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Surface-related triggering of the neutrophil respiratory burst. Characterization of the response induced by IgG adsorbed to hydrophilic and hydrophobic glass surfaces

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SUMMARY

Hydrophilic and hydrophobic glass surfaces precoated with human albumin, fibrinogen, or IgG were investigated with respect to their ability to activate the neutrophil NADPH-oxidase. We found that IgG-coated surfaces induced a substantial and prolonged neutrophil production of reactive oxygen species (ROS). When a hydrophilic surface was used to support protein binding, a somewhat lower neutrophil response (around 35%) was obtained, compared with the response induced by IgG on a hydrophobic surface. The production of ROS was completely eliminated when cytochalasin B was added to the measuring system, suggesting the involvement of the cell cytoskeleton in the activation process. The relation between the intra- and extracellular generation of ROS was further assessed, and we found that most of the ROS produced were released from the cells, in agreement with a model in which the activating surfaces induce a 'frustrated' phagocytic response. Serum totally inhibited 'frustrated' phagocytosis provided that the IgG molecules were sticking to a hydrophilic surface.

Keywords neutrophils surface interaction protein adsorption NADPH-oxidase free radical release

INTRODUCTION

Increased knowledge about the molecular determinants of importance for the inflammatory reaction frequently triggered by implanted biomaterials may permit the development of better materials with respect to their biocompatibility. Neutrophils not only form the front line of our defence against infections induced by bacteria and other microorganisms [1], but they have key functions also in the devastating inflammatory reactions associated with rejection of transplanted grafts and foreign bodies [2]. The neutrophils carry out their bactericidal activities by ingesting (phagocytosing) and killing microorganisms, and the killing arsenal is based on two different weapon systems: one being the destructive enzymes with hydrolytic activity present primarily in the neutrophil azurophil granules [3], the other being the system responsible for the production of toxic oxygen metabolites, the NADPH-oxidase [4]. The NADPH-oxidase, composed of a membrane-bound b-type cytochrome and a number of translocating cytosolic components [5], can be assembled in the plasma membrane as well as in the membrane of the phagosome containing an ingested prey. Accordingly, neutrophil production of reactive oxygen species (ROS) during phagocytosis of an easily ingested prey takes place exclusively inside the phagocytosing neutrophil [6]. As biomaterials are foreign to the host, it is not surprising that they can activate inflammatory cells. This may cause degradation of the implanted material [7] and inflict damage on the surrounding tissue [8,9]. It has been postulated that neutrophils activated through an interaction with a prey that is too large to be engulfed will be 'frustrated' and the ROS formed will be secreted from the cells. However, very little is known about the mechanisms that regulate the neutrophil production and release of ROS during this type of 'frustrated phagocytosis'.

Neutrophil recognition, and response to, biomaterials are not determined solely by the surface of an implanted material [10,11], but to a large extent by the nature of the protein layer that, as an unavoidable necessity, will cover the foreign surface soon after implantation [12,13]. Surface characteristics of the implanted material may, however, be of importance for the 'selection' of proteins allowed to bind to the surface as well as for the way that the adsorbed proteins are presented to the cells. The predominating proteins that bind to biomaterials are albumin, IgG, and fibrinogen [14]. Adsorbed albumin does not attract inflammatory cells and tends to dampen inflammatory responses [15]. Adsorbed IgG on a surface can activate complement [16–18], which in turn induces

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neutrophil activation [19,20]. Fibrinogen has been shown to be of great importance in acute inflammatory responses to implanted polymers, as reflected by a large accumulation of phagocytes on an implanted surface covered with this protein [21].

The mechanisms by which biomaterials mediate their proinflammatory activities are only partly known, but from what is described above, it is obvious that phagocytic cells are directly or indirectly involved in the process. In order to increase further our knowledge about the basis for the proinflammatory response induced by biomaterials, we investigated the neutrophil respiratory burst induced during cell interaction with protein-coated surfaces. We have earlier studied protein adsorption to well characterized solid surfaces [16,17] using optical techniques such as ellipsometry, and silicon has then been used as a supporting surface. Based on the fact that silicon (non-transparent) can not easily be used for the determination of neutrophil respiratory burst activity, we chose to use glass surfaces that largely resemble the silicon surfaces used in our earlier studies [17]. The experimental model used allowed us to determine the influence on neutrophil NADPH-oxidase activity of albumin, fibrinogen, IgG, and serum factors adsorbed to hydrophobic glass and hydrophilic glass, respectively. The neutrophil response was further characterized with respect to the subcellular localization of the NADPH-oxidase activity.

MATERIALS AND METHODS

Chemical reagents and materials

Dextran and Ficoll–Paque were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Luminol, isoluminol, formyl-methionyl-leucyl-phenylalanine (fMLP), and cytochalasin B were obtained from Sigma Chemical Co. (St Louis, MO). Superoxide dismutase (SOD), catalase, and horseradish peroxidase (HRP) were from Boehringer (Mannheim, Germany). Ionomycin was purchased from Calbiochem (La Jolla, CA). The glass tubes used (having a similar surface chemistry as silicon; catalogue no. 110.625-6) were from KEBO Lab (Stockholm, Sweden). Human albumin was bought from Behringwerke AG (Marburg-Lahn, Germany), human fibrinogen purchased from Chromogenix AB (Mölndal, Sweden), and human IgG, γ-globulin, 16·5% solution was obtained from Kabi AB (Stockholm, Sweden). Conjugated MoAbs against complement receptor 3 (CR3; specific for CD11b) were obtained from Dakopatts (Glostrup, Denmark), and antibodies against CD18 were a generous gift from Dr S. Wright (Rockefeller University, NY).

