Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 3566–3570, April 1995
Medical Sciences

Expression of the fms-like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development

ARJA KAIPAINEN*, JAANA KORHONEN*, TUIJA MUSTONEN*, VICTOR W. M. VAN HINSBERGH†, GUO-HUA FANG‡, DANIEL DUMONT‡, MARTIN BREITMAN‡§, AND KARI ALITALO*

*Molecular/Cancer Biology Laboratory, University of Helsinki, PL21 (Haartmaninkatu 3), 00014 Helsinki, Finland; †Netherlands Central Organization for Applied Scientific Research Institute of Ageing and Vascular Research, Gaubius Laboratorium, P.O. Box 430, 2300 AK Leiden, The Netherlands; and ‡Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto, Canada M5G 1X5

Communicated by Judah Folkman, Harvard Medical School, Boston, MA, January 3, 1995 (received for review August 1, 1994)

ABSTRACT **We have recently cloned the human fms-like tyrosine kinase 4 gene *FLT4*, whose protein product is related to two vascular endothelial growth factor receptors FLT1 and KDR/FLK1. Here the expression of *FLT4* has been analyzed by *in situ* hybridization during mouse embryogenesis and in adult human tissues. The FLT4 mRNA signals first became detectable in the angioblasts of head mesenchyme, the cardinal vein, and extraembryonally in the allantois of 8.5-day postcoitus (p.c.) embryos. In 12.5-day p.c. embryos, the FLT4 signal decorated developing venous and presumptive lymphatic endothelia, but arterial endothelia were negative. During later stages of development, FLT4 mRNA became restricted to vascular plexuses devoid of red cells, representing developing lymphatic vessels. Only the lymphatic endothelia and some high endothelial venules expressed FLT4 mRNA in adult human tissues. Increased expression occurred in lymphatic sinuses in metastatic lymph nodes and in lymphangioma. Our results suggest that FLT4 is a marker for lymphatic vessels and some high endothelial venules in human adult tissues. They also support the theory on the venous origin of lymphatic vessels.**

The physiology of the vascular system, embryonic vasculogenesis and angiogenesis, blood clotting, wound healing, and reproduction as well as several diseases involve the vascular endothelium lining the blood vessels (1, 2). In the mouse embryo, certain mesenchymal cells differentiate into endothelial cell precursors *in situ*—e.g., in the head mesenchyme, in the dorsal aorta, and in the cardinal veins (3–5). Blood islands of the yolk sac are sites of extraembryonic vasculogenesis (6, 7). Further development of the vascular tree occurs through angiogenesis (8, 9). According to some theories, the formation of the lymphatic system starts shortly after arterial and venous development by venous sprouting (10, 11).

After the fetal period endothelial cells proliferate very slowly, except during angiogenesis associated with neovascularization (12, 13). Among the factors stimulating angiogenesis, the acidic and basic fibroblast growth factors (FGF-1 and FGF-2) and the vascular endothelial growth factor (VEGF) exert their effects via specific cell surface receptor tyrosine kinases: FGF receptor 1 and the endothelial-specific fms-like tyrosine kinase 1 (FLT1) and KDR/FLK1 receptors, respectively (refs. 14–16; for review, see ref. 17). The protein product of the FLT4 receptor tyrosine kinase cDNA is structurally similar to the FLT1 and KDR/FLK1 receptors (18), but FLT4 does not bind VEGF (19). Here we have analyzed FLT4 mRNA expression during vasculogenesis and angiogenesis in mouse embryos and in endothelia of human adult tissues in normal and pathological conditions.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

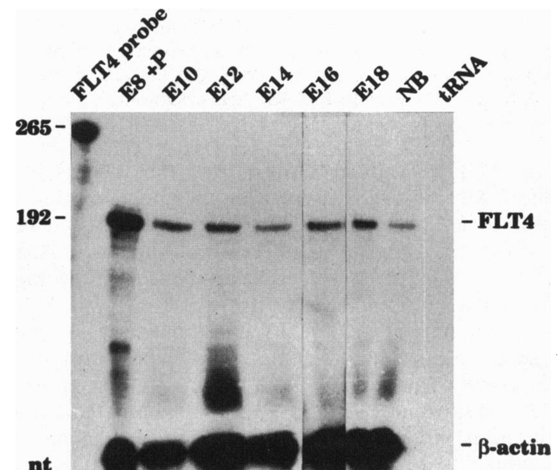

FIG. 1. RNase protection analysis of RNA isolated from mouse embryos of various gestational ages (E8.5–E18.5) and from a newborn mouse. Sample E8+P also contains the placenta. The size of the probe and the protected FLT4 fragment are given in nt; β-actin was used as a control. NB, newborn; E, embryonic day.

