Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 4002–4006, June 1986
Medical Sciences

Monoclonal antibodies to a particulate superoxide-forming system stimulate a respiratory burst in intact guinea pig neutrophils

(phagocytic cells/NADPH oxidase)

G. BERTON*, H. ROSEN†, R. A. B. EZEKOWITZ†, P. BELLAVITE*, M. C. SERRA*, F. ROSSI*, AND S. GORDON†‡

†Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, United Kingdom; and *Institute of General Pathology, University of Verona, Verona, Italy

Communicated by Zanvil A. Cohn, January 2, 1986

ABSTRACT **Monoclonal rat antibodies were produced against a subcellular preparation of phorbol 12-myristate 13-acetate (PMA)-stimulated guinea pig neutrophils that retains NADPH-oxidase activity. Two antibodies, 1A10.4 and IG4, were isolated that bind to a surface antigen restricted to guinea pig neutrophils from bone marrow and peritoneal exudate and to macrophages and that trigger a respiratory burst in neutrophils in the presence of cytochalasin B. Intact antibody 1A10.4, subclass IgG2c, can trigger superoxide anion release directly; $F(ab')_2$ fragments of 1A10.4 and intact IG4 require further cross-linking by $F(ab')_2$ fragments of anti-rat immunoglobulin antibody. Both antibodies recognize the same antigen, a proteolipid of apparent molecular mass 10 kDa. Immunoprecipitation of solubilized oxidase activity with 1A10.4 brings down this activity as part of a macromolecular complex. Surface expression of the antigen is increased on treatment of cells with both PMA and cytochalasin B. 1A10.4 also triggers release of the granule enzyme β-glucuronidase. Triggering of a respiratory burst by the antibodies appears distinct from the PMA and fMet-Leu-Phe signalling systems. These studies indicate that the antigen defined by antibodies 1A10.4 and IG4 becomes associated with the superoxide anion-generating system of neutrophils but may play a more general role in signal transduction in phagocytic cells.**

Phagocytosis of opsonized particles and microorganisms by neutrophils and macrophages (Mϕ) is accompanied by increased cyanide-insensitive oxygen consumption, the respiratory burst (RB) (1, 2). The generation of superoxide anion (O_2^-) during the RB is the crucial and flux-generating step of oxidative killing by these cells. O_2^- dismutes to H_2O_2 and various reactive oxygen intermediates are produced. O_2^- is formed by a poorly characterized electron transfer complex in the membranes of phagocytic cells, the NADPH oxidase (3). Subcellular macromolecular aggregates that retain NADPH oxidase activity have been isolated from phorbol 12-myristate 13-acetate (PMA)-stimulated guinea pig neutrophils (4). To shed light on the components of the oxidase complex and its regulation, we have raised monoclonal antibodies (Ab) to such material. In this report we describe monoclonal Ab to a component of the oxidase preparation that bind to a surface antigen on intact guinea pig neutrophils and trigger a RB.

MATERIALS AND METHODS

Cells. Guinea pig leukocytes were elicited from 350-g Hartley guinea pigs of either sex by intraperitoneal injection of sterile 1% casein in normal saline. Neutrophils were harvested by peritoneal lavage after 20 hr, Mϕ after 4 days. Lymphocytes were prepared by depleting a lymph node suspension of adherent cells. Bone marrow cells were prepared by flushing the femoral marrow cavity with Dulbecco A phosphate-buffered saline (PBS). Erythrocytes were lysed in 0.2% NaCl or distilled water. Differential counts were performed with Giemsa stain. The 20-hr peritoneal exudate contained >95% neutrophils. Rat neutrophils and mouse bone marrow cells were prepared as above.

Production of Monoclonal Ab. AO rats were immunized and boosted four times (5) with fractions of guinea pig neutrophils with NADPH-oxidase activity. These were prepared according to Serra *et al.* (4) by extraction with 0.3% deoxycholate of active subcellular particles from PMA-stimulated neutrophils. The solubilized activity was further purified by chromatography on an Ultrogel AcA 22 column and isopyknic glycerol gradient centrifugation. Monoclonal Ab were obtained from a fusion between one of these rat spleens and the nonsecreting hypoxanthine phosphoribosyltransferase-negative rat myeloma line Y3, the kind gift of C. Milstein (Cambridge, U.K.). The fusion protocol was essentially that of ref. 6. Hybrids were screened for binding to guinea pig myelomonocytic cells as opposed to lymphocytes. The binding of hybridoma supernatants to rat neutrophils and mouse bone marrow cells was also excluded. Hybrids restricted to surface antigens on guinea pig myelomonocytic cells (6 out of 289) were then cloned by limiting dilution. The cloned hybridomas grew readily as ascites in pristane-treated AO rats. Hybridoma tissue culture supernatants were concentrated four times by ultrafiltration and screened for their ability to stimulate O_2^- release by intact guinea pig neutrophils. Of the 6 hybridomas selected, 1, designated 1A10.4, triggered the RB spontaneously. Another, IG4, could be induced to trigger a RB on cross-linking by a $F(ab')_2$ fraction of anti-rat Ab. The remaining 4 had no activity.

