medium A, containing 0.625 mg of ferricytochrome *c*, 1 mM $CaCl_2$ and stimulated with 0.1 μM fMet-Leu-Phe or 100 ng/ml PMA (the concentrations of other agents are indicated elsewhere). Superoxide anion production was continuosly monitored in a Beckman DU-65 spectrophotometer, following the SOD-inhibitable reduction of ferricytochrome *c* at 550 nm. Secretion of lysozyme was determined by evaluating the appearance of the lysozyme activity in the extracellular medium of stimulated cells. Neutrophils were activated as described above, and, after 5 min of incubation at 37°C, the cells were discarded by centrifugation at $1000 \times g$. Lysozyme activity [33] was measured on aliquots of the clear supernatant.

2.5. Binding of monoclonal antibodies to intact cells

Neutrophils ($6 \cdot 10^6$ cells) were resuspended in 3 ml of medium A and activated for 5 min at 37°C with 10^{-7} M fMet-Leu-Phe or 100 ng/ml PMA in the presence of 1 mM $CaCl_2$. After stimulation, 0.5 ml aliquots were centrifuged at 1000 rpm, resuspended in 1 ml of medium A containing 4% paraformaldehyde and incubated for 15 min at 25°C. Cells were then centrifuged and resuspended in 0.1 ml of DME containing 20% FCS; half of each suspension was immediately removed and used as a control, the other half was treated with mAb 72.1 or 188.10 (20 μg/tube) and incubated for 10 min at 37°C. Labelled cells were centrifuged, washed twice with DME and resuspended in 50 μl of fresh DME containing 20% FCS. Finally, the cells were incubated in the dark for 30 min at 25°C with fluoresceinated goat anti-mouse IgG antibodies (Organon Teknica, Durham, NC). Cells were washed twice with medium A and resuspended in 0.5 ml of the same medium containing 2% paraformaldehyde. Fluorescein isothiocyanate-stained cells (FITC-stained cells) were analyzed by flow cytometry using a Becton Dickinson FACScan equipped with a Becton Dickinson Lysys II software.

2.6. Binding of [^{3}H]fMet-Leu-Phe to neutrophils

The binding of the labelled formyl peptide to intact neutrophils was determined by incubating $1 \cdot 10^6$ neutrophils for 5 min at 37°C with $1 \cdot 10^{-7}$ M [^{3}H]fMet-Leu-Phe (specific activity = 56.4 Ci/mmol) in 1 ml of medium A containing 1 mM $CaCl_2$. At the end of the incubation, neutrophils were collected and the radioactivity associated with the cells was counted in a Packard TriCarb scintillation analyzer model 1600 TR. The competition between the formylated peptide and mAb 188.10 or 72.1 was evaluated by measuring the binding of [^{3}H]fMet-Leu-Phe, as indicated above, in the presence of increasing amounts of either of the mAbs.

2.7. Preparation of the neutrophil particulate fractions

Neutrophils ($5 \cdot 10^8$ cells/ml) were suspended in 0.25 M sucrose solution containing 0.1 mg/ml leu-

peptin, 2 mM PMSF, 10 mM NaF, 1 mM Na_3VO_4, 1 mM EGTA, 1 mM β-MSH and lysed in a ice bath with 70–80 strokes in a glass-Teflon homogenizer. When approximately 90% of the cells were broken, the homogenate was loaded on a 55% Percoll solution (8 ml) containing 0.25 M sucrose and 5 mM $MgCl_2$. The sample was centrifuged at $10000 \times g$ for 20 min and fractions were collected from the top of the gradient. The fractions containing cell particles were diluted 10 times with isotonic sucrose solution and centrifuged for 30 min at $100000 \times g$ to remove Percoll. The clear solution on the top of the Percoll gradient was collected and used as the source of the cytosolic fraction, whereas all the other subcelluar fractions were identified by the assay of specific enzyme markers: 5-nucleotidase activity, for plasma membranes [34]; lysozyme activity, for granule particles [35–36]; succinate dehydrogenase activity, for mitochondria [37]; and NADH-ferricyanate reductase, for endoplasmic reticulum [38].

