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Expression of the CD15 differentiation antigen (3-fucosyl-*N*-acetyl-lactosamine, Le^X) on putative neutrophil adhesion molecules CR3 and NCA-160

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The expression of the carbohydrate antigen 3-fucosyl-*N*-acetyl-lactosamine (CD15, Le^X) on human neutrophil glycoproteins has been studied by immunoprecipitation and immunoblotting by using monoclonal antibody MC2. The antigen is expressed on membrane glycoproteins of approximate molecular mass 165 and 105 kDa. These glycoproteins include the complement receptor and adhesion molecule, CR3, in which the β-chain (CD18, 105 kDa) shows much greater expression than the α-chain (CD11b, 165 kDa). Most of the 165 kDa CD15 antigen is accounted for by expression on the carcinoembryonic antigen (CEA)-related molecule NCA160. Other members of this family, NCA95, NCA90 and NCA55, which are also found in neutrophils, do not express the CD15 antigen. There is a marked increase in the surface expression of CD15, CR3 and the antigen recognized by anti-CEA antibodies upon activation of neutrophils by the chemotactic peptide *N*-formylmethionyl-leucylphenylalanine.

Introduction

Neutrophils play a central role in the defence against infection and the generation of inflammation. The primary neutrophil functions of chemotaxis, phagocytosis and degranulation involve complex intercellular interactions which rely on the adhesive properties of the plasma membrane. In the early stages of acute inflammation, specific receptors appear to facilitate adhesion to endothelial cells, control the directed migration through the tissue, and mediate adherence to target organisms, which are subsequently phagocytosed. Many of the neutrophil surface glycoproteins which are essential for these processes remain to be characterized, both structurally and functionally [for a review, see Horejsi & Bazil (1988)].

Most of the neutrophil surface proteins that have been characterized were first identified by means of monoclonal antibodies. Many of these antibodies react with other leucocytes in addition to neutrophils, suggesting functions for the proteins that are shared by other cell types. A number of 'neutrophil-specific' monoclonal antibodies have been reported, nearly all of which are of the IgM class and recognize the carbohydrate antigen 3-fucosyl-*N*-acetyl-lactosamine, sometimes referred to as 'Le^X' or 'X-hapten' (Gooi *et al.* 1983; Huang *et al.*, 1983). Antibodies with these characteristics are classified as 'Cluster of Differentiation (CD)15' by the international Leucocyte Typing Workshops. In addition to recognizing neutrophils in blood and tissues, these antibodies also recognize several other, non-haemopoietic, cell types especially in fetal and neoplastic tissues (Fox *et al.*, 1983, Kerr & McCarthy, 1985; Sanders *et al.* 1988). Recently it has been suggested that 3-fucosyl-*N*-acetyl-lactosamine mediates cell adhesion during embryonic development (Eggens *et al.*, 1989).

CD15 antibodies recognize both glycolipids and glycoproteins from neutrophil membranes. They immunoprecipitate and immunoblot glycoproteins of 140–180 kDa and 95–110 kDa, seen as characteristically broad bands on SDS/polyacrylamide gels (Skubitz *et al.*, 1983; Tetteroo *et al.*, 1984; Albrechtsen & Kerr 1989). CD15 antibodies have been shown to affect a number of neutrophil functions, including the adhesion to endothelium, phagocytosis, stimulation of degranulation and the respiratory burst (Melnick *et al.*, 1985, 1986; Skubitz & Snook, 1987; Forsyth *et al.*, 1989).

Probably the best characterized of the neutrophil surface adhesion molecules are the CD11/CD18 family, which includes the receptors LFA-1, CR3 and p150,95. These proteins, which have a common β-chain (105 kDa) and unique α-chains (150–185 kDa), belong to the integrin superfamily, which includes platelet glycoprotein IIb/IIIa, and fibronectin and vitronectin receptors (Hynes, 1987). This family of glycoproteins has been shown to mediate a range of neutrophil adhesive functions (Detmers & Wright, 1988), and genetic deficiencies in these glycoproteins are associated with impaired neutrophil function (Springer *et al.* 1984; Springer & Anderson, 1986). LFA-1 and p150,95 are expressed only as minor components of the neutrophil plasma membrane, whereas the more abundant CR3, the receptor for complement fragment C3bi, has been shown to bind to endothelial cells through an unknown ligand, as well as to yeast and bacterial cell walls (Harlan *et al.*, 1985: Ross *et al.*, 1985). CR3 is believed to mediate the homotypic aggregation of neutrophils (Buyon *et al.*, 1988). However, neutrophil adhesion has also been shown to occur independently of CD18 (Zimmerman & McIntyre, 1988), and therefore it is likely that there are still adhesion-promoting neutrophil plasma-membrane glycoproteins to be characterized.