Purification of Ab. Ab 1A10.4 was purified from ascites fluid by precipitation with 25% (wt/vol) Na_2SO_4, diethylaminoethylcellulose chromatography, and affinity chromatography on rabbit Ab to rat immunoglobulin (RAR)-Sepharose. 1A10.4 is a rat IgG2c as typed by Ouchterlony gel diffusion using sera supplied by H. Bazin (Louvain, Belgium). The IgG was free from contamination when analyzed on an overloaded sodium dodecyl sulfate/polyacrylamide gel ($NaDodSO_4$/PAGE) stained with Coomassie blue G-250. 1A10.4 $F(ab')_2$ was produced by digestion of purified IgG with 1% pepsin for 30 min at 37°C at pH 4.5 and separated from intact IgG and cleavage fragments by gel filtration chromatography. Analysis on 5–20% polyacrylamide gels

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Ab, antibody (antibodies); CB, cytochalasin B; Mϕ, macrophage(s); PMA, phorbol 12-myristate 13-acetate; RAR, $F(ab')_2$ fragments of rabbit antibodies to rat Fab; RB, respiratory burst; SOD, superoxide dismutase.

‡To whom reprint requests should be addressed.

showed the $F(ab')_2$ to be free of IgG. IG4 is an IgM as shown by $NaDodSO_4$/PAGE after purification from tissue culture supernatants by affinity chromatography.

Stimulation of O_2^- Secretion. The ability of Ab to trigger a RB in guinea pig neutrophils was assayed kinetically as the superoxide dismutase (SOD)-inhibitable reduction of ferricytochrome *c* by cells in suspension (7). The reaction mixture contained 80 μM cytochrome *c*, 2 mM NaN_3, and cytochalasin B (CB) at 5 μg/ml in Krebs/Ringer phosphate buffer, pH 7.4, with 5.5 mM glucose and 0.75 mM Ca^{2+} (KRPG). SOD was added at 15 μg/ml as a negative control, and PMA at 200 ng/ml was used to trigger maximal release of O_2^-. CB and PMA were stored as 2 mg/ml stock solutions in dimethyl sulfoxide, which alone had no effect on O_2^- secretion. Reactions took place at 37°C and O_2^- generation was measured at 550 nm with a full-scale deflection of 0.5. When indicated, cells were incubated with Ab at 4°C for 30–60 min before commencing the assay. Results are expressed as the maximal initial rate of cytochrome *c* reduction.

Indirect Binding Assays. Binding of Ab to cells was assessed by indirect radioimmunoassay (8). Glutaraldehyde-fixed (0.125%) neutrophils (10^5 cells per well) adherent to poly(L-lysine)-coated 96-well microtiter plates were used. The monoclonal Ab was applied first and after washing was detected with an iodinated second-Ab $F(ab')_2$, either RAR or MRC OX12, a monoclonal anti-rat κ-chain Ab (9).

Flow Cytometry. Live cells (10^6) were incubated for 1 hr at 4°C with saturating concentrations of hybridoma supernatant, washed, then incubated with fluorescein isothiocyanate-conjugated OX12 $F(ab')_2$ before analysis on a Becton Dickinson FACS (5).

Site Analysis. The 1A10.4 $F(ab')_2$ was iodinated by using chloramine-T (5). Site number was determined in two independent experiments by a single-step tube binding assay in triplicate. Cells (2×10^6) were incubated for 60 min at 4°C with a trace of iodinated 1A10.4 $F(ab')_2$ and a saturating excess (0.225 mg/ml) of unlabeled 1A10.4 $F(ab')_2$ prior to washing and measuring radioactivity. Maximal competition of unlabeled for iodinated $F(ab')_2$ occurred at Ab concentrations of 80 μg/ml.