All substances were diluted in Krebs-Ringer phosphate buffer supplemented with 10 mM glucose, 1 mM Ca^{2+}, and 1·5 mM Mg^{2+} (KRG, pH 7·3). Proteins used for glass coating were diluted to a final concentration of 1 mg/ml in KRG.

Human serum was collected from blood of five apparently healthy donors using standard techniques and sera were stored at −80°C until use. A serum concentration of 5% was used by dilution in KRG.

Isolation of neutrophils

Human neutrophils were isolated from buffy coats of apparently healthy blood donors. After dextran sedimentation at 1 ***g***, the remaining erythrocytes were lysed hypotonically. The cell suspension was then centrifuged on a Hypaque–Ficoll gradient [22]. The neutrophils were washed twice and resuspended in KRG. Cells were stored on ice until use.

Mobilization of subcellular organelles

Two different protocols were used for mobilization of neutrophil subcellular organelles [23]. In the first protocol, the secretory vesicles were fully mobilized using the chemoattractant fMLP as stimulus. Cells (1×10^7/ml) were incubated at 15°C for 5 min, after which fMLP (10^{-7} M) was added and incubation was continued for another 10 min. The cells were then transferred to a 37°C water bath and allowed to incubate for 5 min. This treatment results in degranulation of secretory vesicles without activating the NADPH-oxidase [24]. In the second protocol, the calcium-ionophore ionomycin was used to mobilize both secretory vesicles and specific granules to the cell surface. After incubation of neutrophils at 37°C for 5 min, ionomycin (5×10^{-7} M) was added and the incubation was continued for 5 min. The release of ROS during granule mobilization was very low [24,25]. Both cell populations were finally washed twice in KRG, and resuspended to a concentration of 1×10^7/ml in KRG. Cells were stored on ice until use.

Determination of CR3 exposure on the cell surface with FACS analysis

Neutrophils were fixed in paraformaldehyde (4% w/v in PBS) on ice for 30 min. The conjugated MoAb (10 μl) specific for CD11b was added to a cell pellet ($\approx$100 μl) of 10^6 cells. The amount of cell-bound probe was determined by flow cytometry (FACScan; Becton Dickinson, Mountain View, CA).

Preparation of hydrophobic and hydrophilic glass surfaces

Glass tubes (40 ×10 mm) were cleaned and made either hydrophilic or hydrophobic as previously described [17]. In brief, the glass tubes were immersed in a basic peroxide solution at 80°C for 5 min (TL1: 2 M NH_4OH and 1·5 M H_2O_2). After rinsing three times with deionized water, the glass tubes were immersed in an acidic peroxidase solution at 80°C for 5 min (TL2: 1·5 M HCl and 1·5 M H_2O_2). This treatment made the glass hydrophilic. Some glass tubes were further methylated by immersion in trichloroethane with 10% dichlorodimethyl silane (DDS) at room temperature for 10 min. This treatment made the glass hydrophobic.

Opsonization of yeast particles with IgG

Yeast particles (*Saccharomyces cerevisiae*, baker's yeast) were heat-killed. IgG opsonization was obtained by mixing 10^8 yeast particles with rabbit anti-yeast IgG. The mixture was incubated at 37°C for 30 min, and the particles were then washed twice and resuspended in KRG.

Chemiluminescence measurement of the neutrophil oxidase activation induced by surfaces

The hydrophilic and hydrophobic glass tubes were coated with human albumin, fibrinogen, or IgG, respectively, at room temperature (22°C) for 30 min. Some of the IgG-coated tubes were further incubated in 5% human serum at room temperature for 30 min. A very sensitive chemiluminescence (CL) technique (well suited for real-time studies) was used for the determination of respiratory burst activity [26]. The glass tubes were filled with 1 ml of reaction mixture containing KRG, neutrophils (1×10^6/ml), and luminol (5×10^{-5} M), after which they were placed in disposable 4-ml polypropylene tubes and transferred into a six-channel Biolumat LB 9505 (Berthold Co., Wildbad, Germany). The light emission was recorded continuously at 37°C for 30 min.

To quantify the intracellular and extracellular generation of ROS, two different reaction mixtures were used. Intracellular ROS

production was measured in a system where a cell-permeable luminol (5×10^{-5} M) was used in combination with SOD (a membrane-impermeable scavenger of O_2^-, 50 U), and catalase (a membrane-impermeable scavenger of H_2O_2, 2000 U) [26]. Extracellular ROS production was assessed by using a cell-impermeable isoluminol (5×10^{-5} M) together with HRP (4 U) [26].

In some experiments, cytochalasin B (5 μg/ml) was employed in the measuring vials, and in yet other experiments fMLP (10^{-7} M final concentration), or opsonized yeast particles (5×10^6/ml) were added to the cells.

The experiments were repeated at least three times, and the curves shown are from representative experiments.

