Journal of Cell Science 110, 1133-1139 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
JCS4390

Myeloperoxidase mediates cell adhesion via the $\alpha_M\beta_2$ integrin (Mac-1, CD11b/CD18)

Mats W. Johansson[1,*], Manuel Patarroyo[2], Fredrik Öberg[3], Agneta Siegbahn[4] and Kenneth Nilsson[3]

[1]Department of Physiological Botany, University of Uppsala, Villavägen 6, S-75236 Uppsala, Sweden
[2]Microbiology and Tumour Biology Centre, Karolinska Institute, PO Box 280, S-17177 Stockholm, Sweden
[3]Department of Pathology, University of Uppsala, University Hospital, S-75185 Uppsala, Sweden
[4]Department of Clinical Chemistry, University of Uppsala, University Hospital, S-75185 Uppsala, Sweden

*Author for correspondence (e-mail: mats.johansson@fysbot.uu.se)

SUMMARY

Myeloperoxidase is a leukocyte component able to generate potent microbicidal substances. A homologous invertebrate blood cell protein, peroxinectin, is not only a peroxidase but also a cell adhesion ligand. We demonstrate in this study that human myeloperoxidase also mediates cell adhesion. Both the human myeloid cell line HL-60, when differentiated by treatment with 12-O-tetradecanoyl-phorbol-13-acetate (TPA) or retinoic acid, and human blood leukocytes, adhered to myeloperoxidase; however, undifferentiated HL-60 cells showed only minimal adhesion. No cells adhered to horseradish peroxidase, and cell adhesion to myeloperoxidase was not decreased by catalase, thus showing that peroxidase activity, per se, was neither sufficient nor necessary for the adhesion activity. Mannan, which has been reported to inhibit the binding of peroxidases to cells, did not affect adhesion to myeloperoxidase. However, adhesion to myeloperoxidase was inhibited by monoclonal antibodies to α_M (CD11b) or to β_2 (CD18) integrin subunits, but not by antibodies to α_L (CD11a), α_X (CD11c), or to other integrins. Native myeloperoxidase mediated dose-dependent cell adhesion down to relatively low concentrations, and denaturation abolished the adhesion activity. It is evident that myeloperoxidase supports cell adhesion, a function which may be of considerable importance for leukocyte migration and infiltration in inflammatory reactions, that $\alpha_M\beta_2$ integrin (Mac-1 or CD11b/CD18) mediates this adhesion, and that the $\alpha_M\beta_2$ integrin-mediated adhesion to myeloperoxidase is distinct from the previously reported ability of this integrin to bind to certain denatured proteins at high concentrations.

Key words: Myeloperoxidase, Cell adhesion, Integrin, Mac-1, CD11b/CD18

INTRODUCTION

Cell adhesion, essential for the function of multicellular organisms, is not only involved in many physiological processes, e.g. development, wound healing and haemostasis, but also in pathological conditions such as metastasis of cancer cells and inflammatory disease (for reviews, see e.g. Hynes, 1992; Hynes and Lander, 1992). At sites of inflammation, leukocyte adhesive events occur at the vessel wall, during extravasation, and during migration into the underlying tissue (Patarroyo et al., 1990; Springer, 1990; Hynes, 1992; Hynes and Lander, 1992).

Adhesion of nucleated white blood cells has also been studied in an invertebrate system. The first cell adhesive ligand from invertebrate blood cells was purified (Johansson and Söderhäll, 1988), and recently cloned (Johansson et al., 1995). This adhesion protein, first obtained and described from freshwater crayfish, is synthesized in granule-containing blood cells and stored in the granules, and following secretion (degranulation) is able to mediate attachment and spreading of the blood cells (Johansson and Söderhäll, 1988). It is also, in addition, an opsonin, i.e. it stimulates phagocytosis (Thörnqvist et al., 1994). Cloning of the crayfish cell adhesion protein revealed that its sequence is significantly similar to a family of animal peroxidases that includes myeloperoxidase (Johansson et al., 1995). As the crayfish protein has peroxidase activity (Johansson et al., 1995), we have named this invertebrate protein, which to our knowledge is the first protein with combined adhesive/peroxidase functions, peroxinectin.

The question now arises as to whether the classical vertebrate peroxidases, which are homologous to invertebrate peroxinectin, are also able, in addition to producing microbicidal agents (Klebanoff, 1991), to support cell adhesion. Such adhesion of, for example, leukocytes to myeloperoxidase released from neutrophils, could be of considerable importance in the inflammatory process.

We show here that myeloperoxidase does, indeed, mediate adhesion of blood leukocytes and that it also mediates adhesion of cells of an established human myeloid cell line, HL-60, following differentiation. We also show that cell adhesion to myeloperoxidase occurs through the $\alpha_M\beta_2$ integrin (Mac-1 or CD11b/CD18).

MATERIALS AND METHODS

Cells

Blood leukocytes from healthy human individuals were isolated, as described (Siegbahn et al., 1987) and obtained in Gey's modified buffer (Siegbahn et al., 1987). The human cell line HL-60 (Lozzio and Lozzio, 1975) was grown in RPMI 1640 medium, supplemented with 10% foetal calf serum (FCS, Gibco, Uxbridge, UK) and with antibiotics (penicillin 100 i.u./ml and streptomycin 50 μg/ml). For differentiation, the cells were grown in the above medium for 3 days in the presence of 10^{-8} M 12-O-tetradecanoyl-phorbol-13-acetate (TPA) (monocytic differentiation) or in the presence of 10^{-6} M retinoic acid (RA) (myeloid differentiation). In some experiments, undifferentiated HL-60 cells were treated for only 30 minutes (at 37°C) with 10^{-8} M TPA. The HL-60 cells were washed three times with Gey's modified buffer by centrifugation at 150 ***g*** for 10 minutes before they were used in the adhesion assay.

