A Unique Microvascular Phenotype Shared by Juvenile Hemangiomas and Human Placenta

Paula E. North, MD, PhD; Milton Waner, MD; Adam Mizeracki; Robert E. Mrak, MD, PhD; Richard Nicholas, MD; Jay Kincannon, MD; James Y. Suen, MD; Martin C. Mihm, Jr, MD

Background: Juvenile hemangiomas are common, benign tumors, distinctive for their perinatal presentation, rapid growth during the first year of life, and subsequent involution. We recently reported that endothelia of hemangiomas highly express GLUT1, a glucose transporter normally restricted to endothelia with blood-tissue barrier function, as in brain and placenta.

Objective: To investigate possible further similarities between hemangioma and placental vessels.

Design: In a retrospective study of a variety of vascular tumors and anomalies, we assessed lesional immunoreactivities for the placenta-associated vascular antigens FcγRII, Lewis Y antigen (LeY), merosin, and GLUT1.

Setting: A university-affiliated pediatric hospital.

Main Outcome Measure: Immunoreactivities scored for each antigen were summarized according to lesional type, compared with those of normal skin, brain, and placentas, and correlated with patient age, sex, and lesional location.

Results: All of 66 hemangiomas (patients aged 22 days to 7 years) showed intense immunoreactivity for FcγRII, merosin, LeY, and GLUT1. No immunoreactivities for these markers were seen in any of 26 vascular malformations, 4 granulation tissue specimens, 13 pyogenic granulomas, or in the tumor vasculature of 6 malignant tumors of nonvascular origin. Microvascular immunoreactivity for all 4 markers was observed in placental chorionic villi, but was absent in microvessels of normal skin and subcutis. Brain microvessels expressed only GLUT1 and merosin.

Conclusions: A distinct constellation of tissue-specific markers is uniquely coexpressed by hemangiomas and placental microvessels. These findings imply a unique relationship between hemangioma and the placenta and suggest new hypotheses concerning the origin of these tumors.

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From the Departments of Pathology (Drs North and Mrak and Mr Mizeracki), Otolaryngology (Drs Waner and Suen and Mr Mizeracki), Orthopedic Surgery (Dr Nicholas), and Dermatology (Dr Kincannon), University of Arkansas for Medical Sciences and Arkansas Children's Hospital, and the Department of Veterans Affairs Medical Center, Little Rock (Dr Mrak); and the Department of Pathology, Harvard University Medical School, and Massachusetts General Hospital, Boston (Dr Mihm).
MATERIALS AND METHODS

SPECIMENS

All vascular lesions excised at Arkansas Children’s Hospital, Little Rock, during the past 3 years (1997-1999), for which both frozen and paraffin-embedded tissue samples were available (111 cases), were reviewed. Hematoxylin-eosin-stained sections were reviewed blindly by 2 of us (P.E.N. and M.C.M.), and pathological diagnoses made independently.1,8 Two intracranial and 4 intramuscular lesions (all malformations) were excluded, leaving 66 juvenile hemangiomas, 26 malformations, and 13 pyogenic granulomas, with greater than 95% diagnostic concordance between reviewers. Diagnostic discrepancies, all minor, were resolved by joint review. Twenty-eight additional cases, for which only paraffin sections were available, were also included: 6 tufted angiomas, 7 epithelioid hemangioendotheliomas, 1 infantile kaposiform hemangioendothelioma, and 14 angiosarcomas. Clinical information was obtained by chart review after immunohistochemical analysis.

Samples of fresh human term placenta were taken within 30 minutes of delivery from central villus parenchyma. Samples of fresh human brain, spinal cord, and truncal skin were collected from 4 patients (aged 1-15 years) at autopsy within 4 to 8 hours post mortem. Four additional samples of normal skin were taken from margins of resected pediatric skin lesions of nonvascular origin. As examples of normal, reactive capillary proliferation, granulation tissue was collected from 1 healing surgical wound, 2 decubitus, and 1 case of ulcerative colitis. Six malignant pediatric tumors of nonvascular origin (to evaluate tumor neovascularization) included renal medullary carcinoma (n=1), desmoplastic small blue cell tumor (n=1), infantile capillary hemangioendothelioma, and 14 angiosarcomas. Clinical information was obtained by chart review after immunohistochemical analysis. In acetone at 20°C for 1 minute, and air-dried prior to rehydration in Tris-buffered isotonic sodium chloride solution containing 0.05% Triton X-100. Paraffin sections were deparaffinized, rehydrated, and subjected to citrate buffer antigen retrieval; all sections were protein-blocked before incubation with primary antibodies under optimal conditions (Table 1). Bound primary antibody was detected using a peroxidase kit (LSAB+; Dako Corporation, Carpinteria, Calif) using diaminobutyric acid chromagen (DAB+, Dako). Negative controls were processed in parallel without primary antibody. Normal tissue immunoreactivities provided internal positive controls, except for LeY, for which sections of LeY-immunopositive oral mucosa were included in each run.