MATERIALS AND METHODS

Analysis of RNA in Mouse Tissues. From a subcloned FLT4 DNA fragment of a λ FIX II genomic library from 129SV mice (Stratagene) PCR was used to amplify and clone exon fragments covering nt 1–192 and 1745–2049 of mouse FLT4 cDNA (20). Total RNA was isolated and analyzed by RNase protection (21) with probes corresponding to nt 1–192 of FLT4 cDNA and 1188–1279 of β-actin cDNA (22). *In situ* hybridization of sections was performed as described (23) and the mouse Tie 1C1D plasmid was used as a control (24).

Analysis of RNA in Human Cells and Tissues. Endothelial cells from humans were isolated, cultured for five to eight passages, and used for isolation of polyadenylylated RNA as described (25, 26). Human tissues sent for routine histopathological diagnosis were used for RNA *in situ* hybridization. Probes covered bp 1–595 of the human *FLT4* cDNA (18), 1–2334 of the von Willebrand factor cDNA (27), and 1–2190 of the *Tie* receptor cDNA (28). The normal *in situ* protocol did not work for human tissue samples routinely fixed in 10% formalin. However, when the proteinase K digestion step was replaced with microwave treatment, specific hybridization signals were obtained (29, 30).

RESULTS

Analysis of FLT4 mRNA in Early Mouse Embryos. Analysis of RNAs collected during different phases of mouse develop-

§Deceased February 13, 1994.
Abbreviations: p.c., postcoitus; HEV, high endothelial venule.

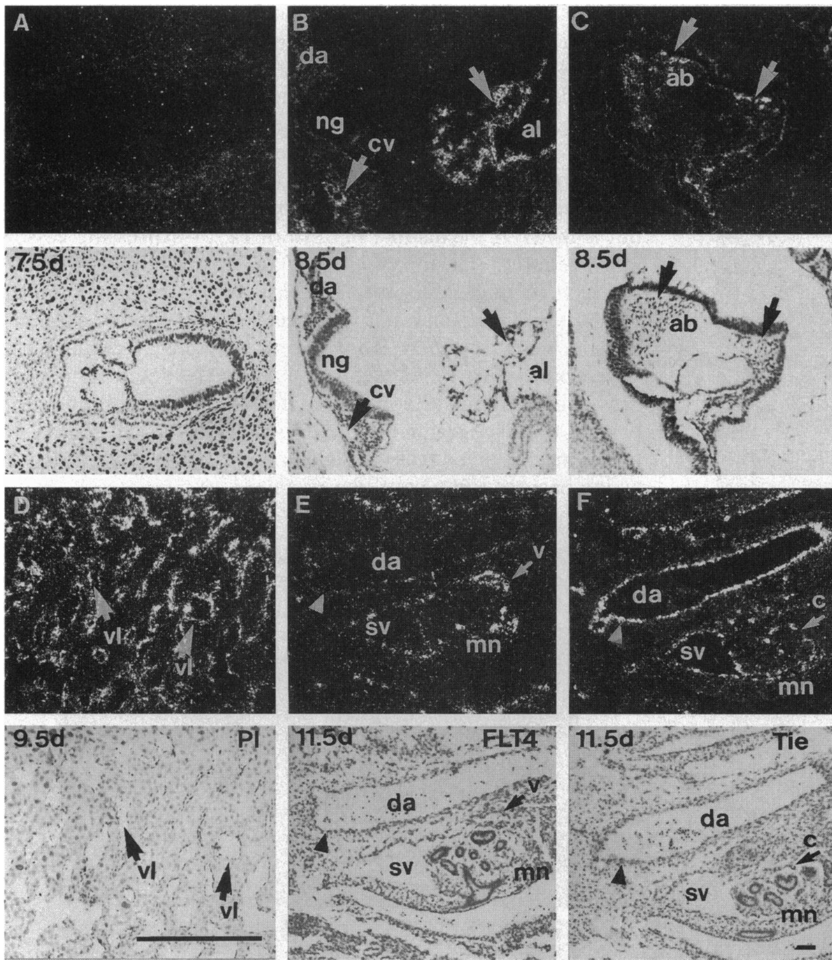

FIG. 2. Expression of FLT4 mRNA in 7.5-, 8.5-, and 11.5-day p.c. embryos. Dark-field and bright-field photomicrographs of *in situ* autoradiograms are shown. No expression of FLT4 mRNA is detected in a 7.5-day p.c. embryo (*A*). FLT4 expression in an 8.5-day p.c. mouse embryo is shown in *B* and *C*. Arrows indicate FLT4-positive cells in the endothelium of posterior cardinal vein (cv), in the allantois (al) in *B* and in angioblasts (ab) of the head mesenchyme in *C*. In a 9.5-day p.c. placenta, FLT4 transcripts can be seen in endothelial cells of venous lacunae (vl) (*D*). (*E* and *F*) Comparison of FLT4 and Tie hybridization signals in 11.5-day p.c. embryos. The region of the developing dorsal aorta (da) (arrowheads) and metanephros (mn) is shown. (×12.) Note that the da is negative for FLT4 but positive for Tie mRNA, whereas both probes hybridize with the endothelium of the subcardinal vein (sv). Also, the FLT4 probe gives a signal from the metanephric vein (v), whereas the Tie probe mostly hybridizes with the developing metanephric capillaries (c, arrows). ng, Neural groove. (Bar = 30 μm.)