Antibodies

The following adhesion-blocking anti-human integrin monoclonal antibodies were used in the adhesion assay: 4B4 (anti-β_1, CD29, Coulter Immunology, Hialeah, FL, USA), IB-4 (anti-β_2, CD18, originally provided by Dr Samuel D. Wright, Rockefeller University, New York, NY, USA), H12 (anti-α_L, CD11a, provided by Professor Hans Wigzell, Karolinska Institute, Stockholm, Sweden), 2LPM19c (anti-α_M, CD11b, Dakopatts, Glostrup, Denmark), SHCL-3 (anti-α_X, CD11c, Becton Dickinson, San Jose, CA, USA) and anti-α_V (CD51, originally provided by Dr James O. Gailit, State University of New York, Stony Brook, NY, USA) (Freed et al., 1989). Flow cytometry was performed with the monoclonals G25.2 (CD11a, Becton Dickinson), 2LPM19c (CD11b, Dakopatts), SHCL-3 (CD11c, Becton Dickinson) and L130 (CD18, Becton Dickinson). Purified polyclonal rabbit anti-human myeloperoxidase antibody was purchased from Dako (Glostrup). Purified polyclonal rabbit anti-human fibronectin antibody was a gift from Dr Erkki Ruoslahti, The Burnham Institute (La Jolla, CA, USA).

Other materials

Human myeloperoxidase was purchased from Sigma (St Louis, MO, USA) or from Calbiochem (La Jolla, CA, USA). Recombinant human myeloperoxidase (Moguilevsky et al., 1991) was a gift from Dr Alex Bollen, Université Libre de Bruxelles (Nivelles, Belgium). Horseradish peroxidase and bovine liver catalase were purchased from Sigma.

Cell adhesion assay

All steps were performed at room temperature. Non-tissue-culture-treated 96-well microtitre plate wells were coated with 100 μl of the sample under study (e.g. myeloperoxidase, usually at 10 μg/ml), dissolved in Gey's modified buffer, for 2 hours. The wells were then washed three times with Gey's modified buffer, blocked by incubation with 3% bovine serum albumin (BSA) in the same buffer overnight, and washed again three times with the buffer before addition of the cells. Control wells were coated only with BSA. Cells were diluted to a concentration of 3×10^5/ml in Gey's modified buffer, and 100 μl of the cell suspension was added to each well. Cells were then incubated for 5-45 minutes (blood leukocytes usually 5 minutes, cultured cells usually 45 minutes). After incubation, the wells were washed once with the buffer, and the attached cells were fixed with 10% formalin in Gey's modified buffer (pH adjusted). The number of attached cells, per well, was calculated by counting the cells in the central object field corresponding to 1/9 of the well area, at ×10 magnification in an inverted microscope. In some experiments, antibodies or chemicals were added to the cell suspension. The cells were preincubated for 15 minutes with the antibody, before the adhesion assay. In another set of experiments, the effect of denaturation on the adhesive activity of myeloperoxidase was tested. Recombinant human myeloperoxidase, horseradish peroxidase or BSA was reduced and alkylated essentially as described (Davis, 1992). The proteins (1 mg/ml) were mixed with 9 volumes of 8 M urea, 50 mM Tris-HCl, pH 7.4, treated first with dithiothreitol (10 mM final concentration) for 2 hours at 20°C, and then with iodoacetamide (60 mM final concentration) for 2 hours at 20°C in the dark. Finally, the samples were dialyzed against Gey's modified buffer and diluted in the same buffer before being used to coat wells.

Flow cytometry

The cell surface expression of proteins was analyzed by indirect immuno-fluorescence using a flow cytometer (FACScan, Becton Dickinson). Cells (10^6) were washed with ice-cold PBS containing 0.5% FCS, incubated on ice for 30 minutes with primary antibody (100 ng, except 200 ng of 2LPM19c), washed twice, further incubated for 30 minutes on ice with fluorescein-isothiocyanate(FITC)-labelled goat anti-mouse $F(ab')_2$ fragments (50 μl of 60 μg/ml) (CLB, Amsterdam, Netherlands), and finally washed twice before analysis. Results obtained with only the secondary antibody determined the lower limit for positive fluorescence.

RESULTS

Adhesion of human blood leukocytes to human myeloperoxidase

The human blood leukocyte suspension consisted of approximately 80% polymorphonuclear cells and 20% mononuclear cells (not shown). When these cells were added to microtitre wells previously coated with human myeloperoxidase (and blocked with BSA), the cells were found to attach to and to spread on this substratum. This adhesion was specific because the cells did not attach to control wells coated with BSA (Fig. 1), and it was dose-dependent (not shown). The variation in leukocyte adhesion to myeloperoxidase was relatively high (Fig. 1). This variation was mostly observed among cells from different individuals, whereas parallel experiments with cells from the same individual showed less variation.