$NaDodSO_4$/PAGE and Immunoblotting. Samples for gel electrophoresis were boiled in 1% $NaDodSO_4$/0.5% 2-mercaptoethanol prior to separation on 5–20% polyacrylamide gels in the system of Wyckoff *et al.* (10). Gels were electrophoretically transferred to nitrocellulose membranes (Schleicher & Schuell BA 85) in 25 mM Tris/192 mM glycine containing 20% (vol/vol) methanol, using a Bio-Rad Transblot cell at 60 V for 3 hr (11). With 0.7 mm gels, almost complete transfer was obtained as assessed by staining gels with Coomassie blue after blotting. The presence of antigen on nitrocellulose blots was demonstrated by indirect immunoassay at room temperature. All buffers contained 0.2% Tween 20. The blot was incubated with 3% bovine serum albumin in PBS for 1 hr, overlaid with hybridoma supernatant for 2 hr, and washed with PBS containing 10 mM NaN_3 and 0.5% bovine serum albumin (five changes over 30 min) before incubation with ^{125}I-labeled OX12 $F(ab')_2$ for 1 hr. After extensive washing, the blot was dried and exposed to prefogged Kodak direct exposure film at −70°C.

Immunoprecipitation of the Oxidase Complex. Detergent-solubilized preparations with NADPH-oxidase activity were incubated overnight at 4°C with various hybridoma supernatants. RAR-Sepharose presorbed with 4% rabbit serum was then added. After 3 hr the beads were separated from supernatant, washed, and assayed for oxidase activity (4).

β-D-Glucuronidase. β-Glucuronidase activity was measured in three independent experiments by the liberation of phenolphthalein from phenolphthalein β-monoglucuronide (Sigma) in 0.2 M sodium acetate, pH 4.5, at 37°C (12). Guinea pig neutrophils at 5×10^7 per ml in KRPG with CB at 5 μg/ml were stimulated at 37°C for 30 min with KRPG, 1A10.4, or PMA at 0.2 μg/ml. Those cells stimulated by 1A10.4 were incubated with saturating concentrations of monoclonal Ab for 30 min at 4°C, then washed before addition to the degranulation buffer. The supernatant was then assayed for β-glucuronidase; the results are expressed as the percentage of total cellular β-glucuronidase activity measured after lysis of unstimulated cells with 0.5% Triton X-100.

Protein Concentrations. These were determined by the method of Lowry *et al.* using BSA standards (13).

Media. All hybridomas were cultured in Iscove's modified Dulbecco's medium supplemented with 5% fetal bovine serum (GIBCO) and gentamicin at 20 μg/ml.

Reagents. PMA, CB, ferricytochrome *c* (type VI), bovine serum albumin, SOD, Tween-20, and Triton X-100 were obtained from Sigma. All other chemicals were of analytical grade.

RESULTS

Cell and Species Specificity of 1A10.4. The cell and species specificity of Ab 1A10.4 were assessed by indirect binding assays and flow cytometry. Fig. 1*A* shows the binding of purified 1A10.4 to glutaraldehyde-fixed guinea pig neutrophils. Ab 1A10.4 also bound to live guinea pig neutrophils in bone marrow and in 20-hr peritoneal exudates, and to peritoneal Mϕ, but not to spleen or lymph node lymphocytes (not shown). The percentage of cells labeled in bone marrow and exudates (40% and 90%, respectively) corresponded closely with differential counts of neutrophils. There was no binding of 1A10.4 to rat, mouse, or human neutrophils. Antigen 1A10.4 was stable to fixation in methanol as well as glutaraldehyde. Indirect absorption assays (14) revealed antigen 1A10.4 in lysates of guinea pig bone marrow cells, peritoneal exudates, and Mϕ, but not lymphocytes, brain, or liver. The antigen was also readily detected in the NADPH-oxidase-containing preparations used for immunization (see below). Similar results were obtained with Ab IG4. These studies indicated that monoclonal Ab 1A10.4 and IG4 were directed against species-restricted antigen(s) found in subcellular fractions derived from guinea pig neutrophils, as well as on the surface of intact myelomonocytic cells, including neutrophils in bone marrow that had not been exposed to an inflammatory stimulus *in vivo*.

