Vascular Biology, Atherosclerosis and Endothelium Biology

Myeloid Cells in Infantile Hemangioma

Matthew R. Ritter,* John Reinisch,[†]
Sheila Fallon Friedlander,[‡] and
Martin Friedlander*

From the Department of Cell Biology, The Scripps Research
Institute, La Jolla; the Department of Plastic Surgery,[†] Children's
Hospital Los Angeles, Los Angeles; and the Division of Pediatric
Dermatology,[‡] Children's Hospital San Diego,
San Diego, California*

Little is known about the pathogenesis of infantile hemangiomas despite the fact that they are relatively common tumors. These benign neoplasms occur in as many as 1 in 10 births, and although rarely life threatening, hemangiomas can pose serious concerns to the cosmetic and psychosocial development of the afflicted child. Ulceration, scarring, and disfigurement are significant problems as are encroachment of the ear and eye, which can threaten hearing and vision. The precise mechanisms controlling the rapid growth observed in the first months of life and the spontaneous involution that follows throughout the course of years remain unknown. In this report we demonstrate the presence of large numbers of hematopoietic cells of the myeloid lineage in proliferating hemangiomas and propose a mechanism for the observed evolution of these lesions that is triggered by hypoxia and involves the participation of myeloid cells. We report the results of experiments using myeloid markers (CD83, CD32, CD14, CD15) that unexpectedly co-labeled hemangioma endothelial cells, providing new evidence that these cells are distinct from normal endothelium. (*Am J Pathol* 2006, 168:621–628; DOI: 10.2353/ajpath.2006.050618)

Hemangioma, also known as infantile hemangioma or hemangioma of infancy, is a relatively common benign tumor. The majority of lesions remain small birthmarks with no serious concerns. However, a fraction of hemangiomas grow to sizes, and in locations, that can be problematic. These lesions appear perinatally, can grow rapidly for months, and regress usually throughout the course of months to years.¹ They often arise in the skin with a large proportion occurring in the head and neck areas. Corticosteroids can be effective at accelerating involution, but the mechanism of this effect is unknown.

These tumors are of interest biologically because of their unusual cellular composition and their predictable course of evolution. Cells with endothelial characteristics comprise a large portion of hemangiomas, making the study of these tumors relevant to the field of vascular biology, particularly because rapid endothelial cell growth is followed by spontaneous regression, providing possible insight into mechanisms regulating angiogenesis and neovascular disease.

We previously identified insulin-like growth factor 2 (IGF2) as a potential mediator of hemangioma growth and demonstrated high expression of angiogenesis-related integrins $\alpha v \beta 3$ and $\alpha 5 \beta 1$ on hemangioma endothelium.² Mast cells have been proposed to have a role in hemangioma progression³ as have endothelial progenitor cells.⁴ Hemangioma endothelial cells were shown to express the lymphatic endothelial marker LYVE-1, suggesting that these cells are arrested in an early vascular differentiation state.⁵ The nonrandom distribution of facial hemangiomas has raised ideas relating to developmental patterning and the deposition of precursor cells.^{6,7} Hemangioma tissue shows evidence of clonality, suggesting that these tumors are derived from a single progenitor cell,^{8,9} although limited sampling of the lesions may indicate that isolated regions are in fact clonal. Despite these advances, a clear understanding of the causes of hemangioma growth remains elusive, and the mechanisms of involution are even less well characterized.

All hematopoietic cells originate in the bone marrow and are derived from a pluripotent hematopoietic stem cell. Two major lineages then diverge in the bone marrow into the lymphoid and myeloid lineages. The common lymphoid progenitor differentiates into B and T cells and the common myeloid progenitor generates the granulocyte/macrophage progenitor. All steps to this point occur in the bone marrow; subsequently in the blood, the myeloid lineage splits into several different cell types collectively known as the polymorphonuclear leukocytes, which includes monocytes. Monocytes exit the peripheral cir-

Supported by the National Eye Institute (grant R01 EY11254 to M.F. and the Kirschstein National Research Service Award F32 EY13916 to M.R.R.) and the Skaggs Scholar in Clinical Science program (to S.F.F.).