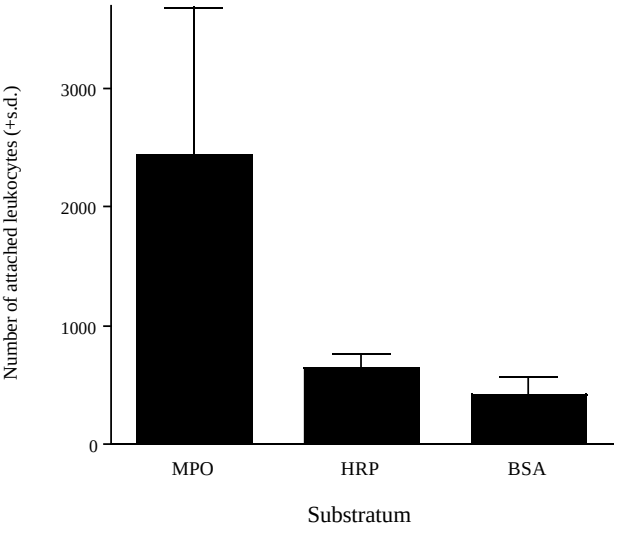

Fig. 1. Leukocyte adhesion to myeloperoxidase. Isolated human blood leukocytes were added to microtitre wells coated with human myeloperoxidase (MPO) or horseradish peroxidase (HRP), both at 10 μg/ml, and then blocked with 3% BSA. Control wells were coated only with BSA. s.d., standard deviation from 13 experiments.

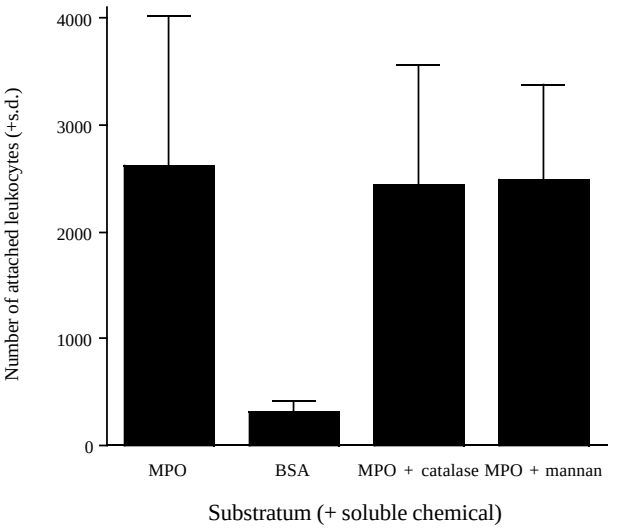

Fig. 2. Leukocyte adhesion to myeloperoxidase in the presence of the peroxide scavenger catalase or mannan. Microtitre wells were coated with 10 μg/ml human myeloperoxidase, and blocked with BSA. Control wells were coated only with BSA. Isolated human blood leukocytes were added in the presence of catalase (0.1 mg/ml) or mannan (1 mg/ml). s.d., standard deviation from 4 experiments.

In order to determine whether the enzyme activity of myeloperoxidase was necessary for cell adhesion to occur, an excess of catalase was added to the cells. This preparation of catalase blocked peroxidase activity in a standard enzyme assay (not shown), but did not decrease adhesion (Fig. 2), thus indicating that the observed cell adhesion activity of myeloperoxidase is independent of its peroxidase activity. Furthermore, horseradish peroxidase, whose sequence is not homologous to myeloperoxidase, did not mediate adhesion (Fig. 1). Thus, in conclusion, peroxidase activity, per se, was neither sufficient nor necessary for cell adhesion.

Because myeloperoxidase has been reported to bind to (and be taken up by) macrophages via its mannose groups, and because this uptake can be inhibited by mannan (Shepherd and Hoidal, 1990), we next tested the effect of mannan on cell adhesion. We found that mannan did not affect attachment (Fig. 2), indicating that cell adhesion to myeloperoxidase is a mannose-independent process, and that the cell binding needed for adhesion is distinct from the uptake by macrophages.

Adhesion of differentiated HL-60 cells to myeloperoxidase

To further study cell adhesion to myeloperoxidase, we tested cells of the HL-60 cell line, undifferentiated and after monocytic differentiation induced by TPA, or myeloid differentiation by retinoic acid (RA) treatment. Undifferentiated HL-60 cells attached minimally to myeloperoxidase (Fig. 3C). HL-60 cells that had been treated for only 30 minutes with TPA (which is known to activate the $\alpha_L\beta_2$ integrin) (Patarroyo et al., 1985a), also attached minimally (Fig. 3D). On the other hand, cells differentiated for a period of 3 days showed significant adhesion (Fig. 3A,B). As with blood leukocytes, the cells did not attach to the non-homologous horseradish peroxidase (Fig. 3A,B) and adhesion to myeloperoxidase was not decreased by an excess of catalase (not shown). Cell adhesion at room temperature, or at 37°C, showed the same general pattern, although cells became more spread at 37°C (not shown). At 4°C, only minimal adhesion was seen (not shown), indicating that metabolic energy is required for firm attachment and spreading and that the adhesion is not due to non-specific sticking to the substratum.

The adhesion of differentiated HL-60 cells to myeloperoxidase was inhibited by a polyclonal anti-human myeloperoxidase antibody, whereas a control polyclonal anti-human fibronectin antibody did not decrease adhesion (not shown). Recombinant human myeloperoxidase, expressed in Chinese hamster ovary cells (Moguilevsky et al., 1991), mediated adhesion to approximately the same degree as the natural protein (Fig. 4).

