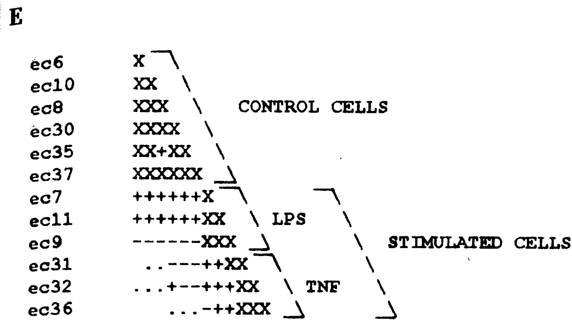

Fig. 2 Computer-assisted cluster analysis shaded matrix. Log 10 of the antigen density was used as the basic parameter for standard computer-assisted cluster analysis of experiments. Identification codes of experiments performed according to different conditions are noted in column 1. Endothelial phenotypes appear as similarities in areas defined by 'X' symbols.

of S-ENDO (E053, E054, E056) and endoglin (E001, E007, E012, E028, E047) antigens that showed similar expression levels on HUVEC can be distinguished on the basis of their density on ECV 304 (Table 2).

In addition, computer-assisted analysis based on antigen densities allowed the clustering of endothelial phenotypes according to experimental culture conditions, distinguishing unstimulated HUVEC from HUVEC stimulated by TNFα or LPS (Fig. 2).

Applied to endothelial cells, the quantitative immunophenotype contributes to a more precise and reproducible definition of antigen expression and modulation, and provides interesting information related to the functional activity of the cell and the effect of culture conditions or of stimulating agents. Antigen density can be considered as a characteristic mAb signature useful for defining the hierarchy of expression of a given epitope on different cell types. In addition to its interest for computer-assisted clustering analysis, antigen density is an interesting parameter for time versus time and laboratory versus laboratory data comparisons and may form the basis for standardization of cell antigen studies.

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E6.9 Expression of sialomucin CD34 by high endothelial venules in human tonsils

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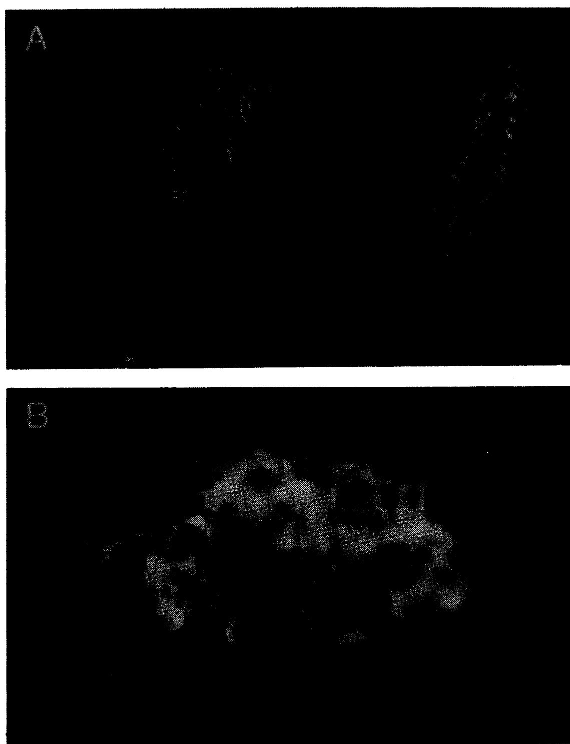
In secondary lymphoid tissues (lymph nodes, Peyer's patches, tonsils), lymphocytes leave the blood by recognizing and binding to specialized postcapillary venules called high endothelial venules (HEV). In contrast to the endothelial cells from other vessels, the high endothelial cells of HEV have a plump, almost cuboidal appearance and express specific ligands for lymphocytes. The initial interaction of lymphocytes with HEV (lymphocyte rolling) is mediated by lymphocyte L-selectin that recognizes ligands of carbohydrates on HEV glycoproteins. Two HEV ligands for L-selectin in mouse, GlyCAM1 and

Table 1 Summary of reactivity of CD34 mAb with HEV

Workshop mAb				
Code	Clone name	Donor	Isotype	HEV staining
MA2	Immu-133	Hirn	IgG1	−
MA3	Immu-409	Hirn	IgG2a	−
MA7	14G3	Lansdorp	IgG1	−
MA8	BI-3C5*	Tindle	IgG1	−
MA11	CD34-9F2	Knapp	IgG1	+
MA22	HPCA2* (8G12)†	+		
MA25	43A1	Bühring	IgG3	+
MA27	681	Guadernack	IgG1	+
MA28	570	Guadernack	IgG1	+
MA29	553	Guadernack	IgG1	+
MA30	563	Guadernack	IgG1	+
MA53	MD34.3	van der Schoot	IgG1	+
MA55	MD34.1	van der Schoot	IgG2b	+
MA56	MD34.2	van der Schoot	IgG1	+
MA65	QBEnd 10* (IOM-34)†	+		
MA74	4A1	Hagiwara	IgG1	+
MA80	9066	Gee	IgG1	+
MA81	9069	Gee	IgG1	+

*Evaluated and verified as CD34 antibodies in the Fourth Workshop [see Civin *et al.* [2] for summary]. mAb BI3C5 published by Tindle *et al.* [3] and mAb QBEnd 10 by Fina *et al.* [4].
†Some of these antibodies are already produced commercially under different names, for example QBEnd 10 (IOM-34) or have commercial names that differ from that in the original publication, for example HPCA2 (8G12).