SCORING OF IMMUNOREACTIVITY

Immunoreactivities were scored blindly by one of us (P.E.N.) as none, weak, moderate, or intense (intense meaning greater than or equal to control immunoreactivity). For LeY, occasional weak immunoreactivity in a perinuclear, hof-type pattern was discounted. Only membranous and/or diffuse cytoplasmic-membranous LeY immunoreactivity was scored as positive. For merosin, occasional immunoreactivity in a discontinuous “stringy” pattern in connective tissue around larger veins and arteries in normal skin and subcutis and malformations, was discounted. Only circumferential, basement membrane–like patterns of merosin (and laminin) immunoreactivity were scored as positive.

IMMUNOHISTOCHEMISTRY

Cryosections were collected at −20°C on sialanized slides, fixed in acetone at 20°C for 1 minute, and air-dried prior to rehydration in Tris-buffered isotonic sodium chloride solution containing 0.05% Triton X-100. Paraffin sections were deparaffinized, rehydrated, and subjected to citrate buffer antigen retrieval; all sections were protein-blocked before incubation with primary antibodies under optimal conditions (Table 1). Bound primary antibody was detected using a peroxidase kit (LSAB+; Dako Corporation, Carpinteria, Calif) using diaminobutyric acid chromagen (DAB+, Dako). Negative controls were processed in parallel without primary antibody. Normal tissue immunoreactivities provided internal positive controls, except for LeY, for which sections of LeY-immunopositive oral mucosa were included in each run.

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RESULTS

PATIENTS

Patients (aged 22 days to 7 years) with both proliferative and involutive phase hemangiomas were well represented, with sex and site distributions similar to those reported in large epidemiological studies15,16 (Table 2). Age ranges for patients with other vascular lesions (excluding angiosarcoma) overlapped that of the hemangioma group.

GLUTI IMMUNOREACTIVITY

Specific microvascular GLUT1 immunoreaction was present in normal brain and placental chorionic villi (and placental trophoblast), but not in normal skin and subcutis, granulation tissue, or neovascularization of nonvascular malignant tumors (Table 2, Figure 2B and H, Figure 3B, and Figure 4B). Intense microvascular, entirely endothelial, GLUT1 immunoreactivity was present in all juvenile hemangiomas tested (Table 2, Figure 4, Figure 5A). No leisonal GLUT1 expression was detected in any of the malformations, pyogenic granulomas, tufted angiomas, or hemangioendotheliomas (Table 2, Figure 6). Weak-to-moderate, focal GLUT1 immunoreaction was present in 5 of 14 angiosarcomas (Table 2, Figure 6K).

LeY and FcγRII IMMUNOREACTIVITY

Vascular immunoreaction for LeY or FcγRII was not present in normal skin and subcutis or brain (except for...
Figure 1. The clinical spectrum of juvenile hemangioma. A, This 18-month-old child’s small cervical hemangioma required no intervention and eventually involuted without noticeable residuum. B, This infant’s periorbital hemangioma, by impinging on her visual axis, placed her at risk for development of amblyopia. Her bulging lesion was largely subcutaneous, but contiguous skin involvement imparted a red surface coloration. C, This 4-month-old infant’s extensive facial and scalp hemangioma infiltrated the eyelids of one eye and threatened damage to nasal and aural cartilage. Note the ulceration of the lower lip, a common complication that usually results in scarring. D, This 3-year-old girl was left with a cosmetically significant atrophic scar despite complete involution of her lower eyelid and cheek hemangioma.