Table 1. Surface expression of β_2 integrins on HL-60 cells analyzed by flow cytometry

Integrin subunit	Control	TPA (30 minutes)	RA (3 days)	TPA (3 days)
α_L (CD11a)	94 (14)	92 (25)	96 (27)	99 (87)
α_M (CD11b)	10 (7.8)	13 (16)	25 (12)	96 (129)
α_X (CD11c)	6 (5.1)	10 (7.4)	17 (6.6)	100 (105)
β_2 (CD18)	92 (38)	91 (42)	100 (68)	100 (243)
Control*	1 (2.6)	1 (5.0)	1 (3.3)	26 (11)

Values show the percentage of positive cells (and mean fluorescence intensity) after different treatments.
RA, retinoic acid; TPA, 12-O-tetradecanoyl-phorbol-13-acetate.
*Without primary antibody.

Cell surface molecules mediating adhesion to myeloperoxidase

Blocking monoclonal antibodies to integrins were tested, in order to identify cell surface molecules involved in the adhesion process. At 1 μg/ml (as well as at higher concentrations), adhesion to myeloperoxidase was inhibited by monoclonal antibodies to the α_M (CD11b), or to the β_2 (CD18) integrin subunits, but not by monoclonal antibodies to other subunits (Fig. 4). Also, a polyclonal antibody to β_3 integrins did not inhibit adhesion (not shown). As in other integrin-mediated adhesion systems, 10 mM EDTA almost completely inhibited the cell adhesion to myeloperoxidase (data not shown). As with the leukocytes, HL-60 cell adhesion to myeloperoxidase was dose-dependent, showing half maximal activity at around 0.01 μg/ml coating concentration (Fig. 5A).

Since it has been reported in the literature that the $\alpha_M\beta_2$ integrin can bind to certain denatured proteins (Davis, 1992), we tested the effect of denaturation on myeloperoxidase. Reduction and alkylation of myeloperoxidase abolished its adhesion activity (Fig. 5A), as did heating (not shown). Also, treatment with urea only decreased this adhesion activity (not shown). Denaturation of horseradish peroxidase by the same methods did not significantly change cell behaviour on this substratum (Fig. 5B) and, thus, did not cause this protein to become adhesive. However, in the same set of experiments, we were able to confirm the results of Davis (1992) as we obtained some cell adhesion (although significantly less than on native myeloperoxidase) on reduced, urea-treated or heated BSA (not shown) at high coating concentrations (100 or 10 μg/ml, i.e.