ment by RNase protection assay showed that FLT4 mRNA was expressed throughout embryogenesis from day 8.5 postcoitus (p.c.) to newborn mice without variations in signal intensity (Fig. 1). Placenta contained enhanced amounts of FLT4 mRNA (lane E8+P; data not shown).

To localize FLT4 transcripts to specific cells and tissues, sections of 7.5- and 8.5-day p.c. mouse embryos were hybridized with labeled FLT4 probes. As shown in Fig. 2, FLT4 mRNA was not expressed in 7.5-day p.c. mouse embryos (Fig. 2*A*), but abundant signals were detected in the posterior cardinal vein and in the angioblasts of the head mesenchyme on day 8.5 of development (Fig. 2 *B* and *C*). In contrast, the dorsal aorta and developing heart (data not shown) did not express FLT4 mRNA. In the extraembryonic tissues, FLT4 was prominent in the allantois (Fig. 2*B*), whereas developing blood islands of the yolk sac were negative (data not shown). In the developing 8.5-day p.c. placenta, FLT4 signal was seen in peripheral sinusoidal veins and in the endothelium of venous lacunae (Fig. 2*D*). Also, giant cells partially fused to the Reichert's membrane (data not shown) expressed FLT4 mRNA.

Thus, although FLT4 expression was very prominent in the earliest endothelial cell precursors, the angioblasts, it appeared to be restricted only to certain vessels of 8.5-day p.c. embryos. This is clear from the comparison of FLT4 with the Tie receptor,

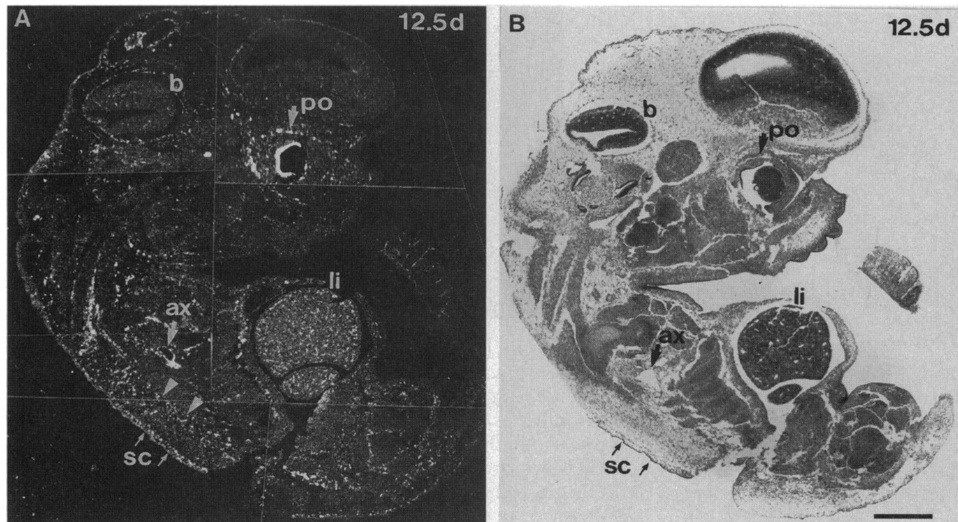

FIG. 3. FLT4 mRNA expression in a 12.5-day p.c. embryo. A parasagittal section through the axillar plane is shown. Note that FLT4 mRNA is prominent in dilated vessels of the axilla (ax), in a plexus-like pattern in the periorbital (po) region, in the paravertebral tissue (arrowheads), and in the subcutaneous (sc) tissue. b, Brain; li, liver. (Bar = 5 μm.)

