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CELL-DEPENDENT CHEMILUMINESCENCE. MODULATION OF THE N-FORMYL CHEMOTACTIC PEPTIDE (FNLPNTL) MEDIATED OXIDATIVE BURST IN HUMAN POLYMORPHONUCLEAR LEUKOCYTES (PMNL) BY MURINE MONOCLONAL ANTIBODY NMS-1

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Binding of purified monoclonal antibody (moAB) IgM NMS-1 to suspended initially spherical living human PMNLs is not associated with the generation of chemiluminescence but was found to enhance the chemiluminescence response to the N-formyl chemotactic peptide FNLPNTL.

We investigated quantitatively the kinetics of oxygen metabolite generation by PMNLs stimulated with FNLPNTL ± moAB NMS-1 using luminol-dependent chemiluminescence as a very sensitive detection system. Chemiluminescence detection allowed the analysis of the time sequence of onset and development of reactive oxygen metabolites following stimulation of PMNLs by FNLPNTL in the presence of moAB NMS-1. The increase of response of PMNLs stimulated with FNLPNTL in the presence of moAB NMS-1 depended on the concentration of the antibody and the sequence of stimulus addition.

Stimulation of human PMNLs by 10 nM FNLPNTL induced a rapid burst of chemiluminescence which peaked ~ 5 min after stimulus addition. The subsequent addition of moAB NMS-1 ($\geq 2\,\mu$g/ml DPBS(+) – 0.1% HSA, 37°C) to FNLPNTL-stimulated PMNLs – after the FNLPNTL-mediated response had already decayed (16–18 min) – without delay induced a second burst of oxygen metabolite generation. The magnitude of this second peak of activation was dose-dependent.

Treatment of PMNLs with moAB NMS-1 ($\geq 1\,\mu$g/ml DPBS(+) – 0.1% HSA, 3 min, 37°C) – prior to FNLPNTL (10 nM) stimulation – increased rate and magnitude of the FNLPNTL-mediated response. This response is biphasic with the first peak at the FNLPNTL position and a second, higher peak ~ 16 min after FNLPNTL addition. The magnitude of response was dose-dependent. The latency (lag time) of the respone was not changed compared to controls which received no moAB NMS-1 treatment.

The observed moAB NMS-1 dependent increase in FNLPNTL-mediated chemiluminescence is transient (50–60 min), persistent activation was not detected.

KEY WORDS: Chemiluminescence, N-formyl chemotactic peptide, monoclonal antibody, human, PMNL, polymorphonuclear leukocytes.

INTRODUCTION

Murine monoclonal antibodies (moAB) directed to various surface epitopes on human polymorphonuclear leukocytes (PMNL) have been obtained which modulate ligand-mediated cell functions or even mimic ligand-induced effects. MoABs have

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been generated which either stimulate or inhibit ligand- or particle-mediated cellular responses. Inhibition of phagocytosis was reported for several moABS (AHN-1, VIM-5, VIM-12, 5.2, 23H7, anti-Mo1)[1–4] whereas moAB L12.2[5] activated phagocytic activity of PMNLs. Chemotaxis was inhibited by moAB NCD1, NCD3, 60.3 and 1–14[6–10] but stimulated by moAB L12.2 and S5.22.[5]

Modulation of the oxidative response of PMNLs to soluble or particulate stimuli by moABs was also reported. Several moABs, including anti-My26, PMN7C3, 23H7, anti-Mo1, 1–14, and 1–15 inhibited the stimulus-generated oxidative response,[3,10–12] whereas moAB L12.2 induced superoxide anion generation.[5] We reported on the generation of IgM moAB NMS-1 which is directed to a periodate-sensitive surface epitope on human PMNLs.[13–16] We observed that interaction of suspended initially spherical PMNLS with moAB NMS-1 induced no detectable superoxide anion or hydrogen peroxide formation.[13–16] Interaction of moAB NMS-1 with human PMNLs, however, was found to modulate the oxidative response to N-formyl chemotactic peptides (FNLPNTL). Rate and magnitude of FNLPNTL-mediated superoxide anion and hydrogen peroxide production were increased in the presence of moAB NMS-1.