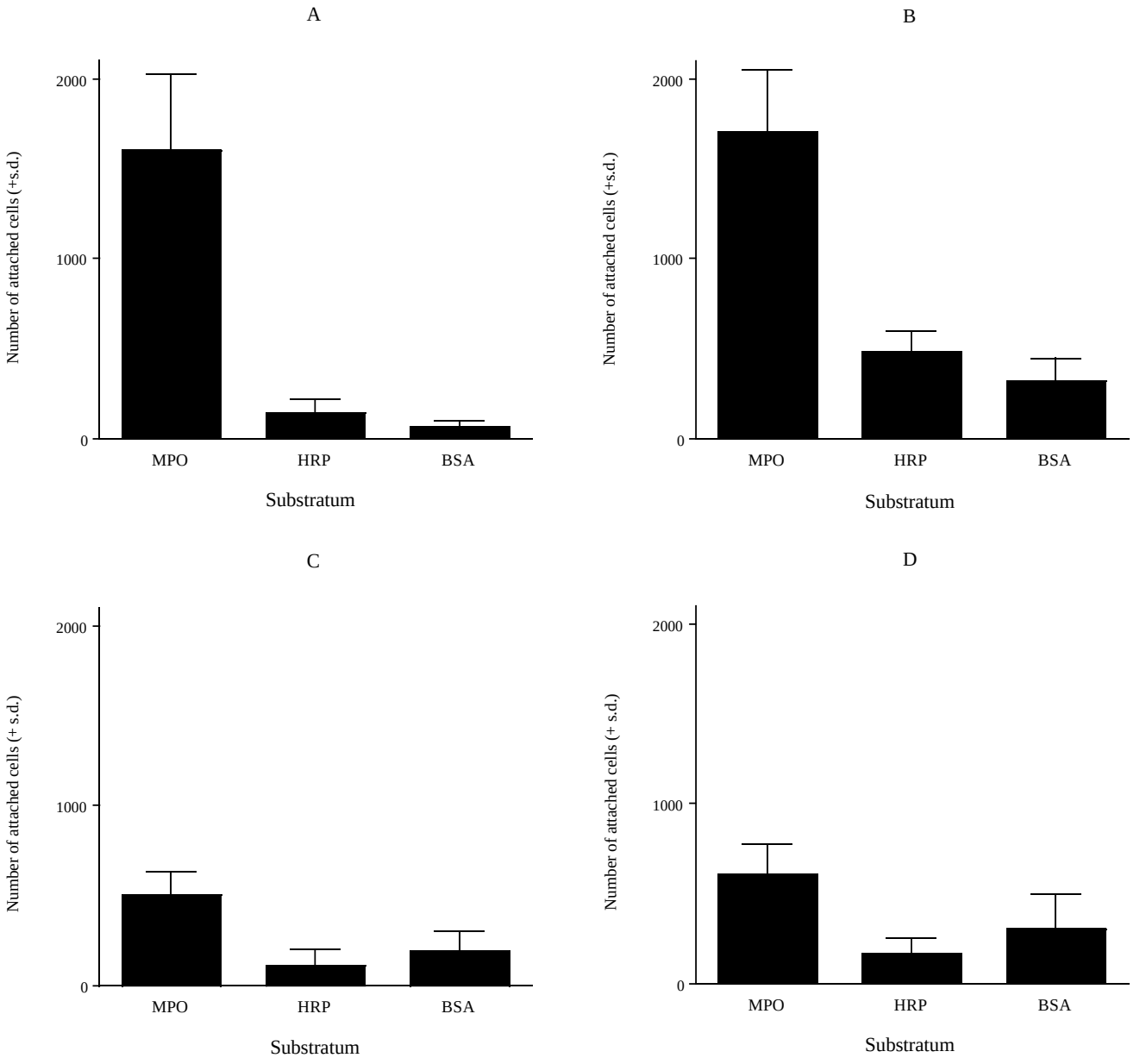

Fig. 3. Adhesion of HL-60 cells to myeloperoxidase. Microtitre wells were coated with human myeloperoxidase or horseradish peroxidase, both 10 μg/ml, and blocked with BSA. Control wells were coated only with BSA. Cells of the human cell line HL-60 were added to the wells. The cells had been treated for 3 days with 10^{-8} M 12-O-tetradecanoyl-phorbol-13-acetate (TPA) (A) or with 10^{-6} M retinoic acid (RA) (B) to induce differentiation. In (C) undifferentiated HL-60 cells were used and in (D) HL-60 cells that had been treated with 10^{-8} M TPA for only 30 minutes. s.d., standard deviation from 8 (A), 9 (B), 10 (C) and 6 (D) experiments.

1,000-10,000 times the concentration required for native myeloperoxidase), whereas we did not observe any adhesion on native BSA.

Flow cytometry studies demonstrated that HL-60 cells expressed β_2 integrins (Table 1). Almost all undifferentiated cells expressed the α_L chain, but only a few of these cells were positive for α_M and α_X. After 3 days of differentiation in the myelomonocytic lineage, when cells had become adhesive to myeloperoxidase, an increased number of cells expressed α_M and α_X (high with TPA, modest with retinoic acid). These results are in accordance with earlier reports (e.g. Prieto et al., 1994).

DISCUSSION

Our results show that human myeloperoxidase can mediate adhesion of differentiated HL-60 cells and human blood leukocytes. Myeloperoxidase is known to be present in very high concentrations in the primary (azurophil) granules of neutrophils; it can also be secreted into the extraceIlular fluid when neutrophils respond to non-phagocytosable antibodies, complement-coated surfaces and/or soluble stimuli, such as chemotactic factors and cytokines (Klebanoff, 1991). This leukocyte protein, which is also found in blood monocytes (Bos et al., 1978), plays a central and critical role in host defence by

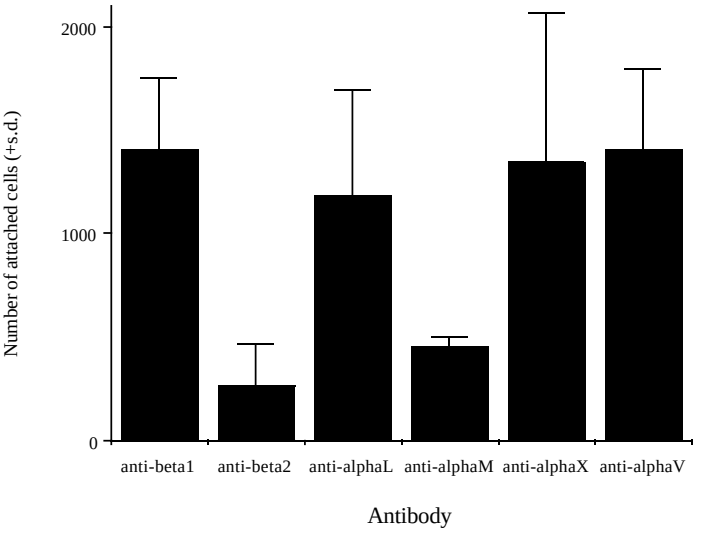

Fig. 4. Inhibition of adhesion of HL-60 cells to myeloperoxidase by monoclonal antibodies to integrin subunits. Microtitre wells were coated with 10 μg/ml recombinant human myeloperoxidase, and blocked with BSA. Retinoic acid-differentiated HL-60 cells were pre-incubated for 15 minutes with 1 μg/ml antibody and then added to the wells in the presence of the antibody. The monoclonal anti-integrin antibodies were: anti-β_1 (CD29), anti-β_2 (CD18), anti-α_L (CD11a), anti-α_M (CD11b), anti-α_X (CD11c) and anti-α_V (CD51). s.d., standard deviation from 6 experiments.

producing powerful antimicrobial agents of broad specificity (Klebanoff, 1991). Cell adhesion to myeloperoxidase, or any other vertebrate peroxidase, has not, to our knowledge, been shown previously.

The results obtained here are similar to those obtained earlier from the study of an invertebrate system, where peroxinectin, a protein purified and cloned from crayfish blood cells, is both a cell adhesion ligand for crayfish blood cells, and a peroxidase (Johansson and Söderhäll, 1988; Johansson et al., 1995). Human myeloperoxidase can also trigger attachment, spreading and degranulation of crayfish blood cells (M. W. Johansson and K. Söderhäll, unpublished).