2.8. Isolation of the protein antigen

The isolation of protein antigen from neutrophil membranes was carried out using the purified mAb 72.1 coupled to activated Sepharose 4B. Purified plasma membranes from $2 \cdot 10^8$ neutrophils were solubilized in 5 ml of 0.1 M $NaHCO_3$, pH 8.0 containing 0.15 M Sodium Chloride and 0.2% Triton X-100. The insoluble material was pelleted by centrifugation at $100000 \times g$ and the clear supernatant was loaded on a column (1×3 cm) containing the mAb-coupled Sepharose. The resin was washed with 30 volumes of the same buffer and the antigen protein was eluted with 50 mM sodium citrate pH 5.5, containing 0.15 M NaCl and 0.2% Triton X-100. The eluted proteins were analyzed in SDS-PAGE.

2.9. Polyacrylamide gel electrophoresis (PAGE) and Western blot analysis

The neutrophil subcellular fractions were precipitated with 8% trichloroacetic acid; the protein pellets were then washed three times with ice-cold aceton and solubilyzed in sample buffer containing 1% SDS [39]. Following heating at 100°C for 3 min, the samples were loaded on a 8% polyacrylamide gel in the presence of SDS. Proteins were then transferred to nitrocellulose according to the method described by Burnette [40]. Protein bands were revealed with horseradish peroxydase conjugate goat anti-mouse IgG.

Table 1
Effects of monoclonal antibodies prepared against human neutrophil plasma membranes on the neutrophil oxidative burst induced bu fMet-Leu-Phe and PMA

Monoclonal antibody	Binding to neutrophil membrane (%)	Inhibition of oxidative burst (%) induced by	
		fMet-Leu-Phe	PMA
40.3	78	0	0
40.13	84	20	26
40.16	100	60	0
40.20	44	21	0
72.1	75	93	100
72.19	80	18	36
72.23	82	0	56
72.29	100	0	38
146.13	100	15	0
146.18	77	20	18
146.19	73	0	0
146.32	59	0	22
188.1	65	0	0
188.10	81	100	0
188.12	84	44	0
188.39	78	0	0

Monoclonal antibodies were prepared as described in Materials and methods. Binding of mAbs to neutrophil membrane proteins, determined in a solid phase radioimmunoassay as described in Materials and methods, are expressed as percentage of maximal binding (the counts obtained using the antibodies 72.29 and 146.13 was taken as 100%). The effect of mAbs on neutrophil activation was evaluated by stimulating the cells with fMet-Leu-Phe (at a concentration of 0.1 μM) or with PMA (at a concentration of 100 ng/ml), following the decrease in the O_2^- production in the presence of 30 μg/ml of the antibodies.

3. Results

3.1. Monoclonal antibodies recognizing neutrophil membrane proteins

In Table 1, the properties of a collection of mAbs are reported, prepared by injecting cytoplasts into mice (see Materials and methods) and characterized by their ability to bind neutrophil membrane protein(s) in a solid phase radioimmunoassay system. All mAbs have also been examined for their ability to inhibit the oxidative burst induced by stimulation of neutrophils with PMA or fMet-Leu-Phe. As shown in Table 1, some of the antibodies promote a partial inhibition when cells were treated with one or the other agonist, but only two of them were found to be particularly effective in promoting an almost complete inhibition. In particular, monoclonal antibody indicated as 72.1 inhibits more than 90% of the oxidative burst induced by fMet-Leu-Phe and almost 100% of that elicited by PMA. Differently, mAb designated as 188.10 produces 100% inhibition of the oxidative response induced by fMet-Leu-Phe but is completely uneffective when PMA is used as an agonist.

Based on their inhibitory properties, mAb 72.1 was selected as a potential marker of a regulatory protein somehow specifically acting on the enzyme complex system that generates O_2^- radicals, whereas mAb 188.10

was used as a tool for a comparative analysis being apparently characterized by a specific cross-reactivity with the formyl peptide receptor.