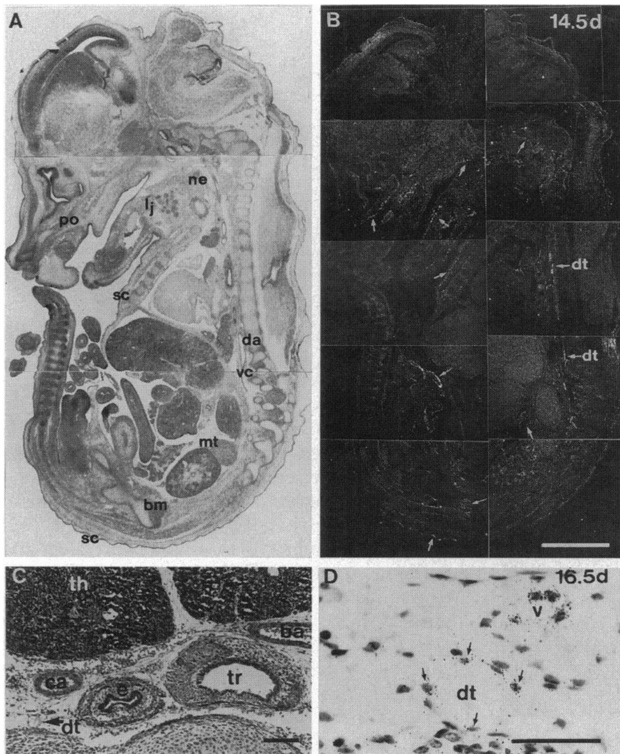

FIG. 4. FLT4 in 14.5- and 16.5-day p.c. embryos. (*A* and *B*) Bright-field and dark-field images of a midsagittal section. (*C*) Transverse section of a 16.5-day p.c. embryo with hematoxylin and eosin staining. (*D*) Higher magnification of the region of ductus thoracicus; autoradiographic grains can be seen over the endothelial cells. (×20.) Also, the small vessel (v) in the upper part of the photograph is positive. da, Dorsal aorta; vc, inferior vena cava; bm, bone marrow; po, periorbital region; lj, lower jaw; ne, neck region; sc, subcutis; mt, mesenterium; dt, thoracic duct; th, thymus; tr, trachea; e, esophagus; ca, carotid artery; ba, brachiocephalic artery. (*A–C*, bar = 10 μm; *D*, bar = 1 mm.)

which is known to be expressed in all endothelial cells of developing mouse embryos and thus provides a marker for these cells (31, 32). Notably, in contrast to the Tie probe, the FLT4 probe hybridized very weakly if at all with arterial endothelia of 11.5-day p.c. embryos—e.g., with the endothelium of the developing dorsal aorta (Fig. 2 *E* and *F*) or the carotid arteries (data not shown). Instead, the FLT4 signal was much more prominent in the developing veins. For example, FLT4 signal was detected in anterior cardinal veins in the neck area (data not shown) and in veins surrounding the developing metanephros (Fig. 2*E*),

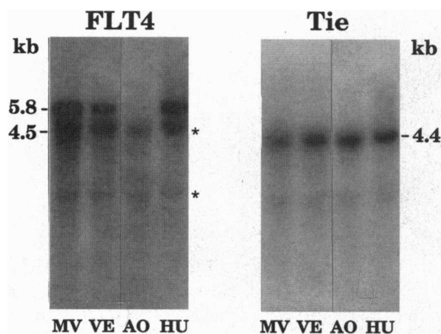

FIG. 5. Comparison of FLT4 and Tie mRNA expression in cultured endothelial cells. Northern blot analysis of polyadenylylated RNA from human foreskin microvascular (MV), femoral vein (VE), aortic (AO), and umbilical vein (HU) endothelial cells. For comparison, the hybridization signal of the Tie receptor tyrosine kinase mRNA is shown. Bands resulting from nonspecific binding of the probe to the rRNA are marked with asterisks.

whereas the Tie probe predominantly recognized developing capillaries within the metanephros (Fig. 2*F*).

FLT4 mRNA in 12.5-Day p.c. Embryos. Fig. 3 illustrates FLT4 signals in a parasagittal section of a 12.5-day p.c. mouse embryo. FLT4 mRNA is distributed in several regions of the embryo, being particularly prominent in a dilated vessel of the axillar region (Fig. 3*A*). Similar FLT4-positive vessel networks were seen in the midsagittal section in the jugular area (data not shown). A plexus-like pattern of FLT4-expressing vessels appeared in the periorbital region and surrounding the developing vertebrae. Also, just beneath the developing skin, an FLT4-positive vascular network was evident. Weaker capillary signals were obtained from several regions, including the developing brain. FLT4 mRNA could also be detected in small vessels of the neck region, of the developing snout, and at the base of the developing tongue as well as in the tail region. In addition, the liver was strongly positive for FLT4 mRNA, which occurred in a spot-like pattern.