MadCAM1, have been found to be mucin-like molecules containing O-linked carbohydrates. More recently, the mouse homologue of CD34, the human haemopoietic stem cell antigen, has also been shown to be a ligand for L-selectin [1]. CD34 is a mucin-like molecule highly O-glycosylated and is known to be expressed in the small vessel endothelium of a variety of human tissues. Therefore, we decided to investigate the expression of CD34 by HEV in human lymphoid tissues.

HEV are well represented in human tonsils in which they constitute a prominent stromal component. Therefore, in an attempt to study the expression of CD34 by human HEV, we have tested a large panel

Fig. 1 *(opposite)* Expression of sialomucin CD34 by human HEV. Immunofluorescence staining of human tonsil frozen sections with (A) HEV-specific mAb MECA 79 and (B) CD34 mAb MA22 (HPCA-2). 8-μm acetone fixed frozen sections of human tonsils were stained with MECA 79 or CD34 mAb for 1 h in a moist chamber at room temperature and binding was detected with fluorescein isothiocyanate (FITC)-labelled secondary antibodies. Magnifications: (A) 125×; (B) 500×.

of Workshop CD34 monoclonal antibodies (mAB) MA2, MA3, MA7, MA8, MA11, MA22, MA25, MA27, MA28, MA29, MA30, MA53, MA55, MA56, MA65, MA74, MA80, and MA81, by immunofluorescence staining on frozen sections of human tonsils (Table 1). We found that most of the CD34 antibodies react with all HEV present in tonsils. The honeycomb-staining pattern of HEV observed with CD34 antibodies was very similar to that obtained with the HEV-specific mAb MECA79 (Fig. 1). In addition to the high endothelial cells from HEV, CD34 mAb exhibited reactivity with endothelial cells from flat-walled vessels and with a small population of scattered cells (fibroblasts ?). Interestingly, the strongest staining of HEV was observed with mAb MA22, MA27, MA29, and MA30, which recognize CD34 epitopes resistant to neuraminidase and O-glycoprotease (class III epitopes), while mAb MA2, MA3, MA7, MA8, which recognize CD34 epitopes sensitive to neuraminidase and O-glycoprotease (class I epitopes), did not react with HEV. Since mAb MA2, MA3, MA7, and MA8, react with CD34 molecules expressed by vascular endothelium and haemopoietic cell lines, these latter results indicate that glycosylation of CD34 is different in HEV and strongly suggest the existence of HEV-specific glycoforms of CD34.

Our study shows that high endothelial cells from HEV in human lymphoid tissues express the CD34 stem cell antigen. CD34 is the first O-glycosylated mucin-like molecule shown to be expressed by human HEV. Interestingly, glycosylation of CD34 differs between HEV and vascular endothelium or haemopoietic cell lines, suggesting the existence of HEV-specific glycoforms of CD34 that could function as HEV ligands for lymphocyte L-selectin in humans, similarly to CD34, GlyCAM1, and MadCAM1 in the mouse. All together, these results indicate that, in addition to its role in haemopoiesis, CD34 could also play a role in lymphocyte recirculation in the human body.

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E6.10 Immunostaining of Kaposi's sarcoma and lymph node tissues with Workshop Endothelial Section mAb

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The nature, origin, and identity of the putative tumour, spindle cells in Kaposi's sarcoma (KS) are still controversial, particularly considering the apparent differences in pathogenic factors of the various clinical tumour forms, that is, sporadic (SKS), endemic (African; EKS), and AIDS-associated (AKS). The histological evolution of the early, highly vascularized KS lesion to the characteristic nodular sarcomatous stage with pathognomonic spindle cells has been taken as evidence for a vascular origin of these cells. However, recent immunohistochemical and *in vitro* studies suggested heterogeneity of the spindle cells [1], possibly related to different types of endothelium. Here the reactivity of monoclonal antibodies (mAb) from the Endothelial Panel was studied by immunostaining acetone-fixed frozen-tissue sections of nodular AKS, EKS skin biopsies, and similarly fixed cell preparations of early passages of cultured human umbilical vascular endothelium (HUVEC).

Table 1 summarizes the overall immunoreactivity of those Workshop Endothelial Section mAb that clearly stained vascular endothelial cells (VEC) in