Detection of reactive oxygen metabolite formation by chemiluminescence is a very sensitive technique which complements results obtained measuring superoxide anion or hydrogen peroxide production and which allows the fine analysis of response generation.[17] In the present study we report that luminol-dependent chemiluminescence can be used as a rapid, highly sensitive detection system to analyze the time sequence of onset and development of PMNL oxidative burst following stimulation by FNLPNTL in the presence or absence of modulating moAB NMS-1.

MATERIALS AND METHODS

Suppliers of chemicals and reagents are indicated in parantheses. FNLPNTL (Bachem, Bubendorf, Switzerland). Human serum albumin (Behring, Marburg, FRG). Bovine serum albumin, silicon solution (Serva, Heidelberg, FRG). Luminol (Sigma, St. Louis, USA). Heparin (Fluka, Buchs, Switzerland. All other reagents were of analytical grade purchased from Merck (Darmstadt, FRG) or Serva (Heidelberg, FRG).

Purification of Human PMNLs

Human PMNLs were purified from heparin-anticoagulated freshly drawn blood obtained from healthy volunteers according to the method described by Keller *et al.*[18] This method of cell purification includes a Ronpacon/Methocel sedimentation step followed by Ronpacon/Ficoll centrifugation. The separation media contained 10 units of heparin/ml. Purified PMNLs were suspended in sterile-filtered 2% HSA-modified Gey's[18] at room temperature. Remaining erythrocytes were not lysed. On the average 3 erythrocytes/PMNL were counted. Only > 90% initially spherical, nonaggregated PMNL preparations (95–98% neutrophils) were used in the chemiluminescence determinations. Contaminations of PMNL preparations by mononuclear cells was < 1%.

Chemiluminescence Assay

PMNLS (100,000/ml) were suspended at room temperature in Dulbecco's PBS (DPBS) containing 0.5 mM $MgCl_2$, 0.9 mM $CaCl_2$, 1 mg glucose/ml (DPBS(+)), and 10 μl luminol-BSA reagent/ml[19] in siliconized glass beakers. 750 μl of cell suspension was preincubated (30 min, 37°C) in siliconized vials before the addition of stimulus. 10 vials were measured repetitively (10 sec each). Chemiluminescence was measured in a computer-interfaced detection system described by Bertoni *et al.*[20] using a Kontron Betamatic I liquid scintillation spectrometer and a Hewlett Packard 98165 microcomputer. Based on the technical conception of the chemiluminescence detection system, there is a short delay (15 sec) in measuring the first time point after stimulus addition.

Generation and Purification of moAB NMS-1

Hybridoma NMS-1 was generated by fusion of the nonsecreter mouse myeloma cell line P3 × 63Ag8 − 653 with spleen cells obtained from a BALB/c-BYJ mouse immunized with highly purified human PMNL plasma membranes.[21] Hybridoma NMS-1 secretes IgM monoclonal antibodies. 10^7 hybridoma NMS-1 were injected per pristane-primed BALB/c-BYJ mouse and 2 weeks later moAB NMS-1 containing ascites fluid was collected. IgM moAB NMS-1 was purified by euglobulin precipitation as described elsewhere.[22] This moAB NMS-1 preparation is pure as judged by HPLC-gel filtration and SDS-PAGE analysis.[22] IgG, transferrin, and serum albumin were not detected. Sterile-filtered purified moAB NMS-1 (5.2 mg/ml TBS) was stored at −80°C and thawed just before chemiluminescence analysis.

RESULTS

Binding of moAB NMS-1 to suspended PMNLs induced no chemiluminescence but increased the response to the N-formyl chemotactic peptide FNLPNTL

Binding of purified moAB NMS-1 (≤ 250 μg/ml, 37°C, 60 min) to PMNLs was not associated with the generation of reactive oxygen metabolites as measured by luminol-dependent chemiluminescence. Formation of superoxide anion or hydrogen peroxide by moAB NMS-1 treated PMNLs was also not detected (data not shown).