Table 1. Antibodies Used for Immunohistochemical Analysis

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antigen Recognized</th>
<th>Supplier†</th>
<th>Type</th>
<th>Tissue Section</th>
<th>Dilution and Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYM</td>
<td>12 Amino acid carboxyl terminal of human GLUT1</td>
<td>DAKO</td>
<td>Polyclonal rabbit</td>
<td>Paraffin</td>
<td>1:500; 30 min (RT)</td>
<td>9</td>
</tr>
<tr>
<td>BM-1</td>
<td>Lewis Y antigen</td>
<td>DAKO</td>
<td>Monoclonal mouse, IgM</td>
<td>Paraffin</td>
<td>1:100; 30 min (RT)</td>
<td>10</td>
</tr>
<tr>
<td>JC70A</td>
<td>CD31 (PECAM)</td>
<td>DAKO</td>
<td>Monoclonal mouse, IgG1</td>
<td>Paraffin</td>
<td>1:100; Overnight (4°C)</td>
<td>11</td>
</tr>
<tr>
<td>KB61</td>
<td>FcγRII (CD32)</td>
<td>DAKO</td>
<td>Monoclonal mouse, IgG1</td>
<td>Frozen</td>
<td>1:500; 30 min (RT)</td>
<td>12</td>
</tr>
<tr>
<td>4C7</td>
<td>Terminal globular domain of human laminin (α1-laminin)</td>
<td>DAKO</td>
<td>Monoclonal mouse, IgG2a</td>
<td>Frozen</td>
<td>1:100; 30 min (RT)</td>
<td>13</td>
</tr>
<tr>
<td>SH2</td>
<td>80-kd Fragment of human merosin (α2-laminin) chain</td>
<td>Chemicon</td>
<td>Monoclonal mouse, IgG1</td>
<td>Frozen</td>
<td>1:1000; 30 min (RT)</td>
<td>13</td>
</tr>
<tr>
<td>PAL-E</td>
<td>Endothelium-specific pinocytotic vesicle antigen</td>
<td>Biomeda</td>
<td>Monoclonal mouse, IgG2a</td>
<td>Frozen</td>
<td>1:1000; 30 min (RT)</td>
<td>14</td>
</tr>
</tbody>
</table>

*PECAM indicates platelet-endothelial cell adhesion molecule; RT, room temperature.
†DAKO Corporation, Carpinteria, Calif; Chemicon International, Inc, Temecula, Calif; and Biomeda Corporation, Foster City, Calif.
perivascular macrophages and microglia17 (Figure 2). Placental intravillous fetal capillaries showed intense endothelial immunoreactivity for LeY and FcγRII (Figure 4). Epithelia of normal and marginal skin showed variable immunoreactivity for LeY (but not for FcγRII), ranging from none (approximately 25% of cases) to moderate (in 10%). Strong LeY immunoreactivity within oral glandular and mucosal epithelia served as positive controls. Patient blood type did not affect LeY immunoreactivity (data not shown). Skin FcγRII immunoreactivity was restricted to dermal macrophages, epidermal Langerhans cells, and inflammatory cells.

Marked lesional endothelial immunoreactivity for FcγRII and LeY, in a diffuse, granular cytoplasmic-membranous pattern, similar to that seen in placenta, was present in all 66 juvenile hemangiomas (Table 2, Figures 4 and 5). No LeY or FcγRII immunoreactivity was found in adjacent native dermal capillaries, or in arterioles or arteries, either within or adjacent to hemangiomas. There was no lesional immunoreaction for LeY or FcγRII in any of the malformations, pyogenic granulomas, granulation tissues, or tumor neovasculatures (Table 2, Figure 3). There was no LeY immunoreactivity in tufted angioima or the kaposiform hemangioendothelioma, and only weak-to-moderate LeY immunoreactivity (in 2%-5% of tumor cells) in 2 of 7 epithelioid hemangioendotheliomas, which showed focal, weak to moderate immunoreactivity for GLUT1 and/or Lewis Y antigen (LeY), involving 2% to 5% of tumor cells, in epithelioid hemangioendotheliomas and 5 of 14 angiosarcomas (Table 2, Figure 6). FcγRII immunoreactivity could not be assessed in these latter lesions, for which frozen material was unavailable.

