Adhesion of eosinophils to E- & P-selectin

RICHARD PRIEST, SALINA NAWAZ, PHIL M GREEN and MICHAEL I BIRD

Glycobiology Research Group, Glaxo R&D Ltd, Greenford Road, Greenford, Middx UB6 OHE, UK.

Tissue eosinophilia is thought to contribute to the pathology of chronic allergic diseases such as asthma and rhinitis (1). It is generally accepted that leukocyte recruitment to activated endothelium involves an initial selectin-mediated 'tethering' and 'rolling', followed by firm adhesion and diapedesis (2). To investigate mechanisms of eosinophil recruitment, we have studied the relative adhesion of human eosinophils and neutrophils to human P- & E-selectins under both static and dynamic flow conditions.

Recombinant selectins, lacking only the transmembrane and cytosolic domains, were produced as C-terminal chimeras with the ZZ-domain of protein A using baculovirus/insect cell expression systems. Purified proteins were adsorbed directly onto 96-well microtitre plates (static assay) or onto IgG-coated microscope slides (flow assay). Human eosinophils and neutrophils were isolated within 4 hours from single donors (3). For static assays, cells were loaded for 30 mins with 50µM BCECF. Following a 30 min incubation period with the immobilised selectin, non-bound cells were removed by 'flick washing' three times with 'adhesion' buffer (HBSS, 25mM-HEPES, pH7.2, 4mM-CaCl₂, 2mM-MgCl₂, 0.1% HSA). Bound cells were solubilised in 1% SDS, and the fluorescence was determined in a Millipore Cytofluor plate-reader. The number of bound cells was calculated from standard curves.

Optimal concentrations of added P- & E-selectins-ZZ (0.38µg per well) and numbers of cells (10⁵ for eosinophils and neutrophils) were determined from concentration-dependence experiments. Similar binding efficiencies were obtained at 4°C and 21°C.

The adhesion of eosinophils and neutrophils was inhibited by EDTA and by a blocking anti-P-selectin antibody (CLBThrom/6), but not by a non-blocking anti-P-selectin antibody (AK6) (Figure 1a). Interestingly, a significantly greater number (2 - 3-fold) of eosinophils than neutrophils bound to P-selectin-ZZ. In contrast, neutrophils bound more effectively than eosinophils to E-selectin-ZZ (1.3-fold increase, Figure 1b). Cell binding to E-selectin-ZZ was also inhibited by EDTA and by a blocking E-selectin F(Ab')₂ antibody (Monosan).

Similar results were obtained when leukocyte tethering and rolling were examined under laminar flow conditions at 4°C. At calculated wall shear stresses of 3.9 dynes cm⁻², the number of eosinophils tethered per unit time on P-selectin-ZZ was 2-fold greater than neutrophils, whereas almost 3-times as many neutrophils than eosinophils were captured onto E-selectin-ZZ. Despite these differences in total cells tethered, rolling velocities remained much less dependent on the type of selectin present.

Preliminary characterisation of the leukocyte carbohydrate ligand for P- or E-selectin indicated a sensitivity of eosinophil and neutrophil adhesion to neuraminidase (C. perfringens, 1.6U/ml, 90 mins) and endo-β-galactosidase (B. fragilis, 0.1U/ml, 90 mins) pre-treatment (Figure 1). However, FACS analysis with CSLEX-1 or CD15 antibodies indicated that in contrast to neutrophils, there was negligible expression of sLeX or LeX antigens on eosinophils. These results are consistent with published findings (4). The nature of the eosinophil ligand for P-selectin is under investigation.

These results indicate that the expression of P- or E-selectin on inflamed endothelium could influence the selective recruitment of leukocytes.