3.2. Characterization of the inhibitory properties of mAbs 72.1 and 188.10

The inhibitory properties of mAbs 72.1 and 188.10, were further investigated by exploring their binding to neutrophil surface antigens. A first approach was directed to the evaluation of the inhibition of the oxidative burst in cells exposed to simultaneous addition of the stimuli and of the antibody. Alternatively, cells were first incubated with the antibody, and then, after extensive washing, stimulated with a proper agonist. As shown in Fig. 1A, mAb 72.1 is very effective in inhibiting O_2^- production by cells stimulated with PMA or fMet-Leu-Phe, as indicated by an almost complete inhibition obtained when 30 μg of the antibody were added together with the stimulus (closed symbols). On the other hand, if cells were incubated in their resting state with the same amount of mAb and then washed before the successive addition of the agonist, no inhibition of the oxidative burst could be observed (open symbols). These results suggest that the antigen interacting with mAb 72.1 is not detectable on resting cells, and that its recognition on the cell surface is obligatorily preceded by cell activation with an agonist. For comparison, we have repeated the same experiments using mAb 188.10 (see Table 1), which is effective in promoting inhibition of O_2^- production only when fMet-Leu-Phe is used as agonist. As shown in Fig. 1B, addition of increasing amounts of mAb promotes a progressive inhibition of the O_2^- production that becomes almost complete when 30 μg of the antibody are added to the cell suspension. If the antibody is added to resting cells, which are then washed before stimulation, inhibition is still observed, being the same as in cell exposed simultaneously to the agonist and to the mAb. These results indicate that the antigen recognized by mAb 188.10 is located on the external surface of neutrophils also in their quiescent state, thus providing a strong indication of the type of the receptor, probably corresponding to that specific for the formylated peptide. This hypothesis is further supported by the competition exerted by the mAb 188.10 on binding of [^{3}H]fMet-Leu-Phe to the neutrophil surface. As shown in Fig. 2, addition of increasing amounts of mAb 188.10 progressively reduces cell binding of the labelled formylated peptide, which is almost completely abolished at a concentration of 30 μg/ml of mAb 188.10. On the contrary, addition of mAb 72.1 has no effect on the binding of the formyl peptide.

The competition between mAb 188.10 and the formylated peptide confirms the hypothesis that the antigen recognized by this mAb is the receptor for the chemotactic peptide, and explains previous observations concerning its inhibitory efficiency (see Fig. 1B).

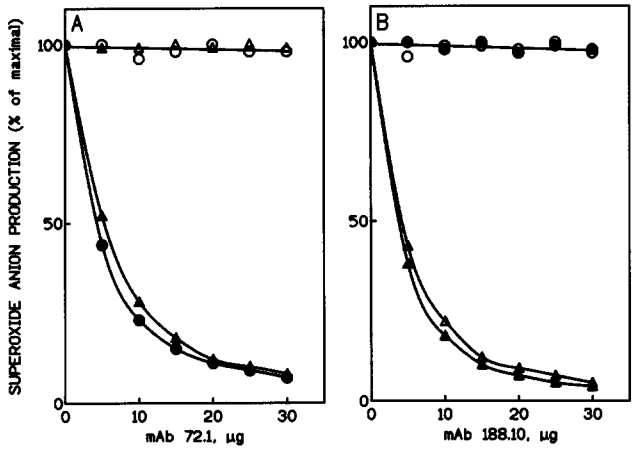

Fig. 1. Effect of monoclonal antibodies on the superoxide anion production by stimulated human neutrophils. (A) Neutrophils (10^6 cells/ml) were suspended in 2 ml of medium A in presence of the indicated amounts of mAb 72.1 and incubated for 5 min at 37°C. The sample was divided into two aliquots; the first one was centrifuged at 1000 rpm for 2 min and washed twice with fresh medium A (open symbols). The other aliquot was used as such (closed symbols). Both samples were then treated with 100 ng/ml of PMA (circles) or 10^{-7} M fMet-Leu-Phe (triangles). Superoxide anion production was monitored as described in Materials and methods. (B) Cells (10^6 cells/ml) were suspended in 2 ml of medium A in presence of the indicated amounts of mAb 188.10 and incubated for 5 min at 37°C. The sample was then divided into two aliquots and the first one was submitted to centrifugation for 2 min at 1000 rpm and washed twice with fresh medium A (open symbols); the other aliquot was used as such (closed symbols). Cells were then stimulated with 10^{-7} M fMet-Leu-Phe (triangles) or 100 ng/ml PMA (circles) and the superoxide anion production was determined as reported in Materials and methods.

