# Deficiency of neutrophil membrane antigen detected by monoclonal antibody in rheumatoid arthritis

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Monoclonal antibodies (mAbs) against cell surface antigens and receptors are instrumental in defining specific membrane markers. mAbs GF 26.7.3 and MF 25.1 against human neutrophils modulated the activation mechanism of superoxide anion production induced by formyl-peptide and PMA in all subjects. However, treatment with mAb MF 25.1 of neutrophils from patients with rheumatoid arthritis did not have any effect. This may suggest that the antigen which MF 25.1 binds is absent in rheumatoid conditions. This confirms our previous data showing that defective expression of membrane components is associated with neutrophil dysfunction.

*Neutrophil activation* *Monoclonal antibody* *Rheumatoid arthritis* *Superoxide production*

## 1. INTRODUCTION

Neutrophils are essential in host defense against microorganisms, viruses and tumours but are also the major effector cells causing tissue damage in a wide variety of autoimmune disorders, such as rheumatoid arthritis. The molecular basis for this effect is not completely clear, but a relationship between defective protein components of the plasma membrane and the altered response of neutrophils to stimulation has been previously detected in rheumatoid subjects [1–4]. Similarly, other authors have shown defects in neutrophil function associated with a deficiency of plasma membrane glycoproteins [5–7]. A number of mAbs have been described as modulating the response triggered by various stimulants [9,10], suggesting that surface neutrophil antigens mediate cellular activation. mAbs GF 26.7.3 and MF 25.1 against human neutrophils, found to bind to cell surface antigens and to inhibit oxidative metabolism [11], were chosen to react with neutrophils from rheumatoid patients as probes of cellular responses under pathological conditions.

*Abbreviations:* mAb, monoclonal antibody; PMA, phorbol 12-*O*-myristate 13-acetate; RA, rheumatoid arthritis; FMLP, *N*-formyl-methionyl-leucyl-phenylalanine; $O_2^-$, superoxide anion; KRPG, Krebs-Ringer-phosphate containing 0.2% (w/v) glucose

## 2. MATERIALS AND METHODS

mAbs GF 26.7.3, and MF 25.1, which specifically react with human PMNs, were produced as described in [12,13]. Ascitic fluids GF 26.7.3 and MF 25.1 were fractionated by 45% ammonium sulfate, dialyzed against PBS (pH 7.4) at 4°C and the concentration determined at 280 nm. Serial dilutions of mAbs were made in PBS. The effect of mAb-neutrophil interaction was studied by exposing $1 \times 10^6$ neutrophils in KRPG for 30 min at

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37°C to antibody concentrations varying from 1 to 100 μg/ml followed by washing the cells. Control PMNs were not treated with mAbs.

Neutrophils were obtained from 20 healthy volunteers, 14 subjects with classical rheumatoid arthritis according to ARA criteria and 3 patients with psoriatic arthritis. All patients had taken no medication for the preceding 3 days. Cells were treated by dextran (Pharmacia) sedimentation of erythrocytes, and further purified by lymphocyte separation medium (Flow Labs); contaminating red cells were lysed with 0.86% $NH_4Cl$. The cells (99–100% granulocytes) were washed twice and resuspended in KRPG (pH 7.4) to give 5–10 × $10^6$ cells/ml.

Superoxide anion release was monitored continuously in a temperature-controlled spectrophotometer (Zeiss PQ 11) as the reduction of ferricytochrome *c*, as described in [14]. At zero time, different amounts of each stimulant were added to 1 × $10^6$ neutrophils/ml and the absorbance change accompanying cytochrome *c* reduction was monitored at 550 nm. The stimulants used were PMA and FMLP. Formyl-peptide was added to cell suspensions after 5 min incubation with 10 μM cytochalasin B. All compounds were from Sigma.

The Wilconxon test was used in statistical evaluation of the differences between groups.

## 3. RESULTS

### 3.1. *$O_2^-$ production by neutrophils from normal and RA subjects*

As shown in [11], no significant differences in $O_2^-$ generation was detected when neutrophils from healthy and RA subjects were stimulated by PMA and FMLP. The PMA concentration ranged from 81 nM to 16.2 μM and that of FMLP from 10 μM to 10 nM; maximal $O_2^-$ production was found to occur at 162 nM PMA and 1 μM FMLP and amounted to 24.6 ± 1 and 10.8 ± 2 nmol $O_2^-$/5 min per $10^6$ neutrophils, respectively.