Treatment of suspended initially spherical human PMNLs with moAB NMS-1, however, was found to modulate the oxidative response to N-formyl chemotactic peptides. We investigated quantitatively the kinetics of reactive oxygen metabolite formation using luminol-dependent chemiluminescence (100,000 PMNLs/ml DPBS(+)−0.1% HSA, 37°C). Stimulation of PMNLs with the N-formyl chemotactic peptide FNLPNTL in the presence of moAB NMS-1 enhanced the oxidative response compared to PMNLs which received no moAB NMS-1 treatment. The magnitude of the response depended on the sequence of stimulus addition.

Quantitative assessment of luminol-dependent chemiluminescence generation in PMNLs stimulated with FNLPNTL ± moAB NMS-1

Stimulation of PMNLs with an optimal dose of FNLPNTL (10 nM) induced a rapid chemiluminescence response which peaked at 5 min after stimulus addition (Fig. 1). This response decayed rapidly within the next 10 min reaching a constant plateau

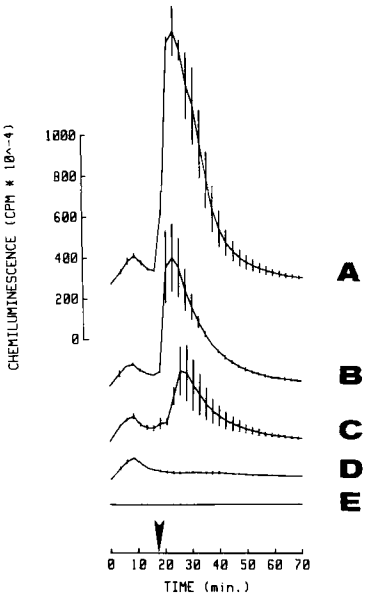
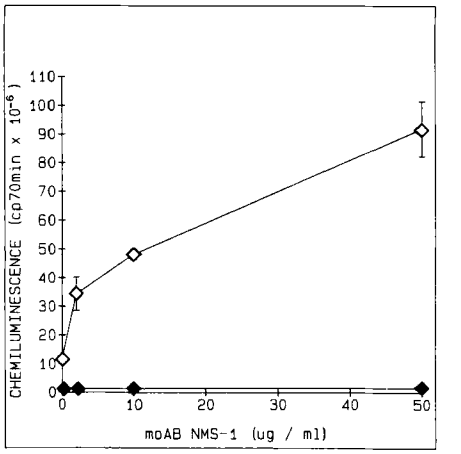