1A10.4 Triggers the RB. Hybridoma supernatants containing saturating levels of Ab 1A10.4 were able to trigger a vigorous RB upon incubation with guinea pig neutrophils in bone marrow and peritoneal exudates, and this activity was exhibited also by purified Ab (Fig. 1*B*). Fig. 2*A* shows O_2^- release after a short lag period when neutrophils were incubated with Ab 1A10.4 in the presence of CB. In the absence of CB O_2^- release induced by the Ab varied in different experiments. More rapid induction and higher rates of O_2^- secretion comparable to the rate elicited by PMA (see below) were obtained when neutrophils were incubated with 1A10.4 for 30–60 min prior to beginning the assay. SOD abolished measurement of this response. Control experiments showed that Ab 1A10.4 stimulated O_2 consumption and hexose monophosphate shunt activity, as well as O_2^- release (not shown). The ability of Ab 1A10.4 to stimulate a RB directly was selective, since several other myelomonocytic Ab at saturation failed to do so.

Further studies were undertaken to examine the interaction of Ab 1A10.4 with live neutrophils by measuring O_2^- release. The rate of O_2^- release triggered by 1A10.4 depended on Ab concentration (Fig. 1*B*). At concentrations below 3 μg/ml in assays using 2×10^6 cells per ml, the Ab did not trigger a RB although binding could be demonstrated. Half-

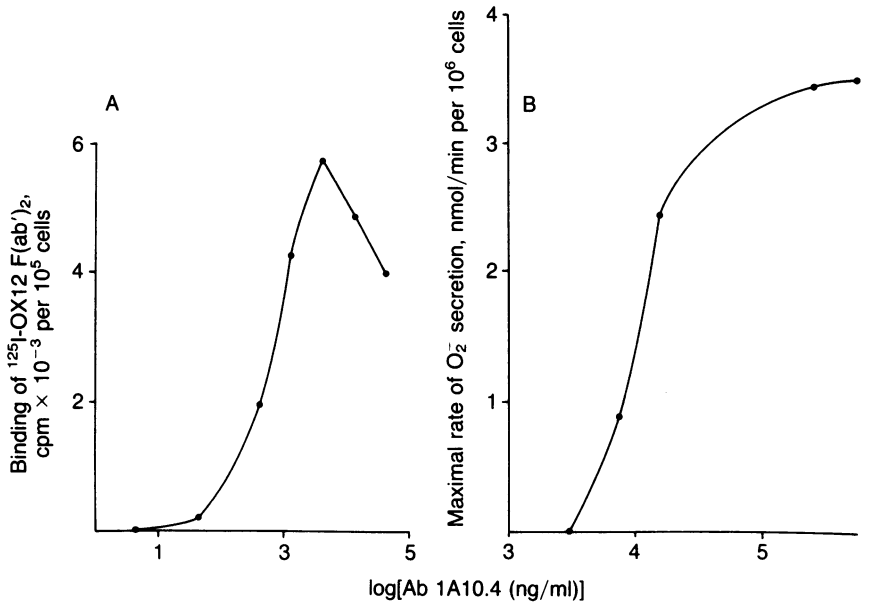

FIG. 1. (*A*) Binding of purified 1A10.4 to glutaraldehyde-fixed guinea pig neutrophils detected by indirect radioimmunoassay. There were 10^5 cells per well, and the initial Ab concentration was 0.32 mg/ml. (*B*) Stimulation of O_2^- release by purified 1A10.4. Dose response in which 10^6 cells were incubated with 1A10.4 for 30 min at 4°C before kinetic assay in the presence of CB at 5 μg/ml.

maximal O_2^- secretion was obtained at Ab concentrations of 15 μg/ml.

To exclude the possibility that Ab 1A10.4 mediated O_2^- release via the Fc receptor, 1A10.4 F(ab')$_2$ was used. Though 1A10.4 F(ab')$_2$ had essentially the same binding characteristics (not shown) as the intact molecule (Fig. 1*A*), binding of the F(ab')$_2$ alone failed to trigger the RB (Fig. 2*B*). However, high rates of O_2^- release were obtained by cross-linking the 1A10.4 F(ab')$_2$ after binding, using OX12 F(ab')$_2$. The second Ab alone had no effect on O_2^- release. The maximal rates of O_2^- secretion triggered by intact 1A10.4 and its F(ab')$_2$ are listed in Table 1. Of the five other Ab selected, IG4 also triggered the RB on cross-linking with OX12 F(ab')$_2$ (Table 1). The other four, incubated at saturating concentrations, had no effect on O_2^- release even with cross-linking.