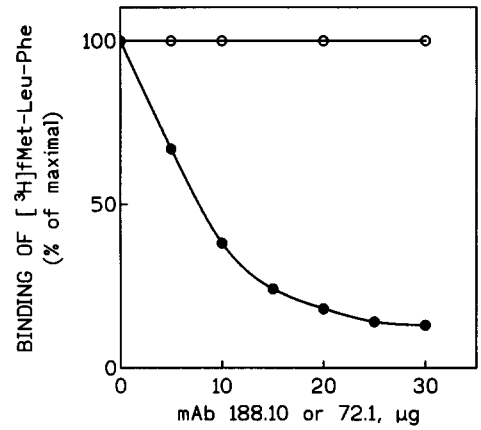

Fig. 2. Competition between fMet-Leu-Phe and mAb 188.10 or mAb 72.1 for the binding to the neutrophil surface. Neutrophils (10^6 cells/ml) were incubated with 0.1 μM [^{3}H]fMet-Leu-Phe in the presence or absence of the indicated amounts of mAb 188.10 (•) or mAb 72.1 (○). The bound radiolabelled peptide was evaluated as described in Materials and methods.

Table 2
Binding of monoclonal antibodies 72.1 and 188.10 to neutrophil surface, evaluated by flow cytometry

mAb	Stimulus	FITC-stained cells (%)
72.1	none	8± 2
72.1	PMA	72±12
72.1	fMet-Leu-Phe	68±10
188.10	none	70±10

Resting neutrophils or activated with 100 ng/ml PMA or 0.1 μM fMet-Leu-Phe were treated with the indicated mAbs as described in Materials and methods. Binding of these mAbs was evaluated by labelling the cells with a fluoresceinated goat anti-mouse antibody. Positive cells were determined by cytofluorimetric analysis. The values refer to the percentage of labelled cells (approximately 10^4 total cells counted) in four different experiments.

3.3. Binding of mAbs to intact neutrophils

To better define the association of these antibodies with the cell surface antigens, their binding properties were analyzed by flow cytometry, using a fluoresceinated goat anti-mouse antibody capable of recognizing those cells having mouse-mAbs exposed on their external surface. As shown in Table 2, when neutrophils were exposed to mAb 72.1, only 8% of total cells become fluoresceinated, thus indicating that only this amount of resting cells possesses the ability to bind mAb 72.1. When neutrophils are stimulated with PMA or with fMet-Leu-Phe before the addition of the antibody mAb 72.1, the number of positive cells increases to approximately 70% of the total cell population, indicating that the binding of the mAb occurs only if cells are exposed to an activating agent. On the contrary, binding of mAb 188.10, is maximal also on resting neutrophils; in these conditions, approximately 70% of the total cells are recognized by mAb 188.10 and become fluorescent without prior activation (see Table 2).

3.4. Effect of mAb 72.1 on granule secretion by activated neutrophils

The effect produced by binding of mAb 72.1 to neutrophil surface on the external secretion of intracellular granule has also been explored. As shown in Table 3, addition of mAb 72.1 produces a significant inhibition (approximately 80–90%) of the O_2^- production regardless of the stimuli employed but has no effect on the secretion of the granule content, revealed by the appearance of lysozyme activity in the extracellular medium. It can be inferred that this mAb interacts with a protein antigen directly involved in the activation of the NADPH oxidase complex. These data are also indicative of a high degree of specificity of the surface antigen protein being its apparent effect limited exclusively to the inhibition of NADPH oxidase system.