Figure 1A



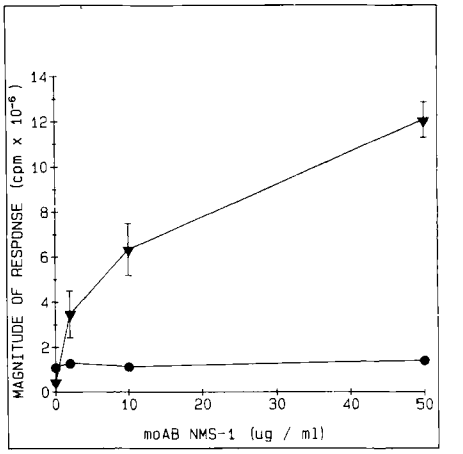


Figure 1B

FIGURE 1 Luminol-dependent chemiluminescence formation by human PMNLs stimulated with FNLPNTL prior to moAB NMS-1 addition. (A) 100,000 PMNLS/ml DPBS(+) − 0.1% HSA + luminol (37°C) were stimulated with 10 nM FNLPNTL and then assessed for chemiluminescence formation in the presence or absence of moAB NMS-1. Trace E: control (DPBS(+) − 0.1% HSA). Trace D: 10 nM FNLPNTL. Trace A–C: 10 nM FNLPNTL, 17 min later moAB NMS-1 was added (arrow). Trace C: 2 μg/ml. Trace B: 10 μg/ml. Trace A: 50 μg/ml. Under these conditions 50 μg moAB NMS-1/ml, without the addition of FNLPNTL, induced no chemiluminescence response (see also Fig. 2). Mean of two determinations ± SD of a single donor. Same donor as in Fig. 2. (B) Calculation of total cpm and maximal magnitudes of response of curves collected in Fig. 1A. Total counts per 70 min generated by PMNLs which were stimulated with 10 nM FNLPNTL in the presence or absence of moAB NMS0-1. –◆–◆– moAB NMS-1, no FNLPNTL. –◇–◇– 10 nM FNLPNTL, after 17 min addition of moAB NMS-1. The magnitudes of response (expressed as cpm) at the FNLPNTL and the moAB NMS-1 position were also calculated and plotted versus moAB NMS-1 concentration. –●–● FNLPNTL peak –▼–▼– moAB NMS-1 peak.

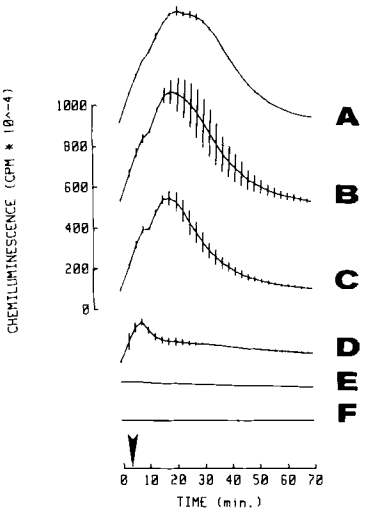
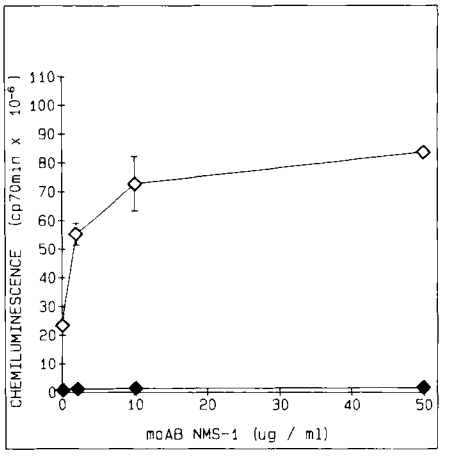


Figure 2A



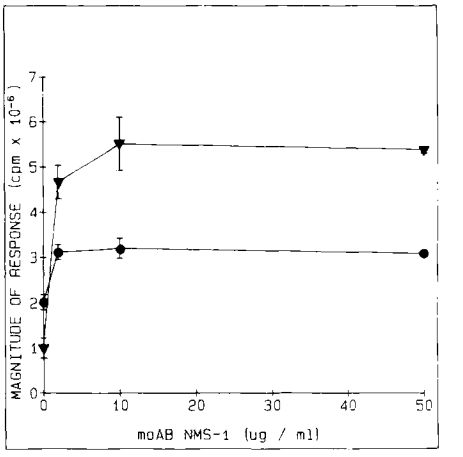


Figure 2B

FIGURE 2 Luminol-dependent chemiluminescence formation by human PMNLs reacted with moAb NMS-1 prior to FNLPNTL stimulation (A) 100,000 PMNLs/ml DPBS(+) – 0.1% HSA + luminol (37°C) were reacted with various concentrations of moAB NMS-1 (3 min) and then assessed for chemiluminescence formation in the presence or absence of 10 nM FNLPNTL (arrow). Trace F: control (DPBS(+)–0.1% HSA). Trace E: 50 μg moAB NSM-1/ml, no addition of FNLPNTL. Trace D: 10 nM FNLPNTL. Trace A–C: PMNLs were reacted with increasing concentrations of moAB NMS-1 (3 min, 37°C) and then 10 nM FNLPNTL was added. Trace C: 2 μg/ml. Trace B: 10 μg/ml. Trace A: 50 μg/ml. Mean of two determinations ± SD of a single donor. Same donor as in Fig. 1. (B) Calculation of total cpm and maximal magnitudes of response of curves collected in Fig. 2A. Total counts per 70 min generated by PMNLs which were reacted with moAB NMS-1 prior to stimulation with 10 nM FNLPNTL. –◆–◆– moAB NMS-1, no FNLPNTL. –◇–◇– moAB NMS-1, after 3 min addition of 10 nM FNLPNTL. The magnitudes of response (expressed as cpm) at the FNLPNTL and the moAB NMS-1 position were also calculated and plotted versus moAB NMS-1 concentration. –●–●– FNLPNTL peak –▼–▼– moAB NMS-1 peak.