Since the intact 1A10.4, but not its F(ab')$_2$, triggered a RB, the role of the Fc portion of this Ab was investigated further. First, it was found that intact 1A10.4 Ab did not trigger O_2^- release by rat bone marrow neutrophils, which do not express the antigen, but which do express receptors for the Fc fragment of certain classes of homologous immunoglobulin. Furthermore, incubation of rat neutrophils with 1A10.4 F(ab')$_2$ followed by OX12 F(ab')$_2$ did not induce a RB. Second, an experiment was designed to ask whether binding

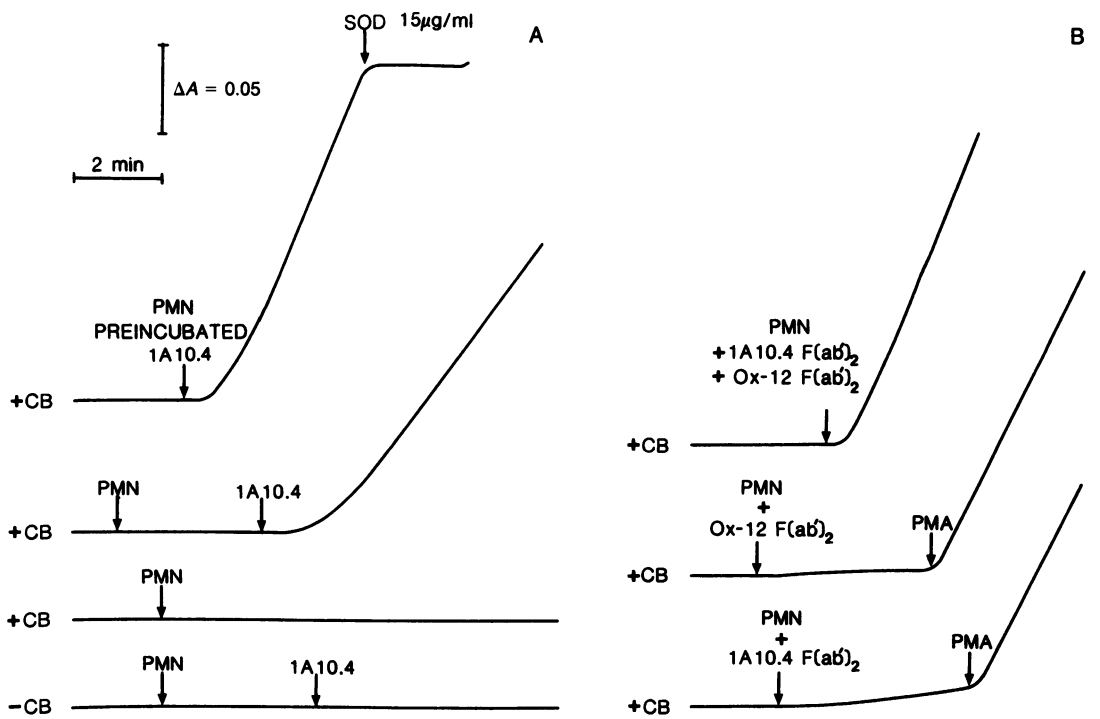

FIG. 2. (*A*) Intact 1A10.4 Ab triggers O_2^- release in the presence of CB. Kinetic assay at 550 nm. PMN, polymorphonuclear leukocytes. (*B*) F(ab')$_2$ fragments of Ab 1A10.4 trigger O_2^- release only after cross-linking with OX12 F(ab')$_2$. All Ab were used at saturating concentrations.

Table 1. Effects of monoclonal antibodies and fragments on O_2^- release by guinea pig exudate neutrophils

Treatment	Maximal rate of O_2^- release, nmol/min per 10^6 cells		
	1A10.4	IG4	PMA
Control (SOD 15 μg/ml)	0.0	0.0	
1st Ab (IgG)	4.1 ± 0.3	0.0	4.2 ± 0.4
1st Ab $(Fab')_2$	0.0	ND	
2nd Ab alone [OX12 $(Fab')_2$]	0.0	0.0	
1st Ab $(Fab')_2$ + OX12 $(Fab')_2$	2.9 ± 0.3	ND	2.8 ± 0.7
1st Ab (IgG) + OX12 $(Fab')_2$	ND	2.8 ± 0.7*	

All Ab were used at saturating concentrations. Results are mean ± 2SD of at least three independent kinetic assays except for that indicated by *, in which two independent experiments were performed. ND, not done.

of 1A10.4 IgG to glutaraldehyde-fixed guinea pig neutrophils would opsonize these targets and induce an Fc-mediated RB in live neutrophils. Target neutrophils were incubated with saturating amounts of 1A10.4 IgG for 1 hr at 4°C. After washing, surface-bound Ab could be detected with an iodinated second Ab. In the reaction mixture 4×10^6 of these coated cells were presented to 10^6 live guinea pig neutrophils at 37°C. Though these cells had a competent RB when challenged either with PMA or 1A10.4 in solution, the surface-immobilized Fc failed to trigger the RB. There was sufficient Ab in the system to trigger a maximal RB on live targets (data not shown). This result, taken with the cross-linking experiments and species specificity of triggering, indicates that 1A10.4 triggers O_2^- release by an antigen–Ab interaction rather than via Fc receptors.