RESULTS

Activation of the neutrophil NADPH-oxidase during interaction with protein-coated glass surfaces

Neutrophil NADPH-oxidase activity induced during interaction with glass surfaces was followed using a very sensitive CL technique. Two surfaces, differing with respect to surface hydrophobicity, were used in the study. The physicochemical properties of the surfaces are summarized in Table 1. Neutrophils were added to glass tubes coated with albumin, fibrinogen or IgG, respectively, and the light emitted during neutrophil interaction with the surfaces was determined. Irrespectively of the physicochemical characteristics of the surface, no neutrophil NADPH-oxidase activity was evoked when either albumin- or fibrinogen-coated glass surfaces were used (data not shown). In contrast, neutrophils interacting with surfaces coated with IgG were activated to produce ROS (Fig. 1). The time course of the neutrophil response to an IgG-coated hydrophobic surface reached a peak value after around 25 min, but the cells were active for a much longer time. The time course of the neutrophil response to an IgG-coated hydrophilic surface was similar, but the activity was lower ($6{\cdot}6 \pm 1{\cdot}3$ Mct/min, compared with $18{\cdot}8 \pm 1{\cdot}7$ Mct/min, the corresponding value on the IgG-coated hydrophobic surface; mean $\pm$ s.d., $n=5$).

Effect of cytochalasin B on neutrophil NADPH-oxidase activation induced during interaction with IgG-coated glass surfaces

Cytochalasin B exerts its effect through interfering with actin filament function. The drug inhibits actin polymerization (F-actin

Table 1. Surface characterizations of the glass surfaces

Surface treatment	Water contact angle ($\theta°$)	Wettability	Dominant chemical groups on surface
TL1 + TL2*	<10	Hydrophilic wetting	OH, O^-
TL1 + TL2 + 10% DDS†	>85	Hydrophobic non-wetting	CH_3

* TL1 and TL2 are the surface treatments making a surface hydrophilic; see Materials and Methods for a detailed description.

† DDS, Dichlorodimethyl silane, which can make the surface hydrophobic.

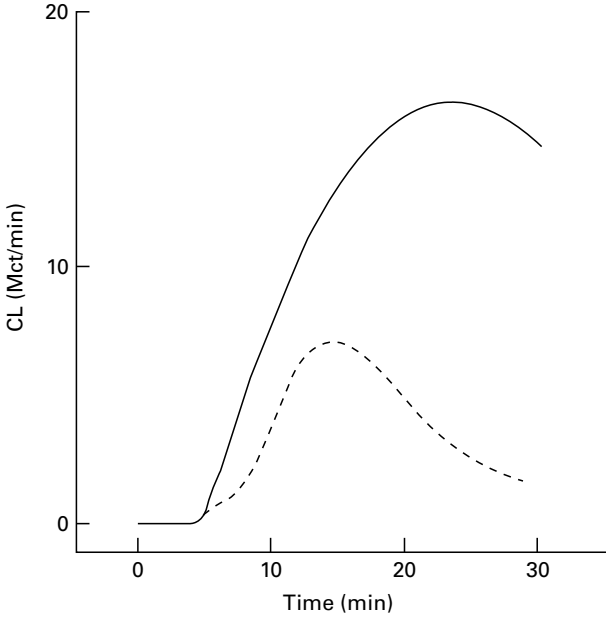

Fig. 1. Neutrophil activation induced by hydrophobic (————) and hydrophilic glass (- - - - -) coated with IgG. The time courses of chemiluminescence (CL) were measured. Abscissa, time of study; ordinate, CL expressed in Mct/min. The curves are from a representative experiment.

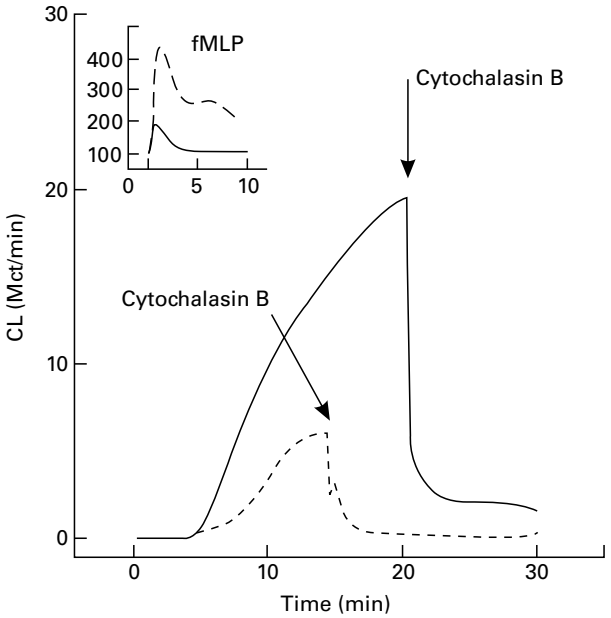

Fig. 2. The effect of cytochalasin B on neutrophil activation induced by hydrophobic (————) and hydrophilic glass (- - - - -) coated with IgG. Cytochalasin B was added at a time point corresponding to the peak response. The time courses of chemiluminescence (CL) were measured. Abscissa, time of study; ordinate, CL expressed in Mct/min. The inset shows the cytochalasin B effect on formyl-methionyl-leucyl-phenylalanine (fMLP)-induced neutrophil respiratory burst where cytochalasin B was added at the beginning of the experiment. In the inset, the solid line represents fMLP-induced CL response without cytochalasin B, and the dashed line represents fMLP-induced CL response with cytochalasin B. The curves are from a representative experiment.