**MEROSIN AND LAMININ**

Strong merosin (α2-laminin) immunoreactivity was seen in vascular basement membranes of brain and placental chorionic villi but not in the vasculature of normal skin

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**Table 2. Vascular Lesional Immunoreactivities and Patient Characteristics**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Specimens</th>
<th>Patient Age Range</th>
<th>Sex Ratio, F:M</th>
<th>Location of Lesion, HN/T/UE/LE %</th>
<th>Vascular Immunoreactivities, No. Positive/Total No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile hemangioma</td>
<td>2</td>
<td>22 d. 3 mo</td>
<td>2:0</td>
<td>100/0/0/0</td>
<td>GLUT1: 2/2, LeY: 2/2, FcγRII: 2/2, Merosin: 2/2</td>
</tr>
<tr>
<td>Early proliferative</td>
<td>27</td>
<td>4-12 mo</td>
<td>3.5:1</td>
<td>89/11/0/0</td>
<td></td>
</tr>
<tr>
<td>Mid-proliferative</td>
<td>12</td>
<td>13-14 mo</td>
<td>1.4:1</td>
<td>83/17/0/0</td>
<td></td>
</tr>
<tr>
<td>Early involutive</td>
<td>19</td>
<td>25-48 mo</td>
<td>2.2:1</td>
<td>95/5/0/0</td>
<td></td>
</tr>
<tr>
<td>End stage</td>
<td>6</td>
<td>51 mo to 7.2 y</td>
<td>6:0</td>
<td>83/0/17/0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>66§</td>
<td>22 d to 7.2 y</td>
<td>2.9:1</td>
<td>89/9/2/0</td>
<td></td>
</tr>
<tr>
<td>Malformations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous</td>
<td>15</td>
<td>16 mo to 36 y</td>
<td>2:1</td>
<td>80/0/13/7</td>
<td>15/15, 15/0/15, 15/0/15, 15/0/15, 15/0/15</td>
</tr>
<tr>
<td>Arteriovenous</td>
<td>4</td>
<td>8-23 y</td>
<td>1.3</td>
<td>75/0/25/0</td>
<td>4/4, 4/4, 4/4</td>
</tr>
<tr>
<td>Lymphatic</td>
<td>2</td>
<td>5-8 y</td>
<td>0.2</td>
<td>100/0/0/0</td>
<td>2/2, 2/0, 2/2</td>
</tr>
<tr>
<td>Mixed venous-lymphatic‡</td>
<td>3</td>
<td>25 mo to 5.5 y</td>
<td>2:1</td>
<td>0/33/67/0</td>
<td>3/3, 3/3, 3/3</td>
</tr>
<tr>
<td>Venulocapillary (port-wine stain)‡</td>
<td>2</td>
<td>14-32 y</td>
<td>1:1</td>
<td>100/0/0/0</td>
<td>2/2, 2/2, 2/2</td>
</tr>
<tr>
<td>Total</td>
<td>26‖</td>
<td>16 mo to 36 y</td>
<td>1.2:1</td>
<td>73/0/15/12</td>
<td>26/26, 26/26, 26/26, 26/26</td>
</tr>
<tr>
<td>Pyogenic granulomas (lobular capillary hemangiomas)</td>
<td>12</td>
<td>4 mo to 16 y</td>
<td>0.3:1</td>
<td>58/33/9/0</td>
<td>12/12, 12/12, 12/12, 12/12</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>1</td>
<td>9 y</td>
<td>0.1</td>
<td>100/0/0/0</td>
<td>1/1, 1/1, 1/1</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>4 mo to 16 y</td>
<td>0.3:1</td>
<td>61/9/0/0</td>
<td>13/13, 13/13, 13/13</td>
</tr>
<tr>
<td>Granulation tissue</td>
<td>4</td>
<td>2-23 y</td>
<td>1:1</td>
<td>25/50/0/25</td>
<td>4/4, 4/4, 4/4</td>
</tr>
<tr>
<td>Tumor neovasculature</td>
<td>6</td>
<td>2-25 y</td>
<td>0.8:1</td>
<td>0/33/17/0</td>
<td>6/6, 6/6, 6/6</td>
</tr>
<tr>
<td>Tufted angioma</td>
<td>6</td>
<td>1 mo to 13 y</td>
<td>1:2</td>
<td>50/17/17/0</td>
<td>6/6, 6/6, NA, NA</td>
</tr>
<tr>
<td>Infantile kaposiform</td>
<td>1</td>
<td>4 mo</td>
<td>1:0</td>
<td>100/0/0/0</td>
<td>0/1, 0/1, NA, NA</td>
</tr>
<tr>
<td>hemangioendothelioma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelioid hemangioendothelioma</td>
<td>7</td>
<td>8 to 17 y</td>
<td>3:4</td>
<td>0/57/14/9</td>
<td>7/27 (Focal), NA, NA</td>
</tr>
<tr>
<td>Angiosarcoma</td>
<td>14§</td>
<td>16-82 y</td>
<td>1:1</td>
<td>71/22/0/7</td>
<td>5/14 (Focal), 5/14 (Focal), NA, NA</td>
</tr>
</tbody>
</table>