Melnick *et al.* (1985) and Skubitz & Snook (1987) by immunoprecipitation of surface-labelled proteins demonstrated that CD15 antibodies reacted with molecules belonging to the LFA1/CR3/p150,95 family. We have recently confirmed, by the more

Abbreviations used: CD, Cluster of Differentiation; CD15 (Le^X), the carbohydrate antigen 3-fucosyl-*N*-acetyl-lactosamine; CR3, complement receptor and adhesion molecule; CD18 and CD11b, the β- and α-chains of CR3; NCAs, non-specific cross-reacting antigens; CEA, carcinoembryonic antigen; fMLP, *N*-formylmethionyl-leucylphenylalanine; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline (8.5 mM-sodium phosphate/0.15 M-NaCl, pH 7.1); DMSO, dimethyl sulphoxide.

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direct technique of immunoblotting, that the CR3 β-chain, and to a lesser extent, the α-chain, do express the CD15 antigen (Albrechtsen & Kerr, 1989). However, in contrast with the results of Skubitz & Snook (1987), our own results show that CR3 can account for only a small percentage of the expression of CD15, consistent with the observation that neutrophils from patients genetically deficient in CR3 still express CD15.

Carcinoembryonic antigen (CEA) is a heavily glycosylated protein found on the surface of many neoplastic tissues, but not their normal counterparts. A family of molecules termed 'non-specific cross-reacting antigens' (NCAs) that are recognized by antisera raised against CEA have been identified in several normal tissues, particularly in neutrophils. Recently, gene-cloning studies have identified the molecular basis for the cross-reaction of these antisera and have shown this family of molecules to be members of the immunoglobin superfamily (reviewed by Thompson & Zimmerman, 1988). Transfection studies have demonstrated CEA to mediate the homotypic adhesion of carcinoma and transfected cell lines (Benchimol *et al.* 1989).

Four members of the CEA family have been shown to be expressed in neutrophils. These are termed NCA50, NCA90, NCA95 and NCA160 from their apparent molecular masses (Audette *et al.*, 1987; Thompson & Zimmerman, 1988). In the present paper we show that NCA160, but not the other NCA molecules, express the carbohydrate determinant CD15, and show that this expression accounts for most of the neutrophil-cell-membrane CD15 of this apparent M_r (160000).

MATERIALS AND METHODS

Antibodies

The monoclonal antibody MC2, specific for the 3-fucosyl-*N*-acetyl-lactosamine carbohydrate antigen (CD15) was produced after immunization of mice with whole human neutrophils, as previously described by McCarthy *et al.* (1985). Monoclonal antibodies MN41 and 44, both specific for the CR3 α-chain (CD11b) were gifts from Dr. N. Hogg, ICRF, Lincoln's Inn Fields, London, U.K. H52 hybridoma, specific for the CR3 β-chain (CD18) was generously provided by Dr. T. August, Department of Pharmacology & Therapeutics, Johns Hopkins University, Baltimore, MD, U.S.A. Purified rabbit immunoglobulins to human CEA were purchased from Dakopatts, Copenhagen, Denmark.

Fluorescein isothiocyanate (FITC)-conjugated goat antibodies against mouse IgM (μ-chain specific) and IgG (whole molecule) for cytofluorimetric analysis were purchased from Sigma Chemical Co., Poole, Dorset, U.K.. FITC-conjugated donkey immunoglobulin against rabbit IgG was obtained from the Scottish Antibody Production Unit, Carluke, Lanarkshire, Scotland, U.K.

Affinity-purified goat anti-mouse IgM (μ-chain specific) and IgG (whole molecule) were purchased from Sigma and conjugated to CNBr-activated Sepharose 4B at 0.8 mg/ml. Affinity purified goat anti-rabbit IgG, conjugated to agarose at 5–10 mg/ml, was purchased from Sigma.