The sequences of human myeloperoxidase (Morishita et al., 1987) and peroxinectin are significantly similar (32% of the residues are identical) (Johansson et al., 1995); both appear to belong to a family of homologous animal peroxidases. The present results, together with the results obtained from the crayfish system, indicate that this family of peroxidases, in addition to producing potent microbicidal agents (Klebanoff, 1991), can also mediate cell-substratum adhesion. Cell adhesion may thus be a conserved function of animal peroxidases; they may form a new family of cell adhesion proteins.

Several results show that, even though the peroxidase and cell adhesive activities are present in the same protein, these activities are independent of each other. The peroxidase scavenger catalase did not affect the adhesive activity to human myeloperoxidase. Horseradish peroxidase did not mediate adhesion of the human cells (this report) or of the crayfish cells (M. W. Johansson and K. Söderhäll, unpublished). Thus, peroxidase

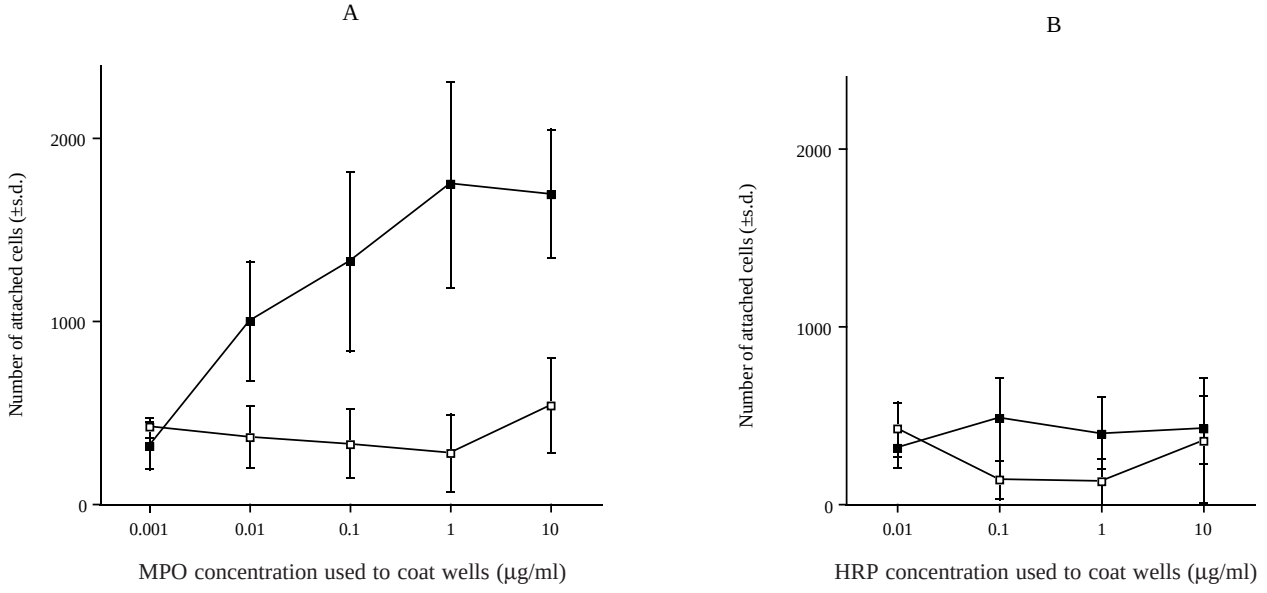

Fig. 5. Dose-dependent adhesion of HL-60 cells to myeloperoxidase and abolishment of its activity by denaturation. Recombinant human myeloperoxidase (MPO) or horseradish peroxidase (HRP) was denatured by reduction and alkylation. HL-60 cells differentiated by treatment with retinoic acid were added to microtitre wells coated with different concentrations of native (filled squares) or denatured (open squares) MPO (A) or HRP (B) and blocked with BSA. s.d., standard deviation from 3 experiments.

activity, by itself, is not sufficient for cell adhesion, and the plant peroxidases, which are not homologous to myeloperoxidase or to peroxinectin, may not be adhesive for cells.

There already are some indications in the literature that myeloperoxidase may play other biological roles apart from its function as an enzyme. The fact that myeloperoxidase comprises up to 5% of the protein content in neutrophils (Schultz and Kaminker, 1962) may well suggest this. Furthermore, myeloperoxidase has been found to induce secretion of certain cytokines, e.g. tumour necrosis factor α, and has been suggested to have an immuno-modulatory role in addition to its enzyme activity (Lefkowitz et al., 1991, 1992, 1993). However, these authors obtained a response with horseradish peroxidase, as well as with mannosylated BSA, indicating that this effect, like the myeloperoxidase uptake by macrophages (Shepherd and Hoidal, 1990), is due to the mannose groups on the peroxidases. In contrast, we did not observe any significant effect by horseradish peroxidase but did observe that the adhesion to myeloperoxidase appeared to be mannose-independent. It thus appears that the mechanism of the proposed immuno-modulatory role of peroxidases, studied by Lefkowitz and colleagues, and the mechanism of cell adhesion to myeloperoxidase are distinct.