Table 3
Effect of mAb 72.1 on responses of human neutrophils stimulated with different agonists

Stimulus ($OD_{550}/10^6$ cells)	O_2^- production ($OD_{450}/10^6$ cells)		Lysozyme secretion	
	−mAb	+mAb	−mAb	+mAb
PMA, 100 ng/ml	0.720	0.122	0.150	0.170
fMet-Leu-Phe, 0.1 μM	0.580	0.075	0.250	0.241
OAG, 0.3 mM	0.422	0.010	0.195	0.208
LTB_4, 0.1 μM	0.362	0.044	0.175	0.193
PAF, 2 μM	0.403	0.058	0.225	0.234

Human neutrophils were incubated with the indicated amounts of stimuli in the absence or in the presence of 30 μg/ml monoclonal antibody 72.1. The O_2^- production and the release of lysozyme were determined as described in Materials and methods. If the excess of monoclonal antibody 72.1 is removed before the addition of the indicated stimuli, the inhibitory effect is completely abolished, indicating that there is no formation of aspecific cross-reactions with the receptors for these stimuli.

3.5. Correlation between the appearance of 72.1 antigen on the neutrophil surface and the onset of NADPH oxidase complex

To define the relationship between the 72.1 antigen and the NADPH oxidase complex, we have compared the kinetics of appearance of the 72.1 antigen on neutrophil surface, measured by flow cytometry, with the onset of the NADPH oxidase activity, measured by the rate of O_2^- production in cells exposed to different concentrations of fMet-Leu-Phe. As shown in Fig. 3, the 72.1 antigen appears on the surface of neutrophils

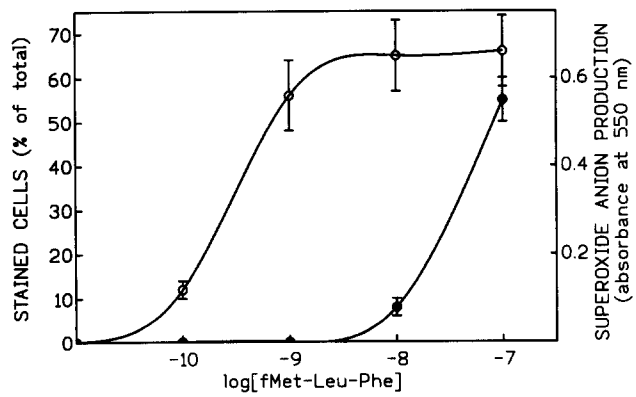

Fig. 3. Correlation between appearance of mAb 72.1 protein antigen on the cell surface and the superoxide anion production by fMet-Leu-Phe stimulated neutrophils. Cells (10^6 cells/ml) were incubated with the indicated concentration of fMet-Leu-Phe and the superoxide anion production (●) was determined as described in Materials and methods. In parallel, aliquots of stimulated cells were incubated with mAb 72.1 (30 μg) and the positive cells (○) were stained with fluoresceinate goat anti-mouse antibody (see Materials and methods).

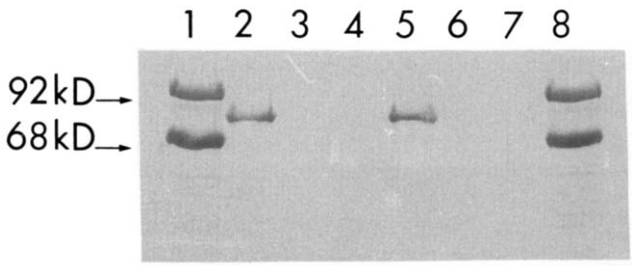

Fig. 4. Identification of the protein molecule recognized by mAb 72.1. Resting and PMA-stimulated neutrophils (see Materials and methods) were lysed and the subcellular fractions were prepared as reported in Materials and methods. An aliqout of each cell fraction (0.1 mg of protein) was loaded on a SDS-PAGE. At the end of the electrophoretic run the proteins were blotted on a nitrocellulose sheet and stained with mAb 72.1. Lane 1 and 8 contains standard proteins (glycogen phosphorylase *b*, molecular mass = 92 kDa; bovine serum albumin, molecular mass = 68 kDa), lanes 2, 3 and 4 contain membrane, cytosolic and granule fractions, respectively, from activated neutrophils; lanes 5,6 and 7 the membrane, granule and cytosolic fractions, respectively, from resting neutrophils.