Isolation of neutrophils

Neutrophils were isolated from heparinized blood from healthy volunteers by discontinuous-density-gradient centrifugation as described previously (Albrechtsen & Kerr, 1989). Briefly, whole blood was layered on to an equal volume of Lymphoprep [ρ (density) 1.077 g/ml; Nycomed, Oslo, Norway] above an equal volume of denser Ficoll/Hypaque (ρ 1.119 g/ml) (Ficoll 400 was from Pharmacia LKB, Uppsala, Sweden, and Hypaque sodium was from Sterling Research Laboratories, Guildford, Surrey, U.K.). Neutrophils were harvested from the interface between Lymphoprep and Ficoll/Hypaque, pelleted, washed, and resuspended in PBS. For neutrophils of the highest purity, the resuspended cells were subjected to a second separation by the same technique. The average yield was 1.2×10^6 neutrophils/ml of blood, and the average purity > 98% neutrophils.

Solubilization of neutrophil membrane proteins

For immunoprecipitation studies, neutrophil membranes were prepared by freezing whole, freshly isolated, neutrophils in buffer containing 0.25 M-sucrose / 100 mM-Hepes / 5 mM-iodoacetamide/2 mM-di-isopropyl fluorophosphate (at −20 °C). Cells were stored at −20 °C for a minimum of 2 h and a maximum of 8 weeks, causing the cells to rupture, the integrity of the nuclear envelope being maintained by the presence of 0.25 M-sucrose. Thawed cells were spun at 11800 ***g*** for 3 min, and the supernatant (containing cytoplasmic and soluble granule proteins in sucrose buffer) removed. The pellet was resuspended in PBS (phosphate-buffered saline) containing 1% Triton X-100 and 10 mM-phenylmethanesulphonyl fluoride and incubated at 4 °C for 60 min. The suspension was then spun at 11800 ***g*** for 3 min, and the supernatant, containing the solubilized neutrophil membrane proteins, was removed.

Indirect immunoprecipitation

Indirect immunoprecipitation of neutrophil membrane antigens was carried out by using monoclonal antibodies MC2, H52 or anti-CEA immunoglobulins. Neutrophil membrane extract was incubated for 2 h at 4 °C with 15 μl of antibody (MC2 and H52 were ascitic fluid containing approx. 2 mg of Ig/ml; the purified rabbit anti-CEA IgG was approx. 10 mg/ml). After this 200 μl of goat immunoglobulin against the primary antibody, coupled to Sepharose 4B, was added and the incubation continued for a further 1 h. The suspension was spun at 2800 ***g*** for 30 s, and the supernatant, containing the unbound proteins, was removed. The Sepharose was then placed in 1 ml columns and washed with 2 × 1 ml of 0.1% Triton X-100 in PBS, 1 ml of 0.75 M-NaCl/0.1% Triton X-100/20 mM-sodium phosphate, pH 7.4, and 2 × 1 ml of 0.1% Triton X-100 in PBS. The bound proteins were eluted with 3 × 200 μl of 0.5 M-acetic acid and neutralized immediately with 75 μl of 2 M-Tris/HCl pH 8.7.

SDS/PAGE and immunoblotting

Discontinuous SDS/PAGE was carried out as described by Laemmli (1970). Unless otherwise stated, gels were 5–10%-linear-gradient acrylamide gels. Proteins were stained with silver as described by Morrissey (1981) or transferred to nitrocellulose membranes (0.2 μm pore size; Schleicher and Schuell) by using the method of Towbin *et al.* (1979), except that the transfer buffer contained only 15% methanol. Staining of the blots was carried out with incubation times of 2 h for the primary antibody and 1 h for the secondary antibody [goat anti-mouse IgG and IgM and goat anti-rabbit IgG conjugated to alkaline phosphatase (Sigma)]. The blots were developed with 5-bromo-4-chloro-3-indolyl phosphate (Sigma) as enzyme substrate. The following high-molecular-mass markers (Sigma) were used: myosin, 205 kDa; β-galactosidase, 116 kDa; phosphorylase *b*, 97.4 kDa; bovine albumin, 66 kDa; egg albumin, 45 kDa; and carbonic anhydrase, 29 kDa.