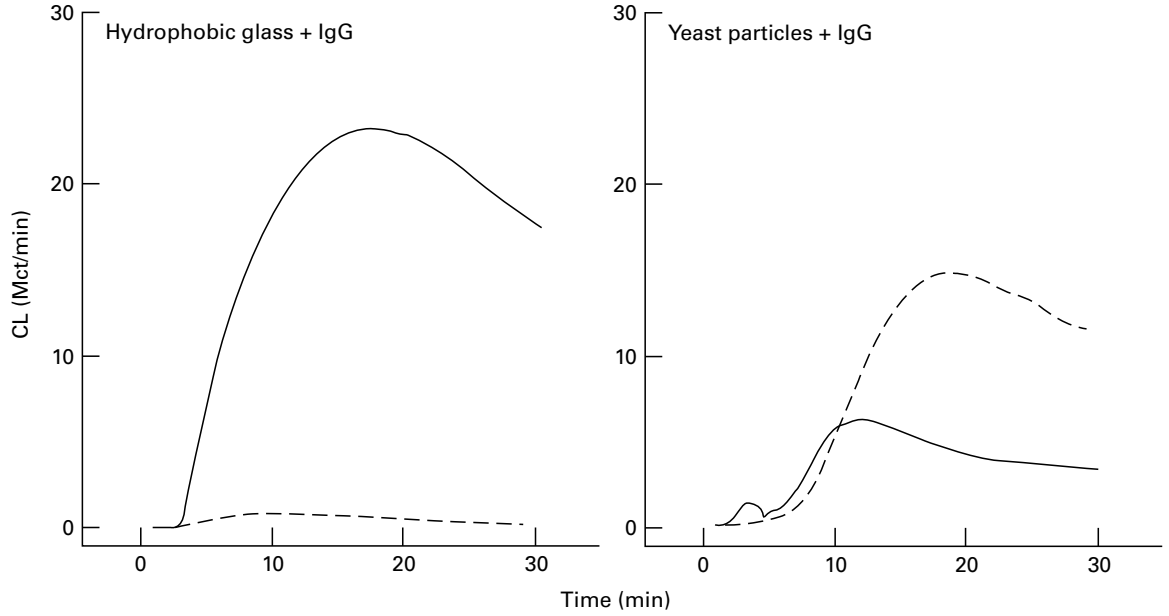

Fig. 3. Localization of the neutrophil NADPH-oxidase activity induced during cellular interaction with hydrophobic glass and yeast particles, which were coated with IgG. The intracellular chemiluminescence (CL; - - - - -) was measured in the presence of superoxide dismutase (SOD) and catalase, whereas extracellular CL (————) was measured in the presence of isoluminol and horseradish peroxidase (HRP). The time courses of CL were measured. Abscissa, time of study; ordinate, CL expressed in Mct/min. The curves are from a representative experiment.

formation), and the dynamic reorganization of the cell cytoskeleton is thus blocked. We found that the ongoing neutrophil NADPH-oxidase activity induced during interaction with the IgG-coated surfaces was rapidly inhibited when cytochalasin B was introduced in the measuring system (Fig. 2). In contrast, cytochalasin B enlarged and prolonged the fMLP-induced burst in neutrophils (Fig. 2, inset) [27,28].

Subcellular localization of the ROS generated during neutrophil interaction with IgG-coated hydrophobic glass

The ROS produced by the neutrophil NADPH-oxidase can either be secreted from the cells (involving mainly the plasma membrane-bound part of the oxidase) or be retained in an intracellular compartment (involving mainly the granule membrane-bound part of the oxidase). The CL technique can be modified to allow determination of the released as well as the intracellularly produced ROS. Yeast particles opsonized with IgG which are phagocytosed by neutrophils induced an intracellular production of ROS (Fig. 3), but when the same opsonin was presented to the cells on a surface that could not be phagocytosed, all the ROS generated were released to the environment (Fig. 3).

Effect of granule mobilization on neutrophil production of ROS induced during interaction with IgG-coated hydrophobic glass

The membrane component of the NADPH-oxidase (the b cytochrome) is localized primarily in the specific granules (around 80%), the rest being in the plasma membrane and the secretory vesicles [29,30]. Most of the receptors for the Fc part of IgG (the FcγIII receptors) [31] and for complement opsonins (CR1 and 3) [32,33] are stored in the same subcellular compartments (the specific granules and the secretory vesicles). The mobilization of intracellular vesicles and granules thus endows the neutrophil plasma membrane with more receptor molecules and oxidase components, and it could be hypothesized that such a mobilization could affect the neutrophil response induced by a receptor binding ligand. Sequential mobilization of vesicles and granules may be obtained *in vitro* by using different mobilization protocols. Three cell populations differing with respect to vesicle/granule mobilization as well as the degree of receptor mobilization, illustrated by