*HN/T/UE/LE% indicates the respective percentages of specimens located on the head and neck, trunk, upper extremities, and lower extremities, respectively. All cases of juvenile hemangiomas, malformations, pyogenic granulomas, tufted angiomas, and the infantile kaposiform hemangioendothelioma were lesions of skin and/or subcutis; 2 of these (1 tufted angioma and the kaposiform tumor) also involved deep skeletal muscle. Epithelioid hemangioendotheliomas involved bone (4 cases) and liver (3 cases). Angiosarcomas involved skin (7 cases), salivary gland (1 case), nasal sinuses (3 cases), liver (1 case), pericardium (1 case), and bone (2 cases).

†All immunopositive cases showed intense immunoreactivity in more than 50% of lesional capillaries, except for angiosarcomas and epithelioid hemangioendotheliomas, which showed focal, weak to moderate immunoreactivity for GLUT1 and/or Lewis Y antigen (LeY), involving 2%-5% of tumor cells, in some cases as shown. PAL-E, CD31, and laminin-positive immunoreactions confirmed preserved endothelial and basement membrane antigenicity in all lesional and control tissue specimens (see the “Results” section). NA indicates not available because of lack of frozen tissue samples.

‡All 3 patients with mixed venous-lymphatic malformations had Klippel-Trenaunay-Weber syndrome; 1 of 2 patients with port-wine stain had Sturge-Weber syndrome.

§Sixty-six juvenile hemangiomas (65 first-time resections and 1 staged) from 64 patients (2 with 2 noncontiguous lesions resected). Of these 64 patients, 7 (11%) had multiple hemangiomas by clinical history (1 with radiological evidence of liver involvement) and 31 (48%) had received prior laser and/or corticosteroid therapy.

‖Sixteen of the malformations (62%) were first-time resections.

*Fourteen angiosarcomas from 12 patients, including 12 primary tumors and 2 metastases.
and subcutis, as reported previously18-21 (Figures 2 and 4). Merosin was highly expressed in nonvascular basement membranes of trophoblast, nerve, skeletal muscle, and dermal-epidermal junctions.

α-Laminin (“classic” laminin), in contrast, decorated all vascular and nonvascular basement membranes (Figures 2 and 4).

Intense merosin immunoreactivity was present in all juvenile hemangiomas, in a broad, continuous, band-like pattern encircling lesional capillaries (Table 2, Figures 4 and 5). Dermal capillaries of normal marginal skin, as well as intralional and extralional arterioles, were immunonegative for merosin, although occasional weak-to-moderate immunoreaction was seen in arterial internal elastic laminae. α-Laminin immunoreactivity was present in all basement membranes, including basement membranes of lesional capillaries and adjacent normal large and small vessels.

No lesional merosin immunoreactivity was present in malformations, pyogenic granulomas, granulation tissue, or tumor neovasculature (Table 2, Figure 3). Intense α-laminin immunoreactivity was present in all lesional and nonlesional vascular and epithelial basement membranes in these specimens (Figure 3). Frozen tissue was not available for tufted angiomas, hemangiendotheliomas, or angiosarcomas, precluding assessment of merosin or laminin immunoreactivity.

**PAL-E AND CD31**

Moderate-to-strong endothelial immunoreactivity for the endothelium-specific, pinocytotic vesicle-associated antigen PAL-E was observed in all specimens with available frozen tissue, except for normal brain, confirming preservation of endothelial antigenicity and findings of previous studies.14,22 Endothelial CD31 immunoreactivity, used to assess antigenicity in paraffin sections, was present in all cases.

**ELECTRON MICROSCOPY**

Juvenile hemangiomas (patients aged 22 days to 3 years) and placental microvessels shared ultrastructural features of (1) continuous, nonfenestrated endothelia with occasional uniform, small, subplasmalemmal vesicles; (2) small numbers of closely apposed, encircling pericytes; and (3) continuous, redundant basement membrane, varying in thickness, adjacent to or encircling interspersed collagen fibrils (Figure 7). Early hemangioma endothelia were plump, whereas those of older (involuting) lesions and of term placental capillaries were relatively thin. Basement membrane multilamination was more developed in hemangiomas of older children.