Stimulation of neutrophils

The chemotactic peptide *N*-formylmethionyl-leucylphenylalanine (fMLP) (Calbiochem, Nottingham, U.K.) was dissolved in dimethyl sulphoxide (DMSO) at 10^{-2} M and diluted to 10^{-7} M with PBS. Freshly collected heparinized whole blood was incubated in 8.5 ml plastic tubes pre-warmed to 37 °C. For experimental samples, 10^{-7} M-fMLP was added to give a final

concentration of 10^{-8} M-fMLP; control samples were incubated with an equivalent volume of PBS containing the same concentration of DMSO as the test sample. The cells were incubated for 40 min at 37 °C, then fixed (and the erythrocytes lysed) by being diluted 1:6 in FACS Lysing Buffer (Becton–Dickinson) containing 1.5% (w/v) formaldehyde and 5% (v/v) diethylene glycol. The lysed whole blood was spun for 5 min at 300 *g* and the supernatant decanted off, a pellet of intact white cells remaining. For experiments examining a time course of up-regulation, samples of blood were removed and lysed at 0, 1, 3, 5, 10, 20, and 40 min after addition of fMLP.

Cytofluorimetry

The white-cell pellet remaining after fixing and erythrocyte lysis was washed once in PBS, spun as described above, and the pellet was resuspended in 200 μl of primary antibody (MC2, MN41 or anti-CEA immunoglobulin) at 1:200 dilution, and incubated for 30 min at room temperature. The cell suspensions were diluted in 6 ml of PBS and spun at 300 *g* for 5 min. The supernatant was then poured off, the pellet resuspended in fluoresceinated secondary antibody and incubated for 20 min at room temperature. The cells were washed twice in PBS, fixed in 0.5% paraformaldehyde in PBS, then analysed by using a Becton–Dickinson FACScan cytofluorimeter. Results are given with gating for neutrophils. Control samples were incubated with PBS instead of primary antibody, giving a measure of background fluorescence attributable to non-specific binding of the secondary antibody.

RESULTS

Neutrophil membrane extracts contain proteins which are recognized by anti-CR3 monoclonal antibodies and express CD15

Complement receptor CR3 was immunoprecipitated from detergent-solubilized neutrophil membranes by using an excess of the anti-(CR3 β-chain) monoclonal antibody H52, which was subsequently bound to goat anti-mouse IgG–Sepharose. When the precipitated proteins were eluted from the resin with 0.5 M-acetic acid, the CR3 α-chain (165 kDa) and the β-chain (105 kDa) were clearly visible, after SDS/PAGE, on the silver-stained gels, although the samples were contaminated with lower-molecular-mass proteins derived from the ascitic fluid (Fig. 1, lane 1). The proteins which did not bind to the monoclonal antibody are shown in lane 2. A second immunoprecipitation of these unbound proteins failed to absorb any further CR3.

Immunoblotting of the anti-CR3-immunoprecipitated proteins using anti-CD15 monoclonal antibody MC2 demonstrated clearly that the 105 kDa CR3 β-chain protein strongly expressed CD15, but the 165 kDa CR3 α-chain expressed very little (lane 4). There was still a strong expression of CD15 in unbound material (lane 5), accounting for much of the reactivity seen in the original extract (lane 3). This shows that CD15 expression on the CR3 β-chain cannot account for all the CD15 on proteins of 105 kDa and that expression on the CR3 α-chain accounts for only a very small proportion of the 165 kDa band. None of the CD15-reactive material was absorbed by a second or third immunoprecipitation of the supernatant with the anti-CR3 antibody (lanes 6–8).

Anti-CEA and CD15 antibodies recognize epitopes on the same neutrophil membrane glycoprotein of molecular mass 165 kDa

On immunoblotting of proteins from a neutrophil membrane extract, antibody MC2 routinely recognized broad bands corresponding to glycoproteins of approx. 165 kDa and 105 kDa. A smaller protein was also detected in some blots, especially when the blots were developed for an extended period of time. This band, which has also been recognized in other laboratories, appears to correspond to a degradation product of the 105 kDa glycoprotein (Fig. 2, lane 1, and Albrechtsen & Kerr, 1989). Two different rabbit anti-CEA immunoglobulin preparations recognize a band corresponding to a glycoprotein of approx. 165 kDa and a broad smear in the range 60–100 kDa (Fig. 2, lanes 7 and 8). The mobility and appearance of the 165 kDa band was similar for both MC2 and CEA antibodies. Monoclonal antibodies recognizing other neutrophil glycoproteins, namely cALLA, CD16 and an uncharacterized anti-neutrophil monoclonal antibody 3D3, gave no staining on the same blot, suggesting that they recognize antigens which are not stable to the conditions of SDS/PAGE.