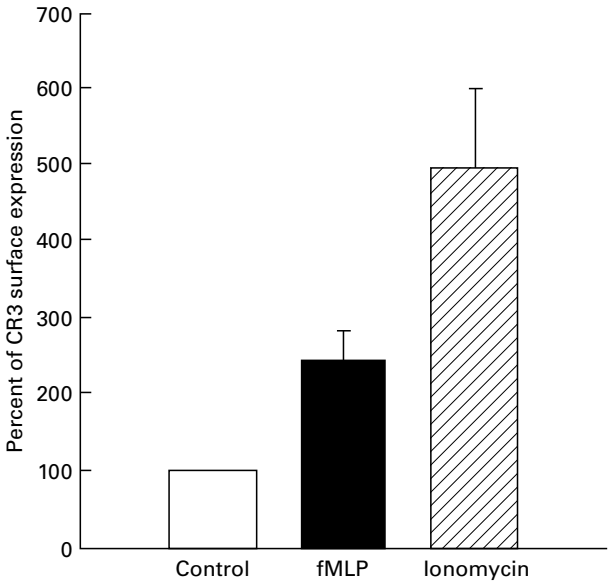

Fig. 4. Mobilization of granules measured as surface expression of CR3. Binding of MoAbs against CR3 was determined with FACS analysis, and the results are expressed as mean ± s.d., $n = 4$. Receptor expression was determined in three cell populations: control cells, formyl-methionyl-leucyl-phenylalanine (fMLP)-treated cells, ionomycin-treated cells.

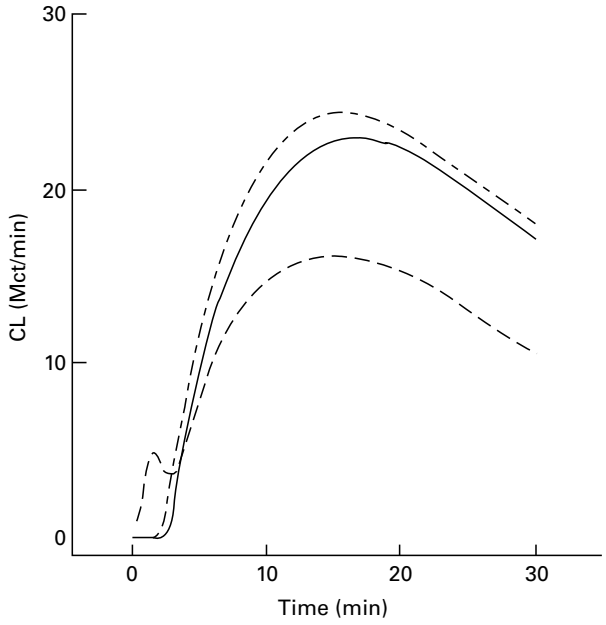

Fig. 5. The extracellular chemiluminescence (CL) response of neutrophils induced during cellular interaction with hydrophobic glass coated with IgG. Three cell populations, control cells (———), formyl-methionyl-leucyl-phenylalanine (fMLP)-treated cells (- – – -), and ionomycin-treated cells (- - - - -) were allowed to interact with surfaces. The extracellular CL was measured in the presence of isoluminol and horseradish peroxidase (HRP). The time courses of CL were measured. Abscissa, time of study; ordinate, CL expressed in Mct/min. The curves are from a representative experiment.

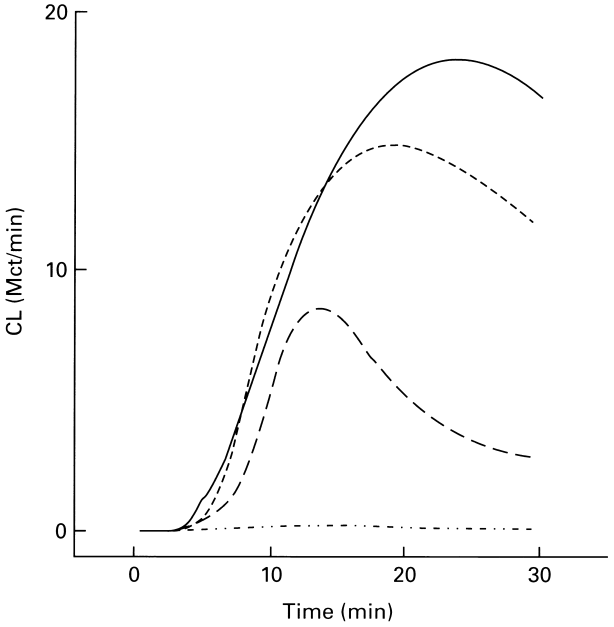

Fig. 6. Neutrophil activation induced by hydrophobic and hydrophilic glass coated with IgG, or IgG combined with serum. The time courses of chemiluminescence (CL) were measured. Abscissa, time of study; ordinate, CL expressed in Mct/min. The curves are from a representative experiment. ————, Hydrophobic glass + IgG; - - - - -, hydrophobic glass + IgG + serum; – – – – –, hydrophilic glass + IgG; – - - –, hydrophilic glass + IgG + serum.

the increased expression of CR3 (the neutrophil receptor for C3bi) on the cell surface as shown in Fig. 4, were used in the study: control cells (not treated with any mobilizing agent), fMLP-treated cells, and ionomycin-treated cells. The three cell populations were allowed to interact with IgG-coated surfaces. The surfaces induced a large, prolonged extracellular release of ROS, but from a quantitative point of view there was no apparent effect of the mobilization protocols (Fig. 5).