Biochemical Analysis of the Antigen Identified by 1A10.4 and IG4. *Nature of the antigen and its role.* The antigen recognized by both Ab 1A10.4 and IG4 has been demonstrated in Triton X-100 lysates (1%) of guinea pig neutrophils and Mϕ. Samples were separated on $NaDodSO_4$/5–20% PAGE and blotted onto nitrocellulose, and the antigen was visualized by indirect radioimmunoassay. Fig. 3 shows a typical blot of the antigen, which has an apparent molecular mass of approximately 10 kDa. Molecular mass did not vary with either reduction or alkylation. Studies to be reported elsewhere (H.R. and S.G., unpublished data) indicate that the antigen is a proteolipid. Specificity controls have shown that the antigen is present in neutrophil membrane preparations made from PMA-stimulated and unstimulated cells and in the immunogen but cannot be demonstrated in lysates of guinea pig erythrocytes or lymphocytes, nor is it present in lysates of rat neutrophils or mouse bone marrow. None of the four other hybrids or polyclonal rat IgGs bound to this 10-kDa species. Immunoblotting is thus specific with respect to Ab, species, and cell type.

1A10.4 precipitates oxidase activity. A close association between 1A10.4 antigen and the oxidase complex was suggested by the ability of 1A10.4 Ab to immunoprecipitate oxidase activity. A detergent-solubilized NADPH-oxidase preparation was incubated with 1A10.4 hybridoma supernatant, supernatant from a nontriggering hybrid, or medium alone. The Ab was precipitated with RAR-Sepharose and the beads were assayed for oxidase activity. In the average of two experiments, precipitated oxidase activity was 1.45 nmol/30 min with 1A10.4, 0.36 nmol/30 min for the other hybrids, and 0.26 nmol/30 min for medium alone. Control experiments showed that Ab 1A10.4 did not influence NADPH-oxidase activity directly. These studies indicated that 1A10.4 antigen was associated with the oxidase complex in deoxycholate-solubilized material.

Surface expression of 1A10.4 antigen increases on treatment with PMA and CB. To learn more about the distribution of antigen 1A10.4 in intact cells, peritoneal exudate neutrophils were stimulated for 90 sec at 37°C in KRPG with PMA at 200 ng/ml, CB at 5 μg/ml, or both agents and site numbers on the cell surface were measured in a single-step binding assay at saturation. For the purposes of calculation, binding was assumed to be monovalent at saturating concentrations of 1A10.4 $F(ab')_2$. A molecular mass of 100,000 kDa was assumed for the $F(ab')_2$. On the average, $3.62 \pm 0.35 \times 10^5$ sites per cell were found on native cells or cells treated with PMA or CB. Cells treated with both PMA and CB had 6.30 $\pm 0.63 \times 10^5$ sites per cell, an increase of 1.75-fold.

1A10.4 also triggers release of β-glucuronidase. To discover whether exocytic phenomena triggered by the binding of 1A10.4 are restricted to O_2^- release, we examined release of the azurophil granule enzyme β-glucuronidase. We found that intact Ab 1A10.4 also triggered β-glucuronidase release directly. Neutrophils incubated for 30 min at 37°C with 1A10.4 Ab released 24 ± 2% of total cellular β-glucuronidase compared with 8 ± 2% in untreated controls and 31 ± 3% after stimulation with PMA. This finding indicates that 1A10.4 antigen can trigger degranulation as well as the RB. Further studies are needed to establish whether Ab 1A10.4 can also influence other neutrophil responses such as phagocytosis and chemotaxis.