Our experiments with monoclonal antibodies suggest that the $\alpha_M\beta_2$ integrin (Mac-1 or CD11b/CD18) functions as a cell adhesion receptor for myeloperoxidase. The $\alpha_M\beta_2$ integrin has been shown to mediate neutrophil adhesion to endothelium, subsequent extravasation to inflammatory sites, and also neutrophil homotypic adhesion and chemotaxis; it is also involved in phagocytosis of opsonized particles (Harlan et al., 1985; Patarroyo et al., 1985b; Anderson et al., 1986; Arnaout et al., 1988, Carlos and Harlan, 1990; Larson and Springer, 1990; Patarroyo et al., 1990). This integrin, which is largely expressed by polymorphonuclear leukocytes (PMNs), monocytes/macrophages and natural killer cells, binds intercellular adhesion molecule 1 (ICAM-1or CD54), iC3b, factor X and fibrinogen (Altieri et al., 1988; Wright et al., 1988; Diamond et al., 1991; Sánchez-Madrid and Corbi, 1993). However, these four established ligands do not account for all $\alpha_M\beta_2$-dependent adhesive activities; for instance, the adhesive ligands for the $\alpha_M\beta_2$ integrin in neutrophil aggregation and chemotaxis are not known. It has recently been suggested that heparin or heparan sulfate may mediate some, or all, of these interactions (Diamond et al., 1995). It is possible that myeloperoxidase is a novel $\alpha_M\beta_2$ adhesive ligand, and that it may be involved in these situations.

One report claims that the $\alpha_M\beta_2$ integrin can also bind and mediate adhesion to a variety of denatured proteins, for instance to denatured bovine serum albumin (but not e.g. to gelatin) (Davis, 1992). However, myeloperoxidase does not belong to this group of proteins, since we found that denaturation in fact abolished its adhesion activity. Furthermore, 1,000-10,000 times higher concentration is required of denatured BSA than of (native) myeloperoxidase to obtain significant adhesion. In conclusion, we believe that adhesion to myeloperoxidase in its native conformation is due to an interaction with the $\alpha_M\beta_2$ integrin that is distinct from the 'scavenger' type of binding to denatured proteins.

We further believe that the adhesive activity of myeloperoxidase, which we have described here, may be of importance in inflammation. We propose that when PMNs and monocytes respond to inflammatory stimuli, they secrete myeloperoxidase as an endogenous adhesive substratum for their migration.

We thank Ms Matilda Johnell and Ms Inger Karlberg for technical assistance. We thank Professor Kenneth Söderhäll, Department of Physiological Botany, University of Uppsala for discussions. This work was supported by the Swedish Council for Forestry and Agricultural Research and the Swedish Cancer Society.

REFERENCES

Altieri, D. C., Morrissey, J. H. and Edgington, T. S. (1988). Adhesive receptor Mac-1 coordinates the activation of factor X on stimulated cells of monocytic and myeloid differentiation: an alternative initiation of the coagulation protease cascade. *Proc. Nat. Acad. Sci. USA* **85**, 7462-7466.

Anderson, D. C., Miller, L. J., Schmalstieg, F. C., Rothlein, R. and Springer, T. A. (1986). Contributions of the Mac-1 glycoprotein family to adherence-dependent granulocyte functions: structure-function assessment employing subunit-specific monoclonal antibodies. *J. Immunol.* **137**, 15-27.

Arnaout, M. A., Lanier, L. L. and Faller, D. V. (1988). Relative contribution of the leukocyte molecules Mo1, LFA-1, and p150,95 (LeuM5) in adhesion of granulocytes and monocytes to vascular endothelium is tissue- and stimulus-specific. *J. Cell. Physiol.* **37**, 305-309.

Bos, A., Wever, R. and Roos, D. (1978). Characterization and quantitation of the peroxidase in monocytes. *Biochim. Biophys. Acta* **525**, 37-44.

Carlos, T. M. and Harlan, J. M. (1990). Membrane proteins involved in phagocyte adherence to endothelium. *Immunol. Rev.* **114**, 5-28.

Davis, G. E. (1992). The Mac-1 and p150,95 β_2 integrins bind denatured proteins to mediate leukocyte cell-substrate adhesion. *Exp. Cell Res.* **200**, 242-252.

Diamond, M. S., Staunton, D. E., Marlin, S. D. and Springer, T. A. (1991). Binding of the integrin Mac-1 (CD11b/CD18) to the third immunoglobulin-like domain of ICAM-1 (CD54) and its regulation by glycosylation. *Cell* **65**, 961-971.

Diamond, M. S, Alon, R., Parkos, C. A., Quinn, M. T. and Springer, T. A. (1995). Heparin is an adhesive ligand for the leukocyte integrin Mac-1 (CD11b/CD18). *J. Cell Biol.* **130**, 1473-1482.

Freed, E., Gailit, J., van der Geer, P., Ruoslahti, E. and Hunter, T. (1989). A novel integrin β subunit is associated with the vitronectin receptor α subunit (α_v) in a human osteosarcoma cell line and is a substrate for protein kinase C. *EMBO J.* **8**, 2955-2965.

Harlan, J. M., Killen, P. D., Senecal, F. M., Schwartz, B. R., Yee, E. K., Taylor, R. F., Beatty, P. G, Price, T. H. and Ochs, H. D. (1985). The role of neutrophil membrane glycoprotein GP-150 in neutrophil adherence to endothelium *in vitro*. *Blood* **66**, 167-178.