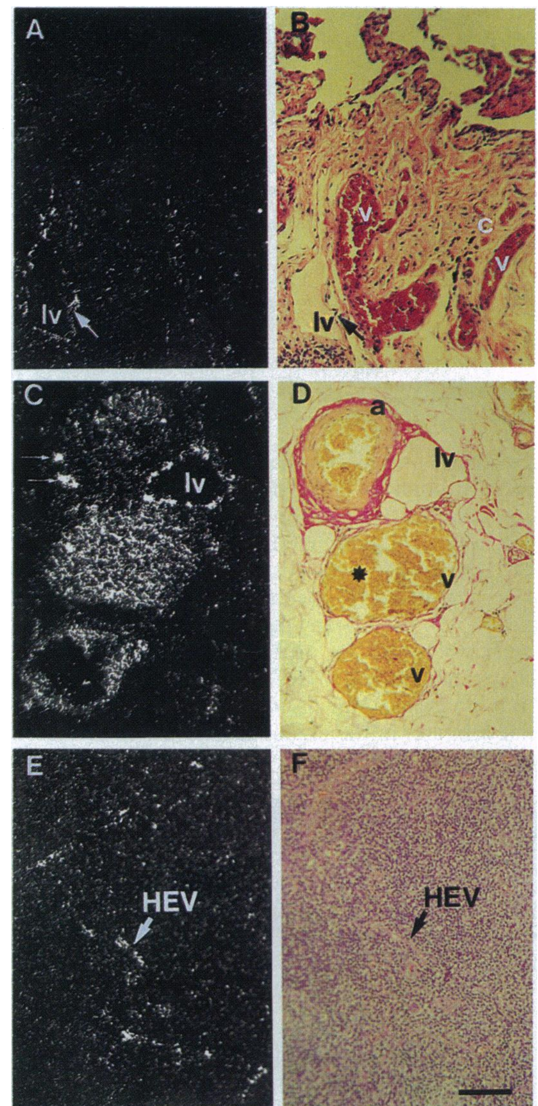

FIG. 6. FLT4 in adult human lymphatic vessels of the lung (*A* and *B*), mesenterium (*C* and *D*), and tonsil (*E* and *F*). Note that only the lymphatic vessels (lv) in *A* and *C* give an FLT4 signal, whereas the veins (v), capillaries (c), and arteries (a) are negative for FLT4 mRNA. Signal within these vessels results from reflection of light from the red cells, which are seen in an adjacent stained section (asterisk in *D*). Arrows in *C* indicate small lymphatic vessels. In the tonsil, the signal is observed in the endothelia of some HEVs. (Bar = 200 μm.)

FLT4 mRNA in 14.5- and 16.5-Day p.c. Embryos. During further development, FLT4 mRNA appeared to become more restricted to certain vessels of the embryo. A 14.5-day p.c. embryo clearly shows this restricted pattern of expression (Fig. 4 *A* and *B*). In the midsagittal section of Fig. 4, the most prominent FLT4 signal is seen along the developing vertebral column on its anterior side. This signal seems to originate from endothelial cells of the thoracic duct. In contrast, the dorsal aorta and inferior vena cava were negative. Dilated vessels in the mesenteric region were also positive for FLT4. Furthermore, as in the 12.5-day p.c. embryos, vessel networks along anatomical boundaries in the periorbital, lower jaw, as well as in the neck region contained FLT4-expressing endothelia. Similar structures were present in the pericardial space and throughout the subcutaneous tissue. Notably, in contrast to FLT4-negative vessels, all FLT4-positive vessels were devoid of red cells in their lumen. This expression pattern suggested that FLT4 becomes confined to the endothelium of lymphatic vessels at this time of development. An additional site where we observed FLT4 expression was the sinusoidal endothelium of the developing bone marrow.

Photographs of a transverse section of the upper thorax of a 16.5-day p.c. embryo hybridized with the FLT4 probe are shown in Fig. 4 *C* and *D*. Higher magnification of the region of the thoracic duct is shown in Fig. 4*D*, where the FLT4 autoradiographic grains can be seen. Endothelial cells of the thoracic duct as well as a small vessel in the vicinity hybridize with the FLT4 probe.

Analysis of FLT4 mRNA in Human Endothelial Cells *in Vitro* and *in Vivo*. Reflecting the selective FLT4 expression in early embryos, cultured human microvascular, venous, and umbilical vein endothelial cells were positive for the FLT4-specific 5.8- and 4.5-kb mRNAs (33), whereas the aortic endothelial cells were negative (Fig. 5). In contrast, *Tie*, another endothelial receptor tyrosine kinase gene was expressed as a 4.4-kb mRNA in all endothelial cell types studied.

We also studied FLT4 in adult human tissues by using the human FLT4 probe. In the lung, mesenterium, and appendix, lymphatic endothelia gave FLT4 signals, while veins, arteries, and capillaries were negative (Fig. 6 *A–D*; data not shown). To determine whether FLT4 is expressed in the high endothelial venules (HEVs), the tonsils were studied. FLT4-specific autoradiographic grains were detected in some, but not all, HEVs in these tissues (Fig. 6 *E* and *F*).

Analysis of FLT4 mRNA in Normal and Metastatic Lymph Nodes and in Lymphangioma. In Fig. 7 a portion of a human mesenteric lymph node is analyzed for FLT4 expression. FLT4 mRNA was present in the lymphatic sinuses and afferent and efferent lymphatic vessels (data not shown). The same pattern is seen in lymph nodes containing adenocarcinoma metastases (Fig. 7 *C* and *D*). Some HEVs in both normal and metastatic lymph nodes were also positive. However, the lymphatic endothelium of metastatic lymph nodes gave an enhanced signal. In Fig. 7*E*, a strong FLT4 mRNA expression is shown in a cystic lymphangioma (compare with the hematoxylin-stained section in Fig. 7*G*). Notably, the specific expression of FLT4 in lymphatic endothelium is evident from comparison with the *in situ* signals for von Willebrand factor mRNA in all blood vessels (Fig. 7*F*).

DISCUSSION

We have shown earlier that the expression pattern of FLT4 in comparison to FLT1 and KDR differs greatly in tissues of 18-week-old human fetuses (23). To understand the role of FLT4 during development, we cloned mouse cDNA probes for FLT4. Using these probes in *in situ* hybridization, we analyzed FLT4 expression during mouse development and confirmed the relevance of our findings in normal and pathological adult human tissues.

Like the *FLK1*, *FLT1*, *TIE*, and *TEK* endothelial receptor tyrosine kinase genes, the *FLT4* gene was not expressed in 7.5-day p.c. embryos. The results of RNase protection suggested that the relative expression level is quite stable after 8.5 days of mouse development. The earliest signals appeared in the angioblasts of the head mesenchyme and veins of the embryo. In contrast, the dorsal aorta, endocardium of the heart, and blood islands of the yolk sac were negative, unlike for FLK1, FLT1, Tie, and Tek mRNAs (refs. 31 and 34; unpublished data). The restriction of FLT4 expression to the venous system was even more clear in samples from 11.5-day p.c. mouse embryos, where Tie mRNA was expressed also in arteries.

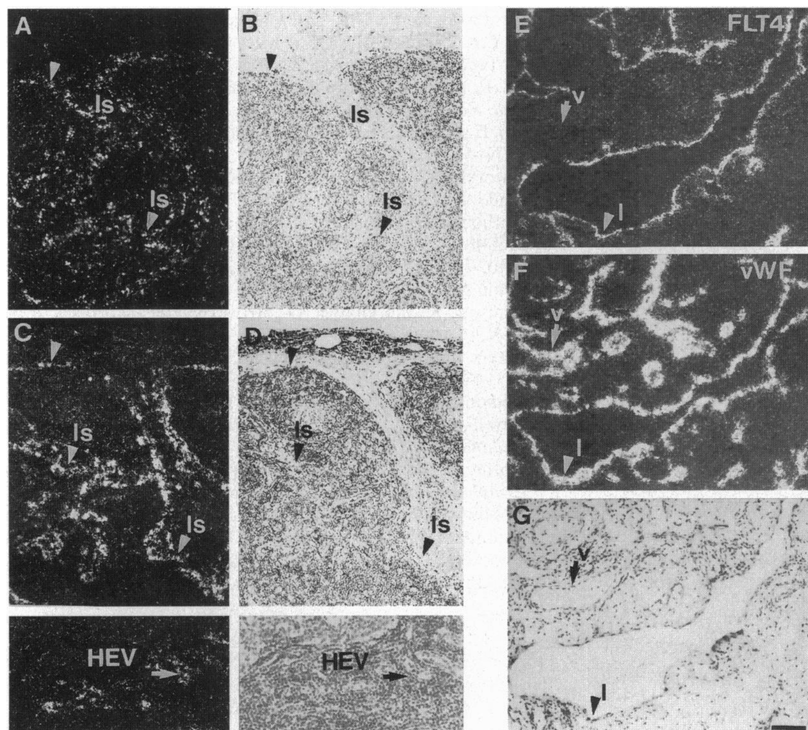

FIG. 7. FLT4 mRNA in normal (*A* and *B*) and metastatic (*C* and *D*) lymph node and in lymphangioma (*E–G*). Arrowheads mark lymphatic sinuses (ls) and HEVs, which are FLT4 positive. A comparison of FLT4 and von Willebrand factor (vWF) signals shows both in the lymphatic endothelium (l) but only von Willebrand factor signal in the capillary and venous (v) endothelia. (*A–D*, bar = 10 μm; *E–G*, bar = 100 μm.)

The idea of lymphatic expression arose during the study of 14.5- and 16.5-day p.c. embryos, because at that stage large arteries and veins as well as small vessels containing red blood cells had no FLT4 signal in their endothelium. However, lymphatic vessels were difficult to identify, because no specific markers are available for them. Since the beginning of the 20th century, three different theories concerning the origin of the lymphatic system have been presented (11, 35, 36). The most widely accepted theory was proposed by Florence Sabin (10), who suggested that during the fetal period lymphatic structures sprout from large central veins in certain locations to form the primordial lymph sacs. Subsequently, the sacs enlarge, coalesce, and form new sprouts, which grow into the periphery of the embryo (11, 12, 35, 36).

The expression of FLT4 in developing mouse embryos coincides with Sabin's model of lymphatic development. According to her theory, the jugular sacs develop from anterior cardinal veins in the neck, and all other lymphatic sacs develop by sprouting from the mesonephric vein and veins in the dorsomedial edge of the Wolffian bodies (36). We found very prominent FLT4 signals in the venous compartment, in the anterior cardinal veins, and mesonephric vein in 11.5-day p.c. embryos, and a day later we could detect the first FLT4-positive sac-like structures in the jugular and axillary areas. The main lymphatic duct, the thoracic duct, is formed by the union of duct primordia (36). Fig. 4 shows such primordia in a 14-day p.c. embryo and a transverse section of the 16.5-day p.c. embryo shows FLT4 signal in the thoracic duct.

At least some of the specificity of FLT4 expression was retained in cultures of human endothelial cells and *in situ* hybridization analysis of adult human tissues confirmed the restriction of FLT4 to the lymphatic system. FLT4 expression was seen in the lymphatic endothelia and in sinuses of human lymph nodes. Interestingly, some of the HEVs, which have a cuboidal endothelium, shown to function in the trafficking of leukocytes to the lymph nodes, were FLT4 positive. FLT4 was also very prominent in lymphangiomas, which are benign tumors composed of connective tissue stroma, and growing endothelium-lined lymphatic channels (37). FLT4 mRNA was restricted to the lymphatic endothelium of these tumors and absent from their arteries, veins, and capillaries. In human lung, we were able to identify lymphatic vessels, which were the only FLT4-positive vessels in this tissue.

In conclusion, our present results show that the uniform venous and capillary expression of FLT4 becomes restricted to lymphatic vessels during mouse development, and in human adult tissues FLT4 is specifically expressed by lymphatic vessels and some HEVs. These results support the theories of the venous origin of lymphatic vessels. The major function of the lymphatic system is to provide fluid return from tissues and to transport many extravascular substances back to the blood (38). In addition, during the process of maturation, lymphocytes leave the blood, migrate through lymphoid organs and other tissues, and enter the lymphatic vessels, from which they return to the blood through the thoracic duct. Specialized venules, HEVs, bind lymphocytes again and cause their extravasation into tissues. Besides providing a marker for lymphatic vessels and some HEVs in human adult tissues, FLT4 may be actively involved in the genesis and maintenance of the lymphatic vessels.

We would like to thank Dr. Daniel C. Lynch for the von Willebrand factor cDNA; Dr. Eero Saksela for expert reviewing of the histology and pathology; Drs. Riitta Alitalo, Sirpa Jalkanen, and Erika Hatva for critical reading of the manuscript; and Kirsti Tuominen and Tapio Tainola for expert technical assistance. This study was supported by the Finnish Cancer Organizations, The Finnish Academy, The Sigrid Juselius Foundation, and The Finnish Cultural Foundation.

1. Klagsbrun, M. & Folkman, J. (1991) in *Peptide Growth Factors and Their Receptors*, eds. Sporn, M. B. & Roberts, A. B. (Springer, New York), pp. 549–586.
2. Folkman, J. & Shing, Y. (1992) *J. Biol. Chem.* **267**, 10931–10934.
3. Pardanaud, L., Altmann, C., Kitos, P., Dieterlen-Lievre, F. & Buck, C. A. (1987) *Development (Cambridge, U.K.)* **100**, 339–349.
4. Coffin, J. D. & Poole, T. J. (1988) *Development (Cambridge, U.K.)* **102**, 735–748.
5. Flamme, I. & Risau, W. (1992) *Development (Cambridge, U.K.)* **116**, 435–439.
6. Noden, D. M. (1989) *Am. Rev. Respir. Dis.* **140**, 1097–1103.
7. Gonzalez-Crussi, F. (1971) *Am. J. Anat.* **130**, 441–460.
8. Folkman, J. & Klagsbrun, M. (1987) *Science* **235**, 442–447.
9. Klagsbrun, M. (1991) *Annu. Rev. Physiol.* **53**, 217–239.
10. Sabin, F. R. (1909) *Am. J. Anat.* **9**, 43–91.
11. van der Putte, S. C. J. (1975) *Adv. Anat. Embryol. Cell Biol.* **51**, 3–60.
12. Hobson, B. & Denekamp, J. (1984) *Br. J. Cancer* **49**, 405–413.
13. Folkman, J. (1992) *Semin. Cancer Biol.* **3**, 65–71.
14. De Vries, C., Escobedo, J. A., Ueno, H., Houck, K., Ferrara, N. & Williams, L. T. (1992) *Science* **255**, 989–991.
15. Terman, B. I., Dougher-Vermazen, M., Carrion, M. E., Dimitrov, D., Armellino, D. C., Gospodarowicz, D. & Böhlen, P. (1992) *Biochem. Biophys. Res. Commun.* **187**, 1579–1586.
16. Millauer, B., Wizigman-Voos, S., Schnürch, H., Martinez, R., Moller, N.-P. H., Risau, W. & Ullrich, A. (1993) *Cell* **72**, 835–846.
17. Mustonen, T. & Alitalo, K. (1994) *J. Cell Biol.*, in press.
18. Pajusola, K., Aprelikova, O., Korhonen, J., Kaipainen, A., Pertovaara, L., Alitalo, R. & Alitalo, K. (1992) *Cancer Res.* **52**, 5738–5742.
19. Pajusola, K., Aprelikova, O., Pelicci, G., Weich, H., Claesson-Welsh, L. & Alitalo, K. (1994) *Oncogene* **9**, 3545–3555.
20. Finnerty, H., Kelleher, K., Morris, G. E., Bean, K., Merberg, D., Kritz, R., Morris, J. C., Sookdeo, H., Turner, K. J. & Wood, C. R. (1993) *Oncogene* **8**, 2293–2298.
21. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) in *Molecular Cloning: A Laboratory Manual*, eds. Ford, N. & Nolan, C. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 7.3–7.78.
22. Tokunaga, K., Taniguchi, H., Yoda, K., Shimizu, M. & Sakiyama, S. (1986) *Nucleic Acids Res.* **14**, 2829.
23. Kaipainen, A., Korhonen, J., Pajusola, K., Aprelikova, O., Persico, M. G., Terman, B. I. & Alitalo, K. (1993) *J. Exp. Med.* **178**, 2077–2088.
24. Korhonen, J., Partanen, J., Armstrong, E., Vaahtokari, A., Elenius, K., Jalkanen, M. & Alitalo, K. (1992) *Blood* **80**, 2548–2555.
25. Van Hinsberg, V. W. M., Binnema, D., Scheffer, M. A., Sprengers, E. D., Kooistra, T. & Rijken, D. C. (1987) *Arteriosclerosis* **7**, 389–400.
26. Van Hinsberg, V. W. M., Scheffer, M. A. & Kooistra, T. (1987) *Thromb. Haemostasis* **57**, 148–153.
27. Bonthron, D. T., Orr, E. C., Mitsock, L. M., Ginsberg, D., Handin, R. I. & Orkin, S. H. (1986) *Nucleic Acids Res.* **141**, 7125–7127.
28. Partanen, J., Armstrong, E., Mäkelä, T. P., Korhonen, J., Sandberg, M., Renkonen, R., Knuutila, S., Huebner, K. & Alitalo, K. (1992) *Mol. Cell. Biol.* **12**, 1698–1707.
29. Shi, E., Kan, M., Xu, J. & McKeehan, W. L. (1991) *J. Biol. Chem.* **266**, 5774–5779.
30. Catoretti, G., Becker, M. H. G., Key, G., Duchrow, M., Schlüter, C., Galle, J. & Gerdest, J. (1992) *J. Pathol.* **168**, 357–363.
31. Korhonen, J., Polvi, A., Partanen, J. & Alitalo, K. (1994) *Oncogene* **8**, 395–403.
32. Sato, T. N., Qin, Y., Kozak, C. A. & Audus, K. L. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 9355–9358.
33. Pajusola, K., Aprelikova, O., Armstrong, E., Morris, S. & Alitalo, K. (1993) *Oncogene* **8**, 2931–2937.
34. Peters, K., Ornitz, D., Werner, S. & Williams, L. (1993) *Dev. Biol.* **155**, 423–430.
35. Zadvinski, D. P., Benson, M. T., Kerr, H. H., Mancuso, A. A., Cacciarelli, A. A., Madrazo, B. L., Mafee, M. F. & Dalen, K. (1992) *RadioGraphics* **12**, 1175–1189.
36. Yoffey, J. M. & Courtice, F. C. (1970) *Lymphatics, Lymph and the Lymphomyeloid Complex* (Academic, London), p. 1–63.
37. Robbins, S. L. & Cotran, R. S. (1979) *Pathologic Basis of Disease* (Saunders, Philadelphia), pp. 593–642.
38. Geneser, F. (1986) *Textbook of Histology* (Munksgaard, Odense), pp. 333–362.