DISCUSSION

In this paper we describe two monoclonal antibodies, 1A10.4 and IG4, that identify a proteolipid surface antigen restricted to guinea pig myelomonocytic cells and that on binding have the ability to trigger a RB. Preliminary experiments using intact neutrophils as an immunogen led to the production of

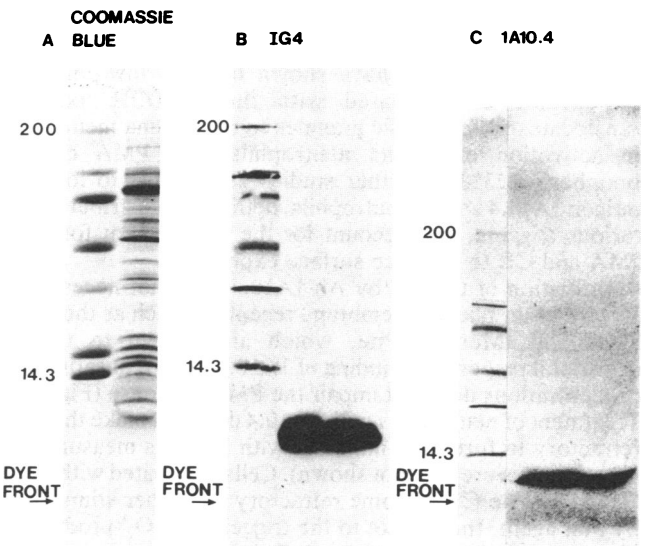

FIG. 3. $NaDodSO_4$/PAGE and immunoblotting of 1A10.4 antigen. All samples are reduced unless otherwise stated. The left track of each panel contains protein standards of molecular mass 14.3–200 kDa, either prestained (Bethesda Research Laboratories) (*A*) or [^{14}C]methylated (Amersham) (*B* and *C*). (*A*) A 15% gel stained with Coomassie blue. The right track shows a $NaDodSO_4$-solubilized neutrophil membrane pellet prepared according to ref. 4. (*B*) Autoradiograph of an IG4 immunoblot of a 15% gel. The center and right tracks contain the oxidase preparation reduced and unreduced, respectively. (*C*) A 1A10.4 immunoblot of a 5–20% gel. The center track contains the guinea pig neutrophil membrane preparation seen in *A*. The right track contains the oxidase preparation. (The antigen always appears as a broader band in uniform compared to gradient gels.) Blots made with 1A10.4 are indistinguishable from those made with IG4. Blots with irrelevant antibodies and lysates of guinea pig erythrocytes, lymphocytes, rat neutrophils, and mouse bone marrows were all negative.

antibodies to many neutrophil surface components, yet none of these antibodies could trigger a RB. It was for this reason that we then used a more restricted immunogen even though the oxidase preparation contained many components (4).

Stimulation of the RB by 1A10.4 is temperature sensitive, CB dependent, maximal at saturating concentrations of antibody, and dependent on an antibody concentration of greater than 3 μg/ml before triggering can take place. These data suggest that a critical threshold of site occupancy or antigen density is required for triggering and that clustering of the antigen might play a role. CB may play a role in aggregation of the antigen.

1A10.4 also requires an Fc fragment to trigger the RB directly, without requiring further cross-linking. The role of the Fc fragment in direct stimulation by the intact Ab is not understood, but it may involve aggregation of Ab on the cell surface, since divalent binding alone is insufficient to trigger the RB. Results obtained by Romeo *et al.* (15) with tetra- and divalent concanavalin A are consistent with our present findings. The nature of the Ab and the phagocytic cell also determines the effect of monoclonal Ab on the RB. Intact IG4, which is directed against the same antigen as 1A10.4 (see below) behaves like the 1A10.4 $F(ab')_2$ in that it requires secondary cross-linking before triggering. Finally, although the antigen 1A10.4 is present on the surface of guinea pig peritoneal Mϕ we have thus far been unable to trigger a RB in these cells with intact 1A10.4, even in the presence of CB and after further cross-linking (not shown).

The increase in site number of 1A10.4 antigen on stimulation with PMA and CB, together with the observation that the antigen is detectable on unstimulated bone marrow neutrophils as well as exudate neutrophils, suggests that there could be an intracellular pool of antigen in addition to that detected on the cell surface. Similar results have been found with different neutrophil markers. For example, Borregaard *et al.* (16) have shown that the low-potential cytochrome *b* associated with the NADPH oxidase translocates from specific granules to the plasma membrane on activation of human neutrophils with PMA or the ionophore A23187. Further studies are needed to localize antigen 1A10.4 within neutrophils, define its redistribution by various triggers, and account for the requirement for both PMA and CB to enhance surface expression.