When the proteins immunoprecipitated from neutrophil membrane extracts using anti-CEA antibodies were analysed by SDS/PAGE and silver-stained, diffuse bands corresponding to proteins of 160 kDa, 85 kDa and 60 kDa were visible, together with the immunoglobulin heavy chain (50 kDa) (Fig. 3, lane 2). MC2-precipitated proteins gave a diffuse band, barely visible on silver-stained gels, corresponding to 165 kDa (Fig. 3, lane 1). The proteins precipitated by MC2, when immunoblotted with the same antibody, gave a diffuse 165 kDa band. The 105 kDa band, which consistently immunoprecipitates poorly with MC2 (Albrechtsen & Kerr, 1989) was barely visible (Fig. 3, lane 3). When the MC2-precipitated proteins were immunoblotted with anti-CEA antibodies, the 165 kDa band was again clearly visible (Fig. 3, lane 5).

Furthermore, when the same neutrophil extract was immunoprecipitated with anti-CEA antibodies, immunoblotting with MC2 also revealed the same 165 kDa band (Fig. 3, lane 4), confirming that CD15 is expressed on the CEA-reactive molecule

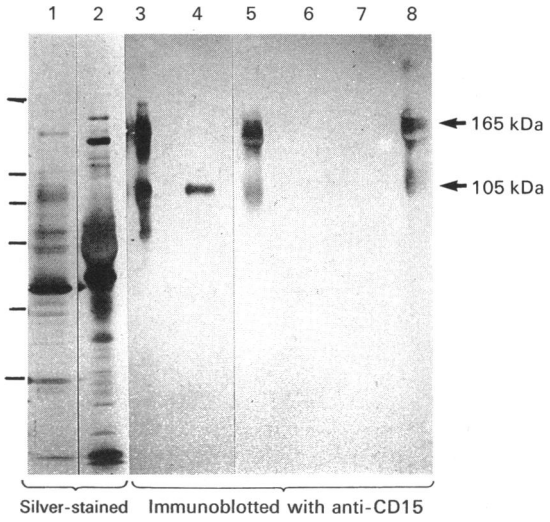

Fig. 1. SDS/polyacrylamide gels (5–15% linear gradient) showing the immunoprecipitation of neutrophil membrane extracts by the anti-(CR3 β-chain) monoclonal antibody H52

Lanes 1 and 2 are silver-stained; lanes 3–8 are immunoblotted with MC2 to detect CD15 expression; lanes 1 and 4 show proteins bound to H52 then eluted with 0.5 M-acetic acid; lanes 2 and 5, proteins not bound upon incubation with excess H52 (the strong bands of 140 and 180 kDa in lane 2 are contaminants from ascitic fluid); lane 3, unabsorbed neutrophil extract; lanes 6–8, second and third immunoprecipitation of the unbound proteins, showing no further binding to the antibody (lanes 6 and 7, material bound and eluted; lane 8, unbound proteins). In this and succeeding Figures, unmarked lanes containing marker proteins (see the text) are also shown.