Effect of serum on the interaction between human neutrophils and IgG-coated glass surfaces

The protein film covering a biomaterial surface could be modified through adsorption of a second protein layer (e.g. deposition of complement components), or replacement by a second protein that binds more strongly to the surface, and by that displaces the protein initially bound. In order to investigate if serum components could modify the activity induced during neutrophil interaction with IgG-coated surfaces, normal human serum was added to the surfaces before the cells were allowed to interact with the surfaces. The IgG molecules adsorbed to the hydrophobic surface evoked a neutrophil response also after serum treatment, and the response was of the same magnitude ($19 \pm 10\%$ inhibition; mean ± s.d., $n = 4$) on serum-treated and non-treated surfaces shown in Fig. 6. In contrast, the neutrophil response was not detectable on IgG-coated hydrophilic glass after serum incubation (Fig. 6). Serum exerted an effect on the neutrophil response induced by IgG on a hydrophilic surface only when the cell-activating IgG molecules were directly in contact with the surface (that is, the inhibitory effect was not obtained if a second anti-IgG antibody was first introduced to build up a sandwich of IgG–anti-IgG; not shown in figure).

DISCUSSION

In the present study we investigated neutrophil interaction with surface-adsorbed proteins, using two surfaces with different physicochemical surface properties. We chose to use glass surfaces that largely resemble the silicon used in our earlier studies dealing with protein adsorption to solid surfaces [17]. Both the silicon and the glass surfaces become highly hydrophilic due to the exposure of OH group following TL1 and TL2 treatment, and they become densely packed with CH_3 groups after immersion in dichlorodimethyl silane (unpublished observations). Consequently, glass and silicon will have the same surface hydrophobicity.

As determined by a direct interaction with the neutrophils, the adsorbed protein, and not the supporting surface determines the outcome of the interaction. A surface covered with IgG activated neutrophils as reflected by a large production of ROS, whereas albumin- or fibrinogen-coated surfaces did not activate the neutrophil respiratory burst activity. The cellular response was higher on IgG-coated hydrophobic glass than on IgG-coated hydrophilic glass. The molecular background to this is not known, but could involve the conformation and orientation of the adsorbed protein (e.g. accessibility of the Fc parts of the molecules on the surface to the corresponding neutrophil receptors FcγRs), or simply the ligand density on the surface, which is determined by the physicochemical property of the supporting surface. Our results on the effect of surface-adsorbed albumin are in agreement with earlier observations [13–15,34–36]. With respect to fibrinogen adsorption, it has been shown that this protein predominates on most

surfaces [37]. Owing to the fact that fibrinogen has been suggested to be of great importance in the acute inflammatory response, reflected by the accumulation of neutrophils on implanted fibrinogen-coated surfaces [21,34,35], we expected some cellular activation during neutrophil interaction with such a surface. However, fibrinogen coating on our glass surfaces failed to activate the neutrophils to any detectable degree. We thus conclude that the proinflammatory character of fibrinogen is not related to a direct effect on neutrophil respiratory burst activity, but may be related to a change in responsiveness to a second stimulation. This conclusion gains support from the fact that the neutrophil NADPH-oxidase is activated by tumour necrosis factor (TNF) when the cells are sticking to a fibrinogen-coated surface [38]. It would be of great interest to know if this adhesion-dependent change in the neutrophil responsiveness to TNF is related to the same small region (amino acids 190–202) in the fibrinogen molecule, which is suggested to be the molecular determinant of acute inflammatory responses to biomaterials [39].

It has been suggested that cytoskeletal elements are involved in the regulation of the NADPH-oxidase, a proposal based mainly on the effects of cytochalasin B. This drug has been shown to decrease cellular adhesion but increase the release of ROS induced by both soluble [27,28] and particulate stimuli [40]. However, in contrast to what has been described in other systems, the production of ROS was abolished when cytochalasin B was present during interaction with an IgG-coated surface, indicating that the surface-induced neutrophil release of ROS is dependent on cellular adhesion events. Opsonized yeast particles were readily ingested by neutrophils, and the ROS produced were retained by the cells. Surface-adsorbed opsonins could activate neutrophils to produce ROS, but no production of ROS occurred intracellularly. Based on these data, we conclude that different signals are induced during activation of the neutrophils through the opsonin receptors, depending primarily on the way in which the ligands are presented to the cells.

Most studies (including biomaterial orientated studies) of neutrophil activation have been conducted on cells isolated from peripheral blood. However, neutrophils exert their function *in vivo* mainly after extravasation, a process that is accompanied by a mobilization of new receptors to cell surface as well as release of different inflammatory mediators [32]. The extravasation process can be mimicked *in vitro* and is associated with qualitative [23] and quantitative [41] changes in the response to external stimuli. We have used two different mobilization protocols to mimic extravasation, but the cellular release of ROS was unaltered even when a large fraction of the specific granules had been mobilized (ionomycin-mediated mobilization) before cellular interaction with the surfaces.

Neutrophil receptors for opsonins, i.e. FcγRII and III (receptors for the Fc part of IgG) or CR1 and CR3 (receptors for the activated complement factor 3), promote phagocytosis of coated particles concomitant with an activation of the respiratory burst. The results presented in this study clearly demonstrate that surface-adsorbed IgG molecules could activate neutrophils to produce ROS, and the ROS generated were released from the cells, making the term 'frustrated phagocytosis' appropriate. Such an 'activating surface' was turned into a non-activating surface if treated with normal human serum, but the inhibitory activity of serum was seen only when the IgG molecules were adsorbed to a hydrophilic surface. It is well known that protein adsorption is more reversible on hydrophilic surfaces than on hydrophobic surfaces [42]. Thus both IgG and C3 are probably displaced on IgG-precoated hydrophilic surface after serum immersion due to the high tendency of protein exchange interaction, and one of the dominating new proteins is high molecular weight kininogen [43], a protein that according to our results lacks the ability to activate neutrophils.