Hynes, R. O. (1992). Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* **69**, 11-25.

Hynes, R. O. and Lander A. D. (1992). Contact and adhesive specifities in the associations, migrations, and targeting of cells and axons. *Cell* **68**, 303-322.

Johansson, M. W. and Söderhäll, K. (1988). Isolation and purification of a cell adhesion factor from crayfish blood cells. *J. Cell Biol.* **106**, 1795-1803.

Johansson, M. W., Lind, M. I., Holmblad, T., Thörnqvist, P.-O. and Söderhäll, K. (1995). Peroxinectin, a novel cell adhesion protein from crayfish blood. *Biochem. Biophys. Res. Commun.* **216**, 1079-1087.

Klebanoff, S. J. (1991). Myeloperoxidase: Occurrence and biological function. In *Peroxidases in Chemistry and Biology* (ed. J. Everse, K. E. Everse and M. B. Grisham), pp. 2-35. CRC Press, Boca Raton, FL.

Larson, R. S. and Springer, T. A. (1990). Structure and function of leukocyte integrins. *Immunol. Rev.* **114**, 181-217.

Lefkowitz, D. L., Mills, K., Castro, A. and Lefkowitz, S. S. (1991). Induction of tumor necrosis factor and macrophage-mediated cytotoxicity by horseradish peroxidase and other glycosylated proteins: The role of enzymatic activity and LPS. *J. Leuk. Biol.* **50**, 615-623.

Lefkowitz, D. L., Mills, K., Morgan, D. and Lefkowitz, S. S. (1992). Macrophage activation and immunomodulation by myeloperoxidase. *Proc. Soc. Exp. Biol. Med.* **199**, 204-210.

Lefkowitz, D. L, Mills, K. C., Moguilevsky, N, Bollen, A., Vaz, A. and Lefkowitz, S. S. (1993). Regulation of macrophage function by human recombinant myeloperoxidase. *Immunol. Lett.* **36**, 43-50.

Lozzio, C. B. and Lozzio, B. B. (1975). Human chronic myelogeneous

leukemia cell-line with positive Philadelphia chromosome. *Blood* **45**, 321-324.

Moguilevsky, N., García-Quintana, L., Jacquet, A., Tournay, C., Fabry, L., Piérard, L. and Bollen, A. (1991). Structural and biological properties of human recombinant myeloperoxidase produced by Chinese hamster ovary cell lines. *Eur. J. Biochem*. **197**, 605-614.

Morishita, K., Kubota, N., Asano, S., Kaziro, Y. and Nagata, S. (1987). Molecular cloning and characterization of cDNA for human myeloperoxidase. *J. Biol. Chem.* **262**, 3844-3851.

Patarroyo, M., Beatty, P. G., Fabre, J. W. and Gahmberg, C. G. (1985a). Identification of a cell surface protein complex mediating phorbol ester-induced adhesion (binding) among human mononuclear leukocytes. *Scand. J. Immunol*. **22**, 171-182.

Patarroyo, M., Beatty, P. G., Serhan, C. N. and Gahmberg, C. G. (1985b). Identification of a cell-surface glycoprotein mediating adhesion in human granulocytes. *Scand. J. Immunol.* **22**, 619-631.

Patarroyo, M., Prieto, J., Rincón, J., Timonen, T., Lundberg, C., Lindbom, L., Åsjö, B. and Gahmberg, C. G. (1990). Leukocyte-cell adhesion: a molecular process fundamental in leukocyte physiology. *Immunol. Rev.* **114**, 67-108.

Prieto, J., Eklund, A. and Patarroyo, M. (1994). Regulated expression of integrins and other adhesion molecules during differentiation of monocytes into macrophages. *Cell. Immunol.* **156**, 191-211.

Sánchez-Madrid, F. and Corbi, A. L. (1993). Leukocyte integrins: structure, function and regulation of their activity. *Semin. Cell Biol.* **3**, 199-210.

Schultz, J. and Kaminker, K. (1962). Myeloperoxidase of the leukocyte of normal human blood. I: Content and localization. *Arch. Biochem. Biophys.* **96**, 465-467.

Shepherd, V. L. and Hoidal, J. R. (1990). Clearance of neutrophil derived myeloperoxidase by the macrophage mannose receptor. *Am. J. Resp. Cell Mol. Biol.* **2**, 335-340.

Siegbahn, A., García, R. C., Kishi, K., Nilsson, K. and Venge, P. (1987). Specific binding of B-CLL cell-derived chemokinetic inhibitory factor (CIF) to human polymorphonuclear leukocytes. *Eur. J. Haematol.* **39**, 172-179.

Springer, T. A. (1990). Adhesion receptors of the immune system. *Nature* **346**, 425-434.

Thörnqvist, P.-O., Johansson, M. W and Söderhäll, K. (1994). Opsonic activity of cell adhesion proteins and β-1,3-glucan-binding proteins from two crustaceans. *Dev. Comp. Immunol.* **18**, 3-12.

Wright, S. D, Weitz, J. I., Huang, A. J, Levin, S. M., Silverstein, S. C. and Loike, J. D. (1988). Complement receptor type three (CD11b/CD18) of human polymorphonuclear leukocytes recognizes fibrinogen. *Proc. Nat. Acad. Sci. USA* **85**, 7734-7738.

(Received 17 December 1996 – Accepted 26 February 1997)