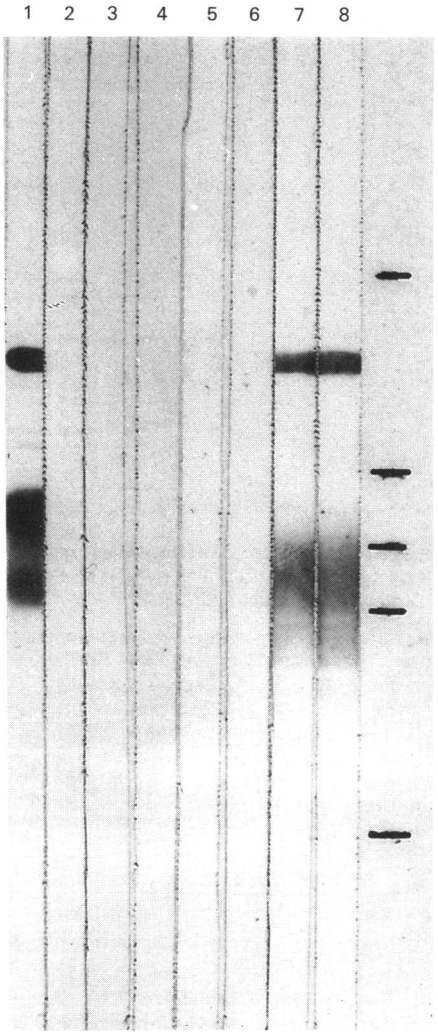

Fig. 2. Immunoblots of neutrophil membrane proteins

Lanes 1, 7 and 8, immunoblot of neutrophil-membrane-extract proteins separated on SDS/5–10% linear-gradient polyacrylamide gels and stained with MC2 (lane 1) or two preparations of anti-CEA antibodies (lanes 7 and 8) Lanes 2–6 were blotted using other anti-neutrophil monoclonal antibodies, namely two anti-cALLA, 3D3 and two anti-CD16.

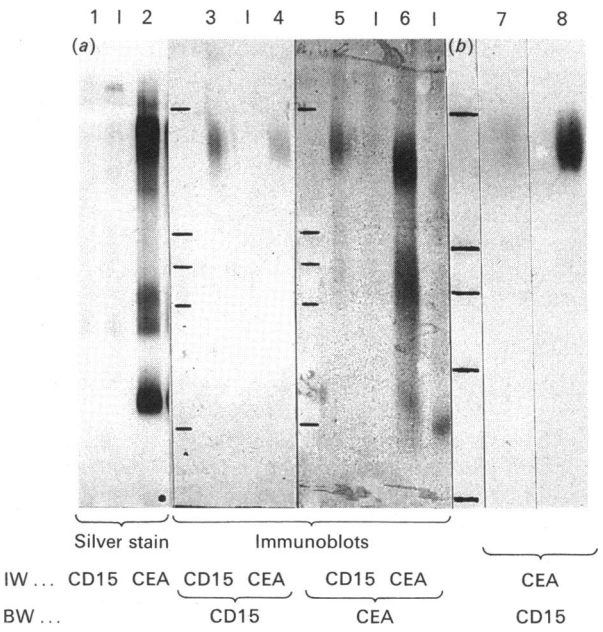

Fig. 3. Immunoprecipitation of neutrophil membrane proteins

(*a*) SDS/polyacrylamide gels of proteins immunoprecipitated with anti-CD15 MC2 (lanes 1, 3 and 5) or anti-CEA antibodies (lanes 2, 4 and 6) detected by silver staining (lanes 1 and 2) or by blotting with MC2 (lanes 3 and 4) or anti-CEA (lanes 5 and 6). (*b*) Immunoblots using antibody MC2 of SDS/polyacrylamide gels showing proteins remaining unbound (lane 7) or immunoprecipitated (lane 8) with an excess of anti-CEA antibodies. Lanes marked 'I' show controls where an irrelevant monoclonal antibody was used. Abbreviations: IW, immunoprecipitated with; BW, blotted with.

previously termed 'NCA160'. Immunoblotting of the anti-CEA-immunoprecipitated proteins using anti-CEA revealed broad bands corresponding to proteins of molecular mass 165 kDa, 85 kDa and 60 kDa. Much more of the CEA-reactive 165 kDa material was precipitated by CEA (Fig. 3, lane 6) than by MC2 (Fig. 3, lane 5), suggesting that only a subpopulation of the NCA160 molecules express CD15. Other molecules immunoprecipitated by anti-CEA antibodies did not express CD15. On careful inspection of the blots it appeared that the protein precipitated by MC2 and recognized by anti-CEA was of a slightly higher average molecular mass than that precipitated by the anti-CEA antibodies (Fig. 3, lanes 5 and 6), suggesting that CD15 is expressed on a more heavily glycosylated subpopulation of NCA160 molecules.

In a similar experiment, an excess of anti-CEA antibodies precipitated most of the 165 kDa glycoproteins which express CD15 (Fig. 3, lane 8). Residual reactivity with MC2 remaining in the supernatant was very low (Fig. 3, lane 7).

Anti-CR3 monoclonal antibodies precipitated proteins of molecular mass 165 kDa and 105 kDa, which were readily visible on silver-stained gels as tight bands, both of which expressed CD15 (Fig. 1). Neither was recognized by anti-CEA antibodies (results not shown).

CR3, CD15, and NCA are up-regulated in response to stimulation by chemotactic peptide

When the chemotactic peptide fMLP was used to stimulate neutrophils and the surface expression of membrane proteins was studied by flow cytofluorimetry, we detected a marked increase in the expression of both CD15- and CEA-reactive antigens after a 40 min incubation at 37 °C (Fig. 4), whereas controls, incubated with PBS instead of fMLP, showed only a marginal increase over 40 min.