Accepted for publication September 26, 2005.

Address reprint requests to Martin Friedlander, The Scripps Research Institute, 10550 North Torrey Pines Rd., MB28, La Jolla, CA 92037. E-mail: friedlan@scripps.edu.

culatation, enter tissues, and differentiate into several cell types with varying functions. Macrophages, the major tissue-resident phagocytic cells of the innate immune system, are derived from monocytes. Macrophages participate in angiogenesis primarily through secretion of proangiogenic growth factors such as vascular endothelial growth factor and fibroblast growth factor-2.^{10,11} Monocytes can also differentiate into immature dendritic cells that enter the tissues and process antigens.¹² When a pathogen is encountered, dendritic cells mature and migrate to lymphoid tissue where they activate antigen-specific T cells. Mast cells also belong to the myeloid lineage and are thought to have roles in orchestrating defense against parasites and recruiting other inflammatory cells.

In this study we demonstrate the widespread presence of myeloid cells in proliferating hemangiomas and suggest a mechanism for myeloid cell-facilitated hemangioma growth involving hypoxia-induced expression of growth factors that drive endothelial proliferation. Other evidence demonstrates similarities between hemangioma endothelial cells and those of placental vessels, which suggests a placental origin and illustrates the unusual characteristics of hemangioma endothelial cells. We also demonstrate that hemangioma endothelial cells co-express myeloid markers, providing another characteristic that serves to distinguish these cells from normal endothelium. These co-expressing cells are not found in other vascular lesions with significant endothelial components, including lymphatic malformation, pyogenic granuloma, or arteriovenous malformation.

Materials and Methods

Specimens

Hemangioma tissue was collected by surgical resection of tumors from children being treated at Children's Hospital San Diego. All specimens were obtained from tissue that would have otherwise been discarded. Procedures conformed to National Institutes of Health guidelines regarding use of human patients and received institutional review board approval from participating institutions. Each case was initially clinically classified as proliferating, plateau, or involuting based on the clinical appearance, history of the lesion, and the age of the patient. Evaluated specimens from involuting cases were then subclassified as early or late involuting using cellular density, measured via 4,6-diamidino-2-phenylindole (DAPI) labeling of cell nuclei, as an additional criterion to stage lesions. Involuting specimens with relatively high density of lesional cells were classified as early involuting whereas those with low densities were termed late involuting. The terms used to describe the lesions in this study were based primarily on histological analysis of specimens.

Figure 1. Hematopoietic cells are abundant in proliferating hemangioma and decrease during involution. **a–c:** Three independent cases of the indicated ages are shown labeled with an antibody against CD45 (leukocyte common antigen), which is expressed on all cells of hematopoietic origin except erythrocytes. Hematopoietic cells (red) are found throughout the lesions. Nuclei are stained blue. **Inset** shows negative labeling with an isotype-matched control antibody. Scale bar, 100 μ m.

Immunohistochemistry

Thirty hemangioma samples were evaluated. Tissue was placed in cold 20% sucrose/phosphate-buffered saline

immediately after resection. Tissue was then embedded in OCT compound, frozen, and sectioned. After blocking, sections were incubated overnight with primary antibody followed by incubation with fluorophore-conjugated secondary antibody. Imaging was performed on a Radiance 2100MP scanning laser confocal microscope (Bio-Rad, Hercules, CA). Antibodies used in these studies included CD31 from Biocare Medical (Concord, CA); GLUT-1 from DAKO (Carpinteria, CA); von Willebrand factor and CD45 from Oncogene (Boston, MA); CD15 from Calbiochem (La Jolla, CA); Ki67 from Abcam (Cambridge, MA); and CD14, CD32, CD83, DC-SIGN, and HLA-DR from Pharmingen (La Jolla, CA). *Ulex europaeus* lectin was obtained from Vector Laboratories (Burlingame, CA). Relative contributions of CD14⁺ cells were estimated by three independent observers who selected representative regions from indicated images and counted the number of positively labeled cells as a percentage of total DAPI labeled cells. Values shown represent averages of the three counts obtained.