(higher than background DPBS(+) – 0.1% HSA) which only slightly decreased within the next 60 min. Addition of moAB NMS-1 to FNLPNTL-stimulated PMNLS – after the FNLPNTL-mediated response had already decayed (~17 min) – without delay induced a second burst of chemiluminescence. The magnitude and position (5–9 min after moAB NMS-1 addition) of this second peak of activation were dose-dependent. This second burst of activation continued for the next 50 min, finally reaching the level of activation of control cells which received no moAB NMS-1 treatment. Persistent activation was not observed. This period of activation was independent of the amount of moAB NMS-1 (2–50 μg/ml) used for activation. The magnitudes and peak positions of moAB NMS-1 induced chemiluminescence responses, however, were dose-dependent. The time course of FNLPNTL activation, calculated values of maximal magnitudes of response, and summarized total counts in the presence and absence of moAB NMS-1 are shown in Fig. 1A, B.

We also observed that short exposure of suspended PMNLs to moAB NMS-1 (3 min, 37°C) enhanced the subsequent chemiluminescence response induced by the addition of 10 nM FNLPNTL, compared to controls which received no moAB NMS-1 treatment (Fig. 2). Magnitude and rate of FNLPNTL-induced response were enhanced by moAB NMS-1 pretreatment. The onset of the response was not changed after moAB NMS-1 pretreatment. This response is biphasic with the first peak at the FNLPNTL position and a second, higher peak 14–16 min after FNLPNTL-addition. The magnitudes of the peaks were dose-dependent (2–50 μg/ml); significant changes in the peak positions were not observed. The time course of activation, calculated values of maximal magnitudes of response, and summarized total counts in the presence or absence of moAB NMS-1 are shown in Fig. 2A,B. The FNLPNTL response ± moAB NMS-1 terminated 70 min after FNLPNTL addition.

We analyzed the accelerating effect of moAB NMS-1 on FNLPNTL-induced chemiluminescence generation in PMNLs in more detail. Results summarized in Fig. 3 demonstrate that the activation curve of chemiluminescence – obtained with FNLPNTL-stimulated PMNLs which had been pretreated with increasing doses of moAB NMS-1 – most probably is the result of two superimposing activation curves. The first peak of activation is caused by the FNLPNTL addition, the second peak of activation is the result of the subsequent moAB NMS-1 addition. This second peak of activation is not observed in PMNLs which received no moAB NMS-1 treatment. Addition of 1 μg moAB NMS-1/ml was sufficient to induce a significant second burst of chemiluminescence formation.

DISCUSSION

Binding of murine moAB NMS-1 to suspended initially spherical human PMNLs (70 min, 37°C) did not induce luminol-dependent chemiluminescence generation. This is in agreement with our previous findings that exposure of moAB NMS-1 to suspended PMNLs did not induce detectable superoxide anion or hydrogen peroxide formation.[13–15] These results suggest that surface-binding of IgM moAB NMS-1 to suspended human PMNLs is not a sufficient trigger to generate measurable amounts of oxygen metabolites. MoAB NMS-1 binds to a periodate-sensitive surface epitope on human PMNLs.[16] Although binding of moAB NMS-1 to PMNL surfaces did not induce oxidative burst formation, we observed a modulating effect of moAB NMS-1 on N-formyl chemotactic peptide-mediated oxidative burst formation.[13–15]