When the kinetics of this up-regulation were studied, CEA expression was seen to increase over a very similar time course to that of CR3, suggesting that NCA comes from the same intracellular pool as CR3. CD15 up-regulation, however, consistently showed a lag in the initial stages of activation.

DISCUSSION

CD15 antibodies are characterized by (i) their restriction to binding to neutrophils among the haemopoietic cells, (ii) their ability to recognize the carbohydrate antigen CD15 and (iii) their ability to immunoprecipitate and immunoblot neutrophil membrane glycoproteins which appear on SDS/polyacrylamide gels as diffuse bands corresponding to proteins with apparent molecular masses of about 165 kDa and 105 kDa. The higher-molecular-mass form is consistently more intensely stained on immunoblots and more readily immunoprecipitated.

We have now shown that the major neutrophil glycoprotein expressing the 165 kDa CD15 antigen is a glycoprotein recognized

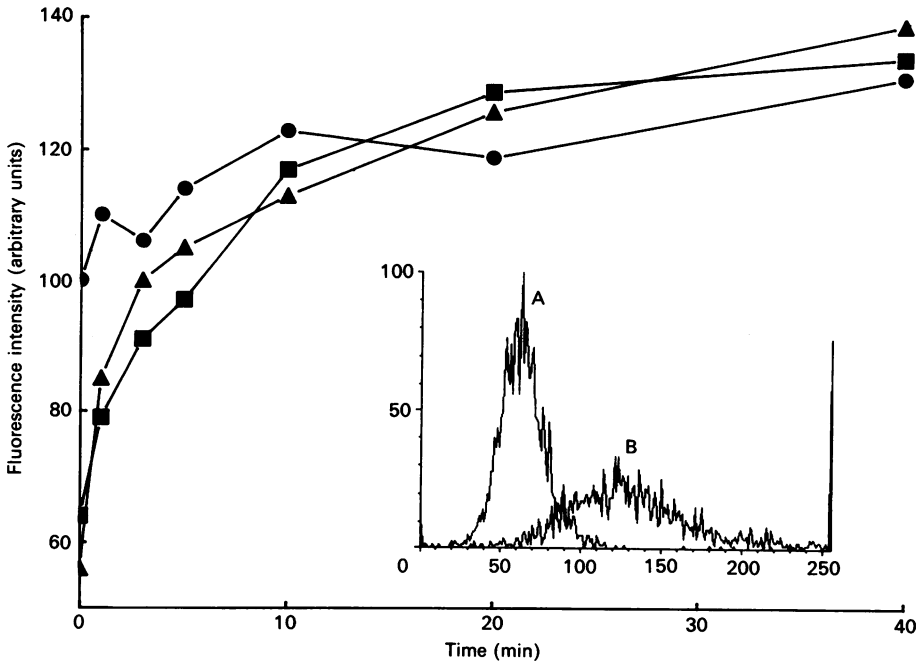

Fig. 4. Time course of the changes in surface expression of CR3 (▲), CD15 (●), and NCA detected with anti-CEA (■) upon stimulation of neutrophils with 10^{-8} M-fMLP at 37 °C

Samples were removed and fixed at various times after addition of the fMLP and analysed by cytofluorimetry. The inset shows cytofluorimeter traces of NCA expression on unstimulated cells (trace A) and stimulated cells (trace B), *y*-axis, cells/channel; *x*-axis, fluorescence intensity (linear scale).

by anti-CEA antisera and previously defined as 'NCA160' (Audette *et al.*, 1987). Those authors identified four neutrophil membrane glycoproteins recognized by anti-CEA antibodies, namely NCA160, NCA95, NCA90 and NCA55. Of these related molecules, only NCA160 appears to express CD15.

The fact that there are a number of neutrophil surface glycoproteins in the molecular-mass region 160–180 kDa has complicated characterization of these molecules. Already identified proteins include aminopeptidase N (CD13, 150 kDa); NCA160 (160 kDa); the α-chains of LFA-1 (CD11a, 180 kDa), CR3 (CD11b, 165 kDa) and p150,95 (CD11c, 150 kDa) and leucocyte common antigen (CD45, 180 kDa). Because of the fact that several of these molecules are heavily glycosylated, resolution by SDS/PAGE is poor, and so their separation is difficult.