Results

Hematopoietic Cells of the Myeloid Lineage Are Prevalent in Proliferating Hemangiomas

As part of our investigation into the role of immune cells in hemangioma progression,¹³ we analyzed hemangiomas for the presence of CD45, which identifies all hematopoietic leukocytes. There was extensive staining throughout the four proliferative lesions analyzed, and seven of eight hemangiomas from all phases were positive for CD45 cells (Figure 1). The case lacking CD45⁺ cells was a late involuting specimen. To determine whether these hematopoietic cells were dividing, we labeled sections with an antibody against Ki67. This revealed that the CD45 cells were almost uniformly not proliferating (Figure 2), suggesting that these cells were recruited from the circulation and not dividing from a smaller population within the lesion. Further immunohistochemical analysis revealed that many of these cells expressed CD14, a marker of monocytes (Figure 3). All proliferating lesions studied ($n = 6$) had large numbers of CD14⁺ cells and 12 of 18 (66%) hemangiomas from all phases were positive to some degree. The number of CD14⁺ cells decreased dramatically in nonproliferative lesions. The cases that had few or no CD14⁺ cells (6 of 18) were primarily late involuting cases ranging in age from 1 year 3 months to 6 years 3 months.

These findings suggest that myeloid cells are involved in the proliferation of hemangiomas and are perhaps not as important during involution. When the percentage of CD14⁺ cells was estimated in hemangiomas, it was found that these cells comprised approximately one-third of the total cells in the lesion in the earliest case studied (Figure 3a). Relative cell numbers dropped quickly in this time series (Figure 3, b–d) and fell further in the most involuted cases (Figure 3, f–h). This indicates that the myeloid cells decrease in number before the overall re-

Figure 2. Hemangioma-associated hematopoietic cells are not dividing. **a:** A proliferating hemangioma was labeled with CD45 (blue) to identify hematopoietic cells, *ulex* lectin (red) to show endothelium, and Ki67 (green) to show dividing cells. These results demonstrate that cellular proliferation is primarily restricted to hemangioma endothelium. **b:** In parallel, an involuting case was labeled showing significant reduction in the number of CD45⁺ cells and Ki67 staining, indicating diminished proliferation and demonstrating a correlation between hematopoietic cells and proliferation. Scale bar, 100 μ m.

gression of the lesion and suggests a role for these cells in promoting hemangioma proliferation.

Hemangioma Endothelium Co-Expresses Myeloid Markers

Throughout the course of this study, we labeled hemangioma sections with several myeloid markers including the dendritic cell marker, CD83, which produced a pattern suggestive of endothelial labeling. The presence of CD83 on hemangioma endothelium was confirmed by co-labeling with *Ulex europaeus* lectin and Von Willebrand

Figure 3. Hemangioma-associated hematopoietic cells are positive for the monocyte marker CD14 and correlate with proliferation. **a–h:** Sections from hemangiomas in all stages of progression were labeled with anti-CD14 antibody (red). The earliest lesions show the largest number of CD14⁺ cells with dramatic reduction in number as the lesions leave the proliferative phase and begin to involute. Note that **e** is chronologically out of sequence in this series, but clearly is less involuted than **f–h**. Nuclei are shown in blue. Percentages indicate estimated content of CD14⁺ cells obtained as described in Materials and Methods. Scale bar, 100 μ m.

factor (Figure 4, a and b). All proliferative lesions studied ($n = 5$) co-expressed CD83 with an endothelial marker and 82% (9 of 11) of hemangiomas from all phases had this type of expression pattern. These co-labeled cells were not observed in arteriovenous malformation