We know from our previous work [17] that IgG molecules adsorbed on a hydrophobic surface activate the complement system in serum to deposit active complement fragments on the solid surfaces. Cells interacting with such a surface should be activated, and we also found this to be the case. At the present stage we do not know, however, whether the signals generated in neutrophils by an interaction with IgG/serum on a hydrophobic surface involve also (or solely) the complement receptors. We investigated if the response could be inhibited by an antibody directed against CD18 (one of the subunits of CR3), but to our surprise we found that the antibody not only inhibited the response induced by the hydrophobic glass coated with IgG combined with serum, but also inhibited the response induced by the hydrophobic glass coated only with IgG (unpublished observations).

In conclusion, we applied a simple experimental system in which neutrophils were allowed to interact with model proteins adsorbed on solid surfaces on which the surface hydrophobicity was varied in a controlled manner. We found that IgG can activate the neutrophil NADPH-oxidase. The activation seems to be dependent on a proper presentation of the ligands on the solid surfaces as well as an actin filament-dependent adhesion step, and the oxygen-free radicals generated are released from the cells.

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REFERENCES

1 Gallin JI, Goldstein IM, Snyderman R, eds. Inflammation. Basic principles and clinical correlates, 2nd edn. New York: Raven Press, 1992.
2 Remes A, Williams DF. Immune response in biocompatibility. Biomaterials 1992; **13**:731–43.
3 Elsbach P, Weiss J. Oxygen-dependent antimicrobial systems of phagocytes. In: Gallin JI, Goldstein IM, Snyderman R, eds. Inflammation. Basic principles and clinical correlates, 2nd edn. New York: Raven Press, 1992:603–36.
4 Klebanoff SJ. Oxygen metabolites from phagocytes. In: Gallin JI, Goldstein IM, Snyderman R, eds. Inflammation. Basic principles and clinical correlates, 2nd edn. New York: Raven Press, 1992: 541–88.
5 Chanock SJ, Benna JE, Smith RM, Babior BM. The respiratory burst oxidase. J Biol Chem 1994; **269**:24519–22.
6 Johansson A, Jesaitis AJ, Lundqvist H, Magnusson KE, Sjölin C, Karlsson A, Dahlgren C. Different subcellular localization of cytochrome b and the dormant NADPH-oxidase in neutrophils and macrophages; effect on the production of reactive oxygen species during phagocytosis. Cell Immunol 1995; **161**:61–71.
7 Sutherland K, Mahoney JRII, Coury AJ, Eaton JW. Degradation of biomaterials by phagocyte-derived oxidants. J Clin Invest 1993; **92**:2360–7.
8 Nelson GD. Complications from the treatment of fibrous capsular contracture of the breast. Plastic Reconstr Surg 1982; **68**:969–70.
9 Stark GB, Gobel M, Jaeger K. Intraluminal cyclosporine A reduces

