AS

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endothelial venules (HEV) in all tissues investigated [Autschbach *et al.*, AS2.5; Zola *et al.* and Cheng and Magnani, unpublished Workshop reports].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].

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AS2.2 CD62E (E-selectin) cluster report

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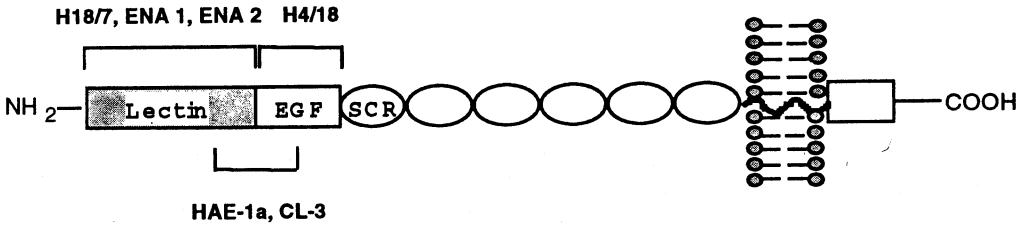

CD62E (E-selectin)

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

Endothelial section, E023 (1F10), E032 (3B7), E033/E034 (7A9), E040 (1.2B6), and E051 (BB11), also belong to the E-selectin cluster. mAb E052 demonstrated cross-reactivity with both E- and P-selectin transfectants in several studies.

Molecular characterization

E-selectin is a glycoprotein that contains an amino-terminal lectin-like domain, a single epidermal growth factor (EGF) like domain, six short consensus repeats (SCR) similar to those found in certain complement-regulatory proteins, a transmembrane domain, and a short C-terminal cytoplasmic domain. cDNA clones encoding this molecule have been isolated and sequenced [3,4]. A single copy of the gene is present in the human genome (long arm of chromosome 1), and contains 14 exons spanning approximately 13 kilobases of DNA [5]. The positions of the exon–intron boundaries correlate with the functional subdivisions of the protein. The CD62E promoter contains an inverted CCAAT box and consensus NF-$\varkappa$B- and AP-1-binding sites. The E-selectin molecule contains 11 potential sites of N-linked glycosylation. E-selectin migrates as a band of 100–115 kDa in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions [1,2].

Epitope analysis and transfectant studies

Evidence for clustering of the CD62E mAb was provided by the use of transfectants. All eight mAb, together with five mAb from the Endothelial Panel, reacted with transfectants expressing the E-selectin molecule but not with cells transfected with either P- or L-selectin. Results for mAb S042 (CL-2) were conflicting, as one of five groups reported cross-reactivity with P-selectin [Diacovo and Springer, AS2, Table 1]. mAb E052 (DA5) was reported to cross-react with activated platelets and P-selectin transfectants by two independent groups [Weitz-Schmidt *et al.*, E6.24; Hoogerwerf *et al.*, E6.12]. The only rat mAb in the panel, S043 (4D10), reacted weakly or not at all with E-selectin transfectants as determined by flow cytometry. This may be due to detection problems with the second reagent. Mapping using chimeric selectin molecules localized mAb epitopes to specific E-selectin domains (see introductory diagram) [Saunders and Tedder, AS2.8].

Cellular expression

E-selectin expression has been well documented on acutely activated endothelium where it participates in the recruitment of leucocytes at sites of acute inflammation [5]. This adhesion molecule has also been identified on endothelium in chronic inflammatory lesions of the skin and synovium [6–8]. The immunohistochemical reactivity of the mAb in the Fifth Workshop E-selectin subpanel was tested on frozen sections of lymph nodes, tonsil, thymus, liver, skin, and small bowel employing the alkaline phosphatase anti-alkaline phosphatase (APAAP) detection method. In addition, flow cytometry was used to evaluate the reactivity on various leucocytes, endothelium, and established cell lines. Immunohistochemical reactivity was restricted to endothelial cells. Staining of high endothelial venules was reported for all mAb [Autschbach *et al.*, AS2.5] or for only two of the mAb [Zola *et al.* and Rizzo *et al.*, unpublished Workshop reports]. Endothelia at sites of mucosal ulceration found in Crohn's disease were strongly stained. Other cell types, including hepatocytes and bone-marrow-derived cells, did not react with the E-selectin antibodies. All of the E-selectin mAb in this subpanel reacted with activated but not resting cultured endothelial cells. Similar results were obtained for the six mAb in the Endothelial Panel. Incubation of cultured endothelium for 24 h with tumour necrosis factor alpha (TNFα), but not interleukins IL-4 or IL-5, membrane cofactor protein, or gamma interferon (IFN-γ) significantly upregulated E-selectin. E-selectin expression was also upregulated by culture supernatant from lipopolysaccharide-stimulated monocytes.

Function

Function-blocking data on the mAb was obtained from the donors and is included in Table 1 of the Subpanel 2 report [Diacovo and Springer, AS2].

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AS2.3 CD62L (L-selectin) cluster report

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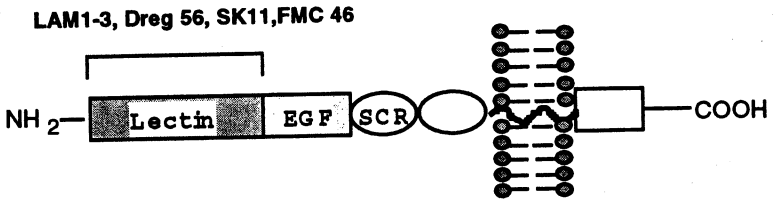

CD62L (L-selectin)

CD62L (L-selectin, LAM-1, Leu 8, TQ1) (introductory figure), a member of the selectin family of adhesion receptors, functions in leucocyte binding to activated endothelium as well as in lymphocyte homing to high endothelial venules (HEV) [1]. The expression of this leucocyte antigen was initially described in the mouse by Gallatin *et al.* [2] using the monoclonal antibody (mAb) mel-14. In the human, L-selectin was characterized with mAb Leu 8 [3] and TQ1 [4]. L-selectin was clustered in the Fifth Workshop as CD62L with four mAb, S054 (LAM1-3), S056 (Dreg 56), S059 (SK11), and S061 (FMC46).

Molecular characterization

The L-selectin molecule is 76 kDa M_r in sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) [5,6]. cDNA clones encoding the L-selectin molecule have been reported [7–9]. The *lyam-1* gene spans more than 30 kb pairs of DNA and is composed of at least 10 exons [10]. The protein encoded by this gene contains an amino-terminal lectin-binding domain, an endothelial growth factor (EGF)-like domain, two short consensus repeat (SCR) sequences similar to those found in complement-binding proteins, a transmembrane region, and a short cytoplasmic region. Seven potential N-linked carbohydrate attachment sites are found in the extracellular region. The human L-selectin protein is 77 per cent identical to mouse L-selectin [7]. It shares considerable amino acid sequence and structure homology with E- and P-selectin and has been mapped to the same region on chromosome 1 (bands q23–25).

Epitope analysis and transfectant studies

The specificity of the four mAb to CD62L was demonstrated using selectin transfectants [Diacovo and Springer, AS2, Table 1]. Epitope analysis using selectin chimeras localized the binding of all of these mAb to the lectin domain of the molecule [Saunders and Tedder, AS2.8].