at a concentration of 0.1 nM fMet-Leu-Phe and becomes almost completely external at a concentration of the formylated peptide at which O_2^- production is still undetectable. This condition induced by both concentrations of fMet-Leu-Phe represents a transition state from a non activated, but highly responsive, to a fully-activated state. Taken together, these results indicate that the appearance of the 72.1 antigen on the neutrophil surface, and the onset of the oxidative burst by activated NADPH oxidase, are sequential but distinct processes, each one elicited by different concentrations of stimulus. 'Primed' neutrophils (cells treated at concentration of formylated peptide insufficient to promote the oxidative burst; see Fig. 3) express the 72.1 antigen at the maximal extent.

3.6. Identification of the 72.1 antigen protein

To identify the protein recognized by the mAb 72.1, neutrophil subcellular fractions were prepared and the localization of 72.1 antigen was investigated in each of the separated cell components. In Western blot analysis the mAb identifies, in large amount, a single protein band with a molecular mass of 82 kDa localized in the plasma membrane fraction (Fig. 4, lanes 2 and 5); because of its molecular mass, this protein becomes easily distinguishable from the components of the NADPH oxidase complex. No cross-reactive material was detected in all other subcellular fractions (Fig. 4, lanes 3,4,6 and 7). This analysis shows that the 72.1 antigen is detectable in plasma membranes of both resting and activated cells (c.f., lanes 2 and 5). At present, no information is available on the mechanism(s) responsible for the appearance of the 72.1 antigen on the external surface of the cell plasma membranes. Preliminary results indicate that this protein undergoes a phosphorylation-dephosphorylation cycle, but the relevance of this reversible covalent modification on the localization of 72.1 antigen has not yet been established.

4. Discussion

Neutrophils, as all other white blood cells, express a number of surface antigens, the functions of many of which are not well defined. Through the action of surface receptors, neutrophils recognize the site of inflammation and trigger the mechanisms leading to their numerous responses [6].

In this paper, we report the identification of a novel neutrophil antigen that appears on the cell surface only following cell stimulation. The binding of a specific mAb to this protein antigen, which has a molecular mass of 82 kDa, completely inhibits the neutrophil oxidative burst, promoted by a number of stimuli. This newly identified protein is involved in the regulation of O_2^- production, but apparently is not part of the NADPH oxidase complex, because of two main reasons: (1) the molecular mass of the protein antigen is different from those of each of the NADPH oxidase components [41–42] and (2) its appearance on the cell surface is not kinetically correlated with the onset of O_2^- production (see Fig. 3). Although the antigen is present in membrane preparations from resting and activated neutrophils, in intact cells interaction between mAb 72.1 and its protein antigen occurs only following cell activation. These data are consistent with the hypothesis that, in an early phase of the activation process, a modification of the 72.1 antigen occurs, capable of inducing its recognition on the surface of neutrophils. Since preliminary observations have shown that this protein can undergo reversible phosphorylation, it might be suggested that this mechanism is involved in its externalization process. Due to the well known importance of the asymmetric organization of NADPH oxidase, it could be suggested a role of the protein in the assembly of the NADPH oxidase components within the neutrophil membrane; a role possibly correlated with the modulation of the activity of the multienzyme system. To explore this possibility, experiments with reconstituted system are now in progress, to evaluate the effect of the 72.1 antigen protein on the isolated NADPH oxidase complex. For comparison, we have studied also the effect of another monoclonal antibody, mAb 188.10, which recognizes the receptor for the formylate chemotactic peptide fMet-Leu-Phe. Also with this antibody, we were able to promote complete inhibition of the O_2^- production, wich is restricted to the stimulation produced by the formylated peptide.

For this reason, it appears that the novel protein recognized by mAb 72.1 may represent an efficient and specific target for the modulation of the neutrophil oxidative burst. In addition, being the 72.1 antigen expressed also in primed neutrophils, its presence could be of potential interest in establishing the activated or preactivated state of neutrophils, a condition that represents a potential toxic side effect in specific human pathologies.

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