### 3.2. *Effect of mAbs on $O_2^-$ production by neutrophils from normal and RA subjects*

The pre-incubation of 1 × $10^6$ neutrophils from healthy individuals with 15 μg/ml of MF 25.1 or GF 26.7.3 inhibited $O_2^-$ production induced by PMA and FMLP. As shown in fig.1, the extent of

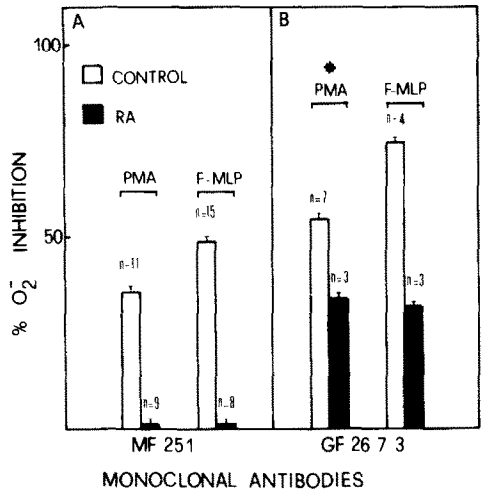


Fig.1. Effect of antibody treatment on $O_2^-$ production induced by PMA and FMLP. 1 × $10^6$ neutrophils from normal subjects (empty columns) and rheumatoid patients (filled columns) were preincubated at 37°C for 30 min with 15 μg/ml MF 25.1 (A) or 15 μg/ml GF 26.7.3 (B). Activation of the $O_2^-$ generating system was induced by 0.162 μM PMA and 1 μM FMLP. Bars represent ± SE of separate experiments performed in duplicate. * Difference between control and RA treated is statistically significant ($p > 0.01$). *n*, number of experiments.

the inhibitory effect depended on both the cell stimulant and the mAb applied. GF 26.7.3 produced 75% inhibition when the enzymatic complex was induced to release $O_2^-$ by 1 μM FMLP. However, when neutrophils from RA patients were treated with MF 25.1 and then stimulated with PMA or FMLP to produce $O_2^-$ no inhibition occurred. After treatment of RA neutrophils with GF 26.7.3 a significant difference in the antibody-mediated inhibitory effect was detectable. It is interesting to note that neutrophils from patients with psoriatic arthritis did not exhibit functional diversity after pre-treatment with either mAb (not shown), in comparison with normal cells.

## 4. DISCUSSION

The two mAbs GF 26.7.3 and MF 25.1, specific for antigens present on the human neutrophil membrane [12,13], were found to inhibit $O_2^-$ release induced by PMA and formyl-peptide [11]. The cell activators used mediate their biological ef-

fects via different mechanisms: FMLP interacts through its proper surface receptor [15] and PMA activates protein kinase C [16]. We have previously shown that GF 26.7.3 and MF 25.1 bind to separate antigen determinants, therefore the inhibition produced by the two mAbs could be attributed to interference with the stimulus-mediated process of activation of NADPH oxidoreductase rather than the functional inactivation of specific receptor sites.

Neutrophils derived from RA patients show a significant decrease in inhibition of $O_2^-$ generation in the presence of GF 26.7.3 and do not respond to pre-treatment with MF 25.1, demonstrating that the superoxide enzymatic complex is unaffected by this antibody. The results may suggest that in rheumatoid disease, most likely the surface antigen determinant reacting with mAb MF 25.1 is lost, this being consistent with previous findings of defective expression of neutrophil membrane proteins in RA. Thus, the mAb MF 25.1 is able to reveal altered neutrophil capacities by not reacting with the defective neutrophil population that characterize RA, an immunological disorder in which neutrophil dysfunction is not completely manifest. Anti-neutrophil mAb MF 25.1 might have some clinical use in diagnosis of RA since neutrophils from psoriatic arthritis, a very similar autoimmune disease, do not share this functional diversity.

In conclusion, mAbs are a very useful tool in the study of neutrophil function in both normal and pathological conditions.

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