capsular thickness around silicon implant in rats. Ann Plast Surg 1990; **24**:156–61.
10 Remes A, Williams DF. Stimulation of a neutrophil respiratory burst by calcium hydrogen phosphate powder. Clin Mater 1992; **9**:71–76.
11 Kaplan SS, Basford RE, Kormos RL, Hardesty RL, Simmons RL, More EM, Cardona M, Griffith BL. Biomaterials associated impairment of local neutrophil function. ASAIO Trans 1990; **36**:M172–M175.
12 Baier RE, Dutton RC. Initial events in interactions of blood with a foreign surface. J Biomed Mater Res 1969; **3**:191–206.
13 Sevastianov VI. Role of protein adsorption in blood compatibility of polymer. CRC Crit Rev Biocompat 1988; **4**:109–54.
14 Anderson JM, Bonfield TL, Zaits NP. Protein adsorption and cellular adhesion and activation on biomedical polymers. Int J Artif Organs 1990; **13**:375–82.
15 Kottke-Marchant K, Anderson JM. Umemura Y, Marchant RE. Effect of albumin coating on the *in vitro* blood compatibility of Dacron arterial prostheses. Biomaterials 1989; **10**:147–55.
16 Elwing H, Ivarsson B, Lundström I. Complement deposition from human sera on silicon surfaces studied *in situ* by ellipsometry, the influence of surface wettability. Eur J Biochem 1986; **156**:359–65.
17 Liu L, Elwing H. Complement activation on solid surfaces as determined by C3 deposition and hemolytic consumption. J Biomed Mater Res 1994; **28**:767–73.
18 Liu L, Elwing H. Complement activation on thiol-modified gold surfaces. J Biomed Mater Res 1996; **30**:535–41.
19 Chuang HYK, Mohammad SF, Mason RG. Prostacyclin (PG12) inhibits the enhancement of granulocyte adhesion to cuprophane induced by immunoglobulin G. Thromb Res 1980; **19**:1–9.
20 Chuang HYK, Sharpton TR, Mohammad SF. Adsorption and reactivity of human fibrinogen and immunoglobulin G on two types of hemodialysis membranes. Inter J Artif Organs 1983; **6**:199–206.
21 Tang LP, Eaton JW. Fibrinogen mediates acute inflammatory responses. J Exp Med 1993; **178**:2147–56.
22 Bøyum A. Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Lab Invest 1968; **21** (Suppl. 97):77–89.
23 Karlsson A, Follin P, Leffler H, Dahlgren C. The human lectin, Galectin-3, activates the NADPH-oxidase in exudated neutrophils. 1997; submitted.
24 Lundqvist H, Gustafsson M, Johansson A, Särndahl E, Dahlgren C. Neutrophil control of formyl-methionyl-leucyl-phenylalanine induced mobilization of secretory vesicles and NADPH-oxidase activation: effect of an association of the ligand–receptor complex to the cytoskeleton. Biochem Biophys Acta 1994; **1224**:43–50.
25 Dahlgren C. Difference in extracellular radical release after chemotactic factor and calcium ionophore activation of the oxygen radical-generation system in human neutrophils. Biochim Biophys Acta 1987; **930**:33–38.
26 Lundqvist H, Dahlgren C. Isoluminol-enhanced chemiluminescence: a sensitive method to study the release of superoxide anion from human neutrophils. Free Radica Biol Med 1996; **20**:785–92.
27 Liu L, Harbecke O, Karlsson A, Dahlgren C. Neutrophil control of formyl-methionyl-leucyl-phenylalanine induced NAPPH-oxidase activity. 1997; submitted.
28 Mukherjee G, Quinn MT, Linner JG, Jesaitis AJ. Remodeling of the plasma membrane after stimulation of neutrophils with f-Met-Leu-Phe and dihydrocytochalasin B: identification of membrane subdomains containing NADPH oxidase activity. J Leuk Biol 1994; **55**:685–94.
29 Borregaard N, Tauber A. Subcellular localization of the human neutrophil NADPH oxidase, b-cytochrome and associated flavoprotein. J Biol Chem 1984; **259**:47–52.
30 Sengeløv H, Nielsen MH, Borregaard N. Separation of human neutrophil plasma membrane from intracellular vesicles containing alkaline phosphatase and NADPH oxidase activity by free electrophoresis. J Biol Chem 1992; **267**:14912–7.
31 Detmers PA, Zhou DH, Powell D, Lichenstein H, Kelley M, Pironkova R. Endotoxin receptors (CD14) are found with CD16 (FcγRIII) in an intracellular compartment of neutrophils that contains alkaline phosphatase. J Immunol 1995; **155**:2085–95.
32 Sengeløv H, Kjelden L, Kroeze W, Berger M, Borregaard N. Secretory vesicles are the intracellular reservoir of complement receptor 1 in human neutrophils. J Immunol 1994; **153**:804–10.
33 Sengeløv H, Kjelden L, Diamond MS, Springer T, Borregaard N. Subcellular localization and dynamics of Mac-1 ($\alpha_m\beta_2$) in human neutrophils. J Clin Invest 1993; **92**:1467–76.
34 Tang LP, Lucas AH, Eaton JW. Inflammatory responses to implanted polymeric biomaterials: role of surface-adsorbed immunoglobulin G. J Lab Clin Med 1993; **122**:292–300.
35 Tang LP, Eaton JW. Mechanism of acute inflammatory response to biomaterials. Cells and Materials 1994; **4**:429–36.
36 Katz DA, Haimovich B, Greco RS. Neutrophil activation by expanded polytetrafluoroethylene is dependent on the induction of protein phosphorylation. Surgery 1994; **116**:446–54.
37 Nimeri G, Nilsson U, Lassen B, Gölander CG, Elwing H. Adsorption of fibrinogen and some other proteins from blood plasma at a variety of solid surfaces. J Biomater Sci Polymer Ed 1994; **6**:573–83.
38 Nathan C. Neutrophil activation on biological surfaces. J Clin Invest 1987; **80**:1550–60.
39 Tang LP, Ugarova TP, Plow EF, Eaton JW. Molecular determinants of acute inflammatory responses to biomaterials. J Clin Invest 1996; **97**:1329–34.
40 Henson PM, Henson JE, Fittschen C, Bratton DL, Riches DWH. Degranulation and secretion by phagocytic cells. In: Gallin JI, Goldstein IM, Snyderman R, eds. Inflammation. Basic principles and clinical correlates, 2nd edn. New York: Raven Press, 1992:511–39.
41 Karlsson A, Markfjäll M, Strömberg N, Dahlgren C. *Escherichia coli*-induced activation of the neutrophil NADPH-oxidase: lipopolysaccharide and formylated peptides act synergistically to induce a release of reactive oxygen metabolites. Infect Immun 1995; **63**:4606–12.
42 Elwing H, Askendal A, Lundström I. Protein exchange reactions on solid surfaces studied with a wettability gradient method. Prog Colloid Polym Sci 1987; **74**:103–7.
43 Elwing H, Liu L, Askendal A, Nimeri G, Brash J. Protein displacement phenomena (the Vroman effect) in blood plasma and serum studied by the lens-on-surface method and the wettability gradient method. In: Thomas AH, Brash JL, eds. Protein at interfaces II. Fundaments and Applications, ACS Symposium Series 602. York: Maple Press, 1995:138–49.