AS2.1 CD62P (P-selectin) cluster report

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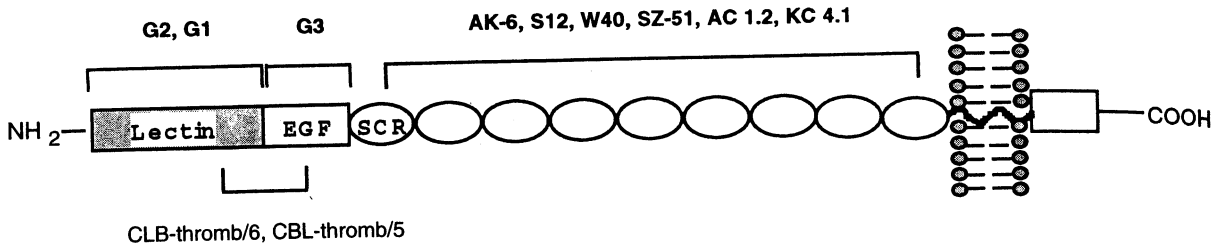

CD62P (P-selectin)

The CD62P (P-selectin, CD62, GMP-140, PADGEM) antigen (introductory figure) was initially characterized with monoclonal antibodies (mAb) including S051 (S12) as part of a biochemical analysis of events associated with platelet activation [1–3]. mAb S057 (CBL-thromb/6) and S062 (CBL-thromb/5), belonging to the CD62 cluster of the Fourth Workshop, were included in the selectin subpanel as reference mAb. Eight additional antibodies, S044 (AK-6), S048 (G2), S049 (G3), S050 (G1), S052 (W40), S053 (SZ-51), S058 (AC 1.2), and S060 (KC 4.1), were clustered as CD62P mAb [Diacovo and Springer, AS2, Table 1]. One mAb included in the Platelet Panel, P094 (P3-38), also clustered to CD62P.

Molecular cloning

P-selectin is an inducible granule membrane protein that is rapidly redistributed to the cell surface of activated endothelium and platelets [1–5]. Its structure [6], similar to that of the other members of the selectin family, consists of an N-terminal lectin-like domain followed by an epidermal growth factor-like domain, nine short consensus repeats (SCR) related to those in complement-binding proteins, a transmembrane region, and a cytoplasmic tail. The gene encoding P-selectin is located on chromosome 1 (q21–24), spans over 50 kilobase pairs, and contains 17 exons [7]. Twelve potential sites of N-linked glycosylation are found in the extracellular region.

Epitope analysis and transfectants

Ten of the 11 mAb included in this cluster reacted specifically with P-selectin-transfected cells as shown in Table 1 of the Subpanel 2 report [Diacovo and Springer, AS2]. This was confirmed by enzyme-linked immunosorbent assay (ELISA) using a chimeric P-selectin molecule. Results for mAb S048 (G2) were conflicting as one group [Nguyen *et al.*, AS2.13] reported cross-reactivity with E-selectin. Four other groups do not support this observation. Epitope analysis using chimeric selectin proteins revealed that the majority of these mAb bound to the SCR domain with the remainder binding to either the lectin domain, EGF-like domain, or the boundary between these two regions [Saunders and Tedder, AS2.8]. Binding of mAb to epitopes located in the lectin or EGF domains was inhibited by protease treatment of activated platelets [de Bruijne-Admiraal and von dem Borne, AS2.7]. Seven different epitopes were recognized in cross-blocking studies [de Bruijne-Admiraal and von dem Borne, AS2.7].

Immunochemistry

Previous studies have shown that all of the CD62P mAb immunoprecipitate a glycoprotein that migrates in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as a band of 140–150 kDa under reducing conditions.

Cellular expression

CD62P is expressed on the surface of activated platelets and endothelium. Frozen sections of normal and reactive lymph nodes, tonsil, small bowel, and skin showed P-selectin expression to be limited to endothelial cells of vessels and high endothelial venules (HEV) in all tissues investigated [Autschbach *et al.*, AS2.5; Zola *et al.* and Cheng and Magnani, unpublished Workshop reports]. The 11 mAb included in the P-selectin subpanel also reacted with activated platelets and with megakaryocytes from bone marrow. Peripheral blood monocytes demonstrated variable reactivity (none to moderate staining) with the mAb. Adhesion of activated platelets to monocytes may account for the discrepancies reported. Other normal cells, with the exception of CD34 positive peripheral blood cells (weak reactivity), do not react with CD62 antibodies.

Function

The ability of CD62 mAb to inhibit platelet–neutrophil interactions has been well described [5,6]. Five mAb submitted to Subpanel 2 were capable of blocking such interactions [Diacovo and Springer, AS2].

References

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AS2.2 CD62E (E-selectin) cluster report

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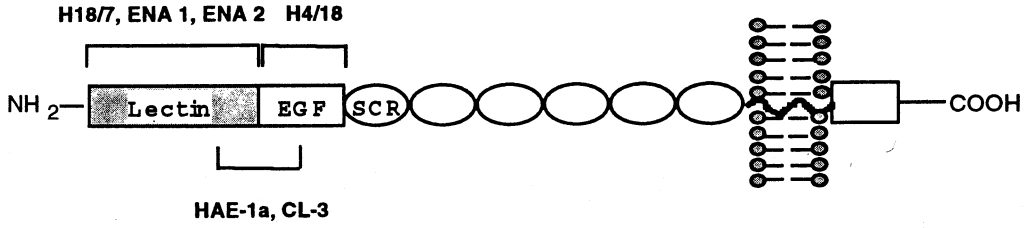

CD62E (E-selectin)

The Fifth Workshop was the first to include monoclonal antibodies (mAb) to CD62E (E-selectin, ELAM-1) (introductory figure). E-selectin was first identified as a cytokine-inducible surface antigen on endothelial cells with the mAb S064 (H4/18) [1]. Another mAb, S045 (H18/7), was subsequently reported that blocked neutrophil adhesion to E-selectin on activated endothelium [2]. These and six additional mAb from the Adhesion Structure section, S042 (CL-2), S043 (4D10), S046 (ENA 2), S047 (ENA 1), S055 (HAE-1a), and S065 (CL-3), were clustered as E-selectin mAb. Five mAb included in the