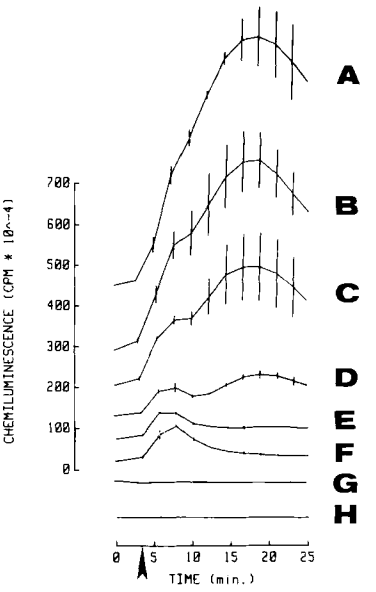


Figure 3A

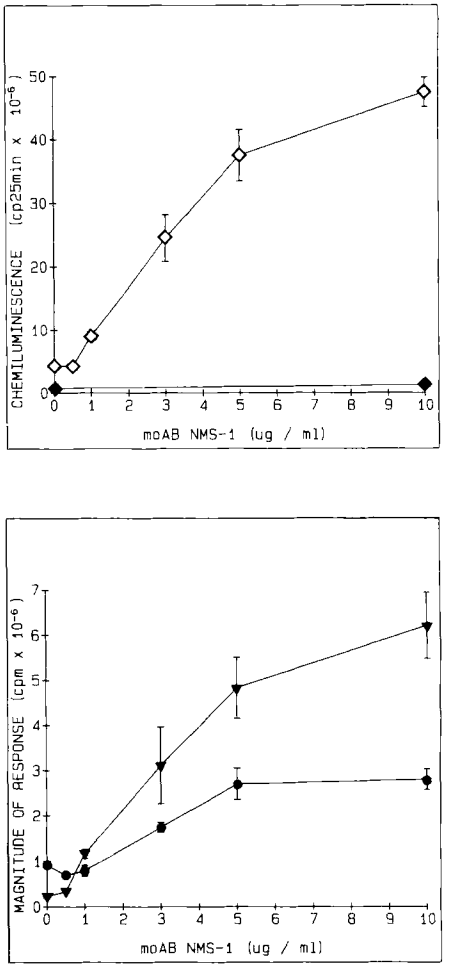


Figure 3B

FIGURE 3 Biphasic luminol-dependent chemiluminescence formation by human PMNLs reacted with moAB NMS-1 prior to FNLPNTL stimulation. (A) 100,00 PMNLs/ml DPBS(+)−0.1% HSA + luminol (37°C) were reacted with various concentrations of moAB NMS-1 and then assessed for chemiluminescence formation in the presence or absence of 10 nM FNLPNTL. Trace H: control (DPBS(+)−0.1% HSA). Trace G: 10 μg moAB NMS-1/ml, no addition of FNLPNTL. Trace F: 10 nM FNLPNTL. Trace A–E: PMNLs were reacted with increasing concentrations of moAB NMS-1 (3 min, 37°C) and then 10 nM FNLPNTL was added (arrow). Trace E: 0.5 μg/ml. Trace D: 1 μg/ml. Trace C: 3 μg/ml. Trace B: 5 μg/ml. Trace A: 10 μg/ml. Mean of two determinations ± SD of a single donor. Different donor than in Fig. 1 and 2. (B) Calculation of total cpm and maximal magnitudes of response of curves collected in Fig. 3A. Total counts per 25 min generated by PMNLs which were reacted with moAB NMS-1 prior to stimulation with 10 nM FNLPNTL. –◆–◆– moAB NMS-1, no FNLPNTL. –◇–◇– moAB NMS-1, after 3 min addition of 10 nM FNLPNTL. The magnitudes of response (expressed as cpm) at the FNLPNTL and the moAB NMS-1 position were also calculated and plotted versus moAB NMS-1 concentration. –●–●– FNLPNTL peak –▼–▼– moAB NMS-1 peak.