Activation of the RB by Ab 1A10.4 does not necessarily involve other plasma membrane receptors, such as those for PMA and fMet-Leu-Phe, which are known to trigger neutrophil responses. Binding of 1A10.4 $F(ab')_2$ at saturating concentrations does not impair the PMA response (Fig. 2*B*). Treatment of neutrophils with 1A10.4 does not make the cells refractory to further stimulation with PMA as measured by superoxide secretion (not shown). Cells stimulated with 1 μM fMet-Leu-Phe (17) become refractory to further stimulation by that agent, though not to the triggering of O_2^- production by PMA or 1A10.4. (not shown). This suggests that the signal transduction mechanism of 1A10.4 is distinct from that mediated by these other ligands, although it does not exclude a role for antigen 1A10.4 in RB stimulation via these receptors.

Several studies have reported Ab directed against surface molecules that modulate phagocytic cell functions, including the RB (18). For example, Lopez and Vadas described a monoclonal Ab to human neutrophils that enhanced killing of Ab-coated tumor target cells (19), and Cotter *et al.* reported Ab that inhibit chemotaxis by human neutrophils (20, 21). Our strategy differed in that having found that immunization with whole cells was ineffective in generating Ab with the specific function of triggering O_2^- release, we elected to use a more restricted antigen. We suggest that this approach is more likely to allow efficient production of Ab with predefined function. In the event, we identified an antigen that is physically associated with the macromolecular complex with oxidase activity but is also involved in the triggering of other neutrophil responses such as degranulation. The nature of the antigen, its association with components of the RB complex, and its role in signal transduction in phagocytic cells remain to be determined.

We thank Mr. Jim Kent for invaluable technical assistance and Mrs. Pam Woodward and Mrs. Elwena Gregory for typing the manuscript. This work was supported in part by grants from the Medical Research Council (U.K.), the Wellcome Foundation, and the National Research Council of Italy and fellowships from the European Molecular Biology Organization (G.B.), and the Royal Commission for Exhibition of 1851 (H.R.).

1. Badwey, J. A. & Karnovsky, M. L (1980) *Annu. Rev. Biochem.* **49,** 695–726.
2. Rossi, F., Romeo, D. & Patriarca, P. L. (1972) *J. Reticuloendothel. Soc.* **12,** 127–149.
3. Babior, B. M., Curnutte, J. T. & McMurrich, B. J. (1976) *J. Clin. Invest.* **56,** 1035–1042.
4. Serra, M. C., Davoli, A., Bellavite, P., Bannister, J. V. & Rossi, F. (1984) *Biochim. Biophys. Acta* **788,** 138–146.
5. Austyn, J. & Gordon, S. (1981) *Eur. J. Immunol.* **11,** 805–818.
6. Galfre, G., Milstein, C. & Wright, B. (1979) *Nature (London)* **277,** 131–133.
7. Cohen, H. J. & Chavaniec, M. E. (1978) *J. Clin. Invest.* **61,** 1081–1087.
8. Mason, D. W. & Williams, A. F. (1980) *Biochem. J.* **187,** 1–20.
9. Hunt, S. V. & Fowler, M. M. (1981) *Cell Tissue Kinet.* **14,** 445.
10. Wyckoff, M., Rodbard, D. & Chrambach, A. (1977) *Anal. Biochem.* **78,** 459–482.
11. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
12. Fishman, W. H., Kato, K., Anstiss, C. L. & Green, S. (1967) *Clin. Chim. Acta* **15,** 435.
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
14. Lee, S. H., Starkey, P. M. & Gordon, S. (1985) *J. Exp. Med.* **161,** 475–489.
15. Romeo, D., Zabuchi, G., Berton, G. & Schneider, C. (1978) *J. Membr. Biol.* **44,** 321–330.
16. Borregaard, N., Heiple, J. M., Simons, E. R. & Clark, R. A. (1983) *J. Cell Biol.* **97,** 52–61.
17. McPhail, L. C., Clayton, C. C. & Snyderman, R. (1984) *J. Biol. Chem.* **259,** 5768–5775.
18. Rossi, F., Zatti, M., Patriarca, P. L. & Cramer, R. (1971) *J. Reticuloendothel. Soc.* **9,** 67–85.
19. Lopez, A. F. & Vadas, M. A. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1818–1821.
20. Cotter, T., Priscilla, S. & Henson, P. (1981) *J. Immunol.* **127,** 1355.
21. Cotter, T., Keeling, P. & Henson, P. (1981) *J. Immunol.* **127,** 2241–2245.