**COMMENT**

We report a constellation of shared markers of cellular specialization that imply a unique relationship between juvenile hemangioma and placental fetal microvessels. These findings characterize juvenile hemangioma as a distinct pathological entity, intrinsically different from other vascular tumors and anomalies to which it has been compared. In light of the perinatal or congenital presentation...
Figure 3. Vascular immunoreactivities of reactive vascular proliferations (granulation tissue and pyogenic granuloma) and tumor neovasculature. Microvessels of ischial decubitus ulcer granulation tissue (A-F), of an eruptive pyogenic granuloma from the cheek of an 8-year-old girl (G-L), and of a renal medullary carcinoma metastatic to ovary (M-R) were immunonegative for GLUT1 (B; H; and N, solid arrow), Lewis Y antigen (LeY) (C; I; and Q, solid arrow), FcγRII (D; J; and P, arrow), and merosin (E; K; and Q, arrow). There was normal antigenicity for laminin in vascular basement membranes (C, L, R), for merosin in epidermal basement membranes (K, arrow), for CD31 in endothelia (not shown), for GLUT1 in erythrocytes (B, arrows; and H), and in keratinocytes (H), and for FcγRII in tissue macrophages (D and J, arrows). Carcinoma cells were focally immunopositive for GLUT1 (N, open arrows) and LeY (Q, open arrow). H&E indicates hematoxylin-eosin. Original magnifications ×200 (C-E, G-Q, R) or ×400 (A, B, F, P).
Figure 4. Lesional immunoreactivities in full-term placenta and in proliferative and involutive phase juvenile hemangiomas. Intravillous fetal microvessels of human full-term placenta (A-F) and lesional capillaries of proliferative (G-L) and involutive (M-R) phase hemangiomas showed strong endothelial immunoreaction for GLUT1 (B, solid arrows; H, double arrows; and N, arrow), for Lewis Y antigen (LeY) (C, I, and O, arrows), for FcγRII (D, arrows; J, arrowheads; and P, arrow), and for merosin (E, K, and Q, solid arrows). There was also GLUT1 immunopositivity in syncytiotrophoblasts lining chorionic villi (B, open arrow) and dermal perineurium (H, single arrow); and strong merosin immunopositivity in trophoblastic basement membranes (E, open arrow) and dermal periarterial nerve twigs (Q, arrowheads). Native capillaries at the normal margins of hemangiomas were immunonegative for all 4 markers, as shown for FcγRII in panel J (single arrow). There was normal antigenicity for laminin in the basement membranes of all capillaries, dermis, and smooth muscle (F, solid arrow; L, R); and of trophoblast (F, open arrow). H&E indicates hematoxylin-eosin. Original magnifications ×200 (A, D, G-R) or ×400 (B, C, E, F).
of hemangiomas and their distinctive pattern of limited growth and involution, the antigenic similarities between these lesions and placental microvessels led us to hypothesize 2 possible pathogenic mechanisms: (1) an origin from invading angioblasts that aberrantly differentiate toward the placental microvascular phenotype in the mesenchyme of skin and subcutis or (2) an origin from embolized placental cells.

The first hypothesis proposes colonization of receptive (possibly abnormal) mesenchyme by angioblasts aberrantly “switched” toward the placental endothelial phenotype by genomic alterations or abnormal inductive influences. These initially dormant cells, perhaps reflected in the blush or blanched spot of nascent hemangiomas, might be driven to proliferate on loss from the fetal circulation, coincident with late gestation or birth, or in response to emerging positive angiogenic influences in the perinatal period. Alternatively, as delineated by our second hypothesis, embolic placental endothelial cells, already committed to the GLUT1/merosin/LeY/FcγRII phenotype, might reach fetal tissues from chorionic villi through right-to-left shunts characteristic of the normal fetal circulation. Because intravascular shedding of placental cells is likely increased by placental injury, this hypothesis could explain the heightened incidence of hemangiomas seen following chorionic villus sampling, as well as the observation that intentional placental trauma, produced during embryoscopy prior to elective pregnancy termination, results in rapid development of fetal ecchymotic lesions.