In the present report we used the very sensitive chemiluminescence assay system to analyze quantitatively the onset and formation of N-formyl chemotactic peptide FNLPNTL-mediated chemiluminescence generation in the presence or absence of moAB NMS-1. We found that simultaneous interaction of suspended PMNLs with moAB NMS-1 and FNLPNTL increased the oxidative response compared to controls which received no moAB NMS-1 treatment. The magnitude of the increase of the chemiluminescence response depended on the sequence of stimulus addition. We observed that pretreatment of PMNLs for 3 min (37°C) with 1 μg moAB NMS-1/ml was sufficient to result in a significant increase of rate and magnitude of the FNLPNTL-induced chemiluminescence response. This response is biphasic with a maximum at a position different from the FNLPNTL-induced peak position. The first peak of activation was found at the FNLPNTL position ($\sim$ 5 min after FNLPNTL addition). The second, higher peak was detected $\sim$ 16 min later after FNLPNTL addition. The latency of the response was not changed by moAB NMS-1 pretreatment.

We observed that pretreatment of suspended PMNLs with FNLPNTL was a sufficient trigger to induce an increase in chemiluminescence formation mediated by moAB NMS-1 binding. Addition of moAB NMS-1 to PMNLs – which were prestimulated with FNLPNTL – after the FNLPNTL-induced oxidative response had already decayed ($\sim$ 17 min) resulted into an immediate second burst of chemiluminescence formation. The described augmentation of the FNLPNTL-induced chemiluminescence formation by moAB NMS-1 was transient, persistent activation was not observed. Increase of N-formyl chemotactic peptide-induced oxidative burst formation by the sequential addition of N-formyl peptide and a different heterologuous stimulus to suspended human PMNLs has been described for phorbol myristate acetate, lectins, cytochalasins, calcium ionophores, leukotriene B4, and opsonized zymosan.[23–27] In addition, we observed that binding of moAB NMS-1 to suspended human PMNLs may also serve as a PMNL-directed priming stimulus in the activation of the oxidative burst by N-formyl chemotactic peptide-treated PMNLs. The so far described PMNL-directed moABs did not enhance N-formyl peptide-induced oxidative burst formation.[1–12]

Several explanations may be at hand to explain the apparent moAB NMS-1-mediated increase in FNLPNTL-induced oxidative burst formation: (1) Binding of moAB NMS-1 to the ligand-binding site within the receptor pocket or to nonligand-binding entities of the receptor area (2) Change of affinity of N-formyl peptide receptor after moAB NMS-1 treatment (3) Inhibition of ligand-receptor complex internalization (4) MoAB NMS-1 stimulated incorporation of new receptor entities into the plasma membrane from intracellular stores (upregulation). (5) Aggregation or cross-linking of surface determinants by moAb NMS-1 (6) Direct activation of the NADPH-oxidase system. (7) Effect on membrane-associated GTP-binding protein (8) Activation of phosphoinositide turnover, phospholipase C, and protein kinase C.

MoAB NMS-1 is not directed to the FNLPNTL-binding site of the N-formyl peptide receptor.[16] Upregulation of moAB NMS-1 specific surface epitopes by FNLPNTL treatment has not been observed.[16] We have already shown that binding of purified nonaggregated moAB NMS-1 to suspended PMNLs is not a sufficient stimulus to induce detectable oxidative burst formation. Cross-linking of PMNL surface-bound IgM moAB NMS-1 by anti-IgM, however, resulted in significant oxidative burst generation.[13] The possibility of direct activation of the FNLPNTL-specific signal transduction pathway by moAB NMS-1 may be screened by measuring

phosphoinositide – protein kinase C turnover or GTP-binding protein activity. Changes in ligand affinity, formation of the cytoskeleton-bound high affinity receptor complex or on- and off-rates of ligand-receptor interaction may also be responsible for the accelerating effect of moAB NMS-1. Direct activation of the plasma membrane-associated NADPH-oxidase system or inhibition of receptor-ligand internalization may also explain the moAB NMS-1 dependent enhancing effect. We are currently investigating these possibilities.

Acknowledgements

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