We have shown by immunoblotting that the CD15 antigen is expressed on both chains of the CR3 molecule, with more expression on the β-chain. In contrast with results obtained from immunoprecipitation of radiolabelled surface proteins, we were able to show that CR3 α-chain accounted for very little of the expression of CD15. Immunoprecipitation of CR3 by monoclonal antibodies specific for either the α- or the β-chain results in co-precipitation of the other chain; it is therefore not possible by this technique to identify which chain expresses the antigen.

CR3 and CD15 are expressed on the surface of neutrophils and in the membranes of intracellular granules as a pool which can be brought to the surface upon activation of the neutrophils with chemotactic peptides such as fMLP. Although CD15 is expressed on other granule-membrane proteins and on a limited number of soluble granule proteins (Albrechtsen & Kerr, 1989), surface-labelling studies, and our own studies using cytoplast preparations, show that only the 165 kDa and 105 kDa CD15-reactive proteins can be brought to the surface. CEA-reactive glycoproteins have previously been detected in granules and on the surface of neutrophils (Audette *et al.*, 1987; Heikinheimo *et al.*, 1987). We now show that CEA-reactive glycoprotein can also be brought to the surface in a manner and to an extent similar to that of CR3. The apparently slower up-regulation of CD15 remains unclear, but it should be noted that this is not in conflict with our findings from Western blotting and immunoprecipitation, since the subpopulations of NCA and CR3 which express CD15 may behave differently from the larger populations, which do not express CD15.

The up-regulation of intracellular pool of glycoproteins is associated with a marked increase in the adherence of neutrophils (Arnaout *et al.*, 1982). However, the processes involved in this increase in adherence are not well characterized. Although CR3 has been shown to mediate adherence reactions, its role is not fully understood. Recent work has shown that aggregation of stimulated neutrophils occurs much more rapidly than the increase in the surface expression of CR3 (Philips *et al.*, 1988) and that it is not newly exposed CR3 that mediates aggregation of neutrophils, but the population present on the surface of resting cells (Buyon *et al.*, 1988). It has also been shown that adherence of neutrophils to cultured human endothelial cells is not completely blocked by certain anti-CD18 antibodies. In the same study, anti-CD11b antibody caused only a 50% fall in the adhesive capability of human neutrophils (Zimmerman & McIntyre, 1988). It is therefore considered likely that other molecules are involved in the mediation of adherence reactions.

The CD15 antigen was first identified not in neutrophils but as a stage-specific antigen, SSEA-1, appearing during embryogenesis at the eight-cell stage (Solter & Knowles, 1978). The antigen has been shown to be essential for the compaction of the embryo. Although its exact nature has not yet been identified, the antigen has been shown to be expressed on high-molecular-mass glycoproteins (Childs *et al.*, 1983). Recent studies have shown that the 3-fucosyl-*N*-acetyl-lactosamine (CD15) moiety is an important mediator of the cation-dependent homotypic adhesion of mouse embryonic cells (Eggens *et al.*, 1989).

It is noteworthy that CEA, a protein that, as its name suggests,

is preferentially expressed on fetal and neoplastic tissues, has also been shown to mediate cation-dependent homotypic adhesion in transfection experiments (Benchimol *et al.*, 1989). The immunoglobulin superfamily of proteins, which includes the CEA family, also includes many other well-characterized adhesion molecules, such as N-CAM and LFA3 (Williams & Barclay, 1988).

One way in which CR3 mediates adhesion is via the Arg-Gly-Asp tripeptide, present in C3bi (Wright *et al.*, 1987), a mechanism which is common to the integrin family of adhesion receptors. The Arg-Gly-Asp sequence is widely distributed. It is found in several members of the CEA gene family, including pregnancy-specific β-glycoprotein (Streyido *et al.*, 1988), and FL-NCA (Khan & Hammarstrom, 1989). It is therefore feasible that members of the CEA family could interact with integrins in the mediation of adhesion.

The up-regulation of CD15, NCA and CR3 is significant in that it suggests that they have a particular role to play on the surface of the activated neutrophil. Because of the fact that CD15 is associated with a known adhesion molecule, CR3, and a putative adhesion molecule, NCA160, we anticipate future research to elucidate the role of CD15 in neutrophil adhesion.

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