These hypotheses invite consideration of angiogenic control mechanisms specifically manifest in utero to explain the explosive perinatal growth phase of hemangiomas. Whereas profound angiogenesis occurs within placental villi until near term, little angiogenesis occurs in maternal tissues or in fetal tissues after organogenesis. Factors that may orchestrate this control include vascular endothelial growth factor (VEGF), placental growth factor (PIGF, a placenta-derived structural homologue of VEGF), Flt-1 (a high-affinity transmembrane receptor for both PIGF and VEGF), and sFlt-1 (a placenta-derived, truncated, soluble form of Flt-1 produced by alternative splicing). Both PIGF and VEGF induce angiogenesis in vivo and induce mitotic activity in cultured endothelial cells. sFlt-1 is released into maternal blood and amniotic fluid during gestation, and may, as a competitive sink for both PIGF and VEGF, protect maternal and fetal tissues from overvascularization. Loss of these and other placental factors at birth is likely to dramatically alter the balance of negative and positive angiogenic factors in the fetus, possibly allowing unrestrained proliferation of embolized or aberrant rests of cells expressing the placental endothelial phenotype.
idea recalls the suggestion of Boon et al.\textsuperscript{32} that hemangioma growth might correlate with decreased levels of trophoblast-derived interferon. The limited period of growth and subsequent involution of hemangiomas could reflect a programmed limit to mitosis in placental-type endothelial cells, appropriate to the 9-month life span of human placenta, or perhaps vascular stabilization imposed by maturing pericytic-endothelial associations and basement membranes.

Hypotheses concerning the pathogenesis of juvenile hemangiomas must also address the strong prediction of these lesions for skin and subcutis (rarely visceras), and for the head.\textsuperscript{15,16} Tissue- and region-specific mesenchyme receptive for angioblasts or embolic placental cells might reasonably be invoked. Head mesenchyme is derived from neural crest,\textsuperscript{33} and, unlike the mesoderm-derived mesenchyme found elsewhere, does not show intrinsic angioblast development, relying instead on invasion by exogenous, migrating angioblasts and vascular sprouts from surrounding tissues for vascularization.\textsuperscript{34} Angioblast migration in chick-quail chimeras is unusually prominent in the head, suggesting heightened production of motility or chemotactic factors in that region.\textsuperscript{35}

The vascular antigens co-expressed by juvenile hemangioma and placenta are probably best regarded as markers of cellular specialization, rather than of cellular proliferation or immaturity, since they persist in late-stage hemangiomas and are not associated with other forms of neovascularization. Ultrastructural studies reported herein and by others\textsuperscript{56} support this contention by demonstrating well-developed basement membrane structure and pericytic-endothelial associations, consistent with vascular stability,\textsuperscript{37} in both proliferative- and involutive-phase hemangiomas. GLUT1 is widely expressed early in embryonic development, but fetal endothelial GLUT1 expression rapidly disappears except in microvessels of developing neuroepithelial tissues, where expression increases.\textsuperscript{3,38,39} In mature brain, endothelial zonula occludens–type junctions, a component of the blood-brain barrier, necessitate high GLUT1 expression for adequate blood-brain glucose transport.\textsuperscript{35} In placenta, GLUT1 expression is required throughout gestation for adequate maternal-fetal glucose transfer,\textsuperscript{4,41} and is independent of developmental stage.\textsuperscript{42} High GLUT1 expression by juvenile hemangiomas may permit tumor growth by providing fuel for mitosis.

Merosin (\(\alpha_2\)-laminin) expression has been previously reported for 2 (of 3) juvenile hemangiomas.\textsuperscript{40} Our results show universal expression of merosin in a large series of GLUT1-positive hemangiomas representing all stages of proliferation and involution and a wide spec-

### Table

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<th>Lesional Immunoreactivities in Tufted Angiomas, Infantile Kaposiform and Epithelioid Hemangiendothelias, and Angiosarcomas</th>
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<td><strong>Tufted Angioma</strong></td>
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**Figure 6.** Lesional immunoreactivities in tufted angiomas, infantile kaposiform and epithelioid hemangiendothelias, and angiosarcomas. A-C, Tufted angioma from the neck of a 13-year-old boy with a 4-month history of violaceous papules arising at the site of a congenital “birthmark” that had faded, shown by hematoxylin-eosin (H&E) staining (A), and by immunoreaction for GLUT1 (B) and Lewis Y antigen (LeY) (C). Review of the previous “birthmark,” having undergone biopsy when the patient was 13 months old and originally diagnosed as “hemangiendotheloma with pericytic component,” showed similar dermal involvement by tufts of proliferating capillaries in a distinct “cannonball” distribution. Note the lack of immunoreactivity within the capillary tufts for both GLUT1 and LeY (B and C, respectively). Positive internal controls for GLUT1 were provided by adjacent perineurium (B, arrow) and intravascular erythrocytes (B).

D-F, Infantile kaposiform hemangiendotheloma (IH-KHE) from the head and neck of a male infant who died of Kasabach-Merritt syndrome at 6 months of age, shown by H&E staining (D) and by immunoreaction for GLUT1 (E) and LeY (F). Note the characteristic slitlike spaces containing erythrocytes and lined by spindled cells, in association with abnormal, dilated lymphatic spaces (D). Intravascular erythrocytes (E, arrow), but not tumor cells, were GLUT1 immunoreactive. Tumor cells were also LeY immunonegative (F). G-I, Epithelioid hemangiendotheloma (HE) resected from the liver of a 1-month-old girl, shown by H&E staining (G) and by immunoreaction for GLUT1 (H) and LeY (I). This example demonstrated no lesional immunoreactivity for GLUT1 (H) or LeY (I), although focal colonies of extramedullary hematopoietic cells (H, arrow) and mature erythrocytes were intensely GLUT1 immunoreactive. Some epithelioid hemangiendothelomas (2 of 7 tested) showed focal lesional LeY (but not GLUT1) immunoreactivity in less than 5% of tumor cells (see Table 2). J-L, Angiosarcoma from the maxillary sinus of a 62-year-old man by H&E staining (J) and by immunoreaction for GLUT1 (K) and LeY (L). Focal tumor cell immunoreactivity for GLUT1 (K, arrow) and LeY (L, arrows) was present in this particular angiosarcoma, as was the case in 5 of the 14 angiosarcomas tested (see Table 2). Original magnifications \(\times 400\) (B, C, F, H, K, L). Frozen tissue was not available for these lesional types, precluding immunoreactions for merosin, laminin, or \(\beta_2\)-RRI.
trum of patient ages. Vascular basement membrane merosin expression is normally restricted to the nervous system and eye and placenta. The merosin (α2) chain, 1 of at least 10 genetically distinct laminin chains, is also present in certain nonvascular basement membranes, including those of placental trophoblast, Schwann cells, striated muscle, and skin, and is abundant in the mesangial matrix of mature renal glomeruli. The known heterogeneity of laminin subtypes in basement membranes and the highly restricted expression of merosin suggest tissue-specific and developmental stage–specific functional diversity. Merosin is essential, for instance, for stability and survival of fused myoblasts in vitro, apparently due to apoptosis inhibition, and merosin deficiency results in severe muscular dystrophy. This suggests that merosin might facilitate growth of hemangiomas by inhibiting apoptosis.

The selective, persistent expression of LeY and FcγRII we find in endothelia of hemangiomas and placental vessels is of unclear functional significance. LeY is a difu-
cosylated type 2 chain oligosaccharide expressed on cell surfaces in a tissue- and stage-specific manner. Changing glycosylation patterns, mediated by differential display of LeY and other specific cell surface oligosaccharides, appear to be important in cell-cell recognition and adhesion during development and have been associated with malignant transformation and progression.49,51 Expression of LeY correlates with apoptosis in some studies52,53 (but not others54); the constant LeY expression by hemangiomas throughout proliferation and involution reported herein does not support a close association with apoptosis in these lesions. FcγRII is a low-affinity Fc receptor that is normally expressed on macrophages, Langerhans cells, platelets, and various leukocytes12,55 and binds only aggregated IgG. Normal endothelial FcγRII expression is limited to placenta56 and hepatic sinusoidal lining cells (possibly Kupffer cells).57 This suggests that endothelial FcγRII expression may be uniquely advantageous in placenta, perhaps assisting clearance of immune complexes and/or maternal-fetal IgG transport.12,56,58 Its potential effect on the natural biology of hemangiomas remains obscure.

In summary, we report a distinct immunophenotypic pattern consisting of 4 functionally unrelated markers of cellular specialization uniquely coexpressed by fetal microvessels of human placenta and juvenile hemangiomas at all stages. We present 2 hypotheses that might explain the shared molecular differentiation of placental vessels and juvenile hemangiomas, and that might explain some of the behaviors and known associations of the latter common, sometimes devastating, lesions. These findings suggest possible new preventive strategies and therapeutic avenues, in particular the pharmacological use of angiogenic modulators that may normally decrease in the fetal circulation on separation from the placenta at birth.

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Corresponding author and reprints: Paula E. North, MD, PhD, Department of Pediatric Pathology, Arkansas Children’s Hospital, 800 Marshall St, Little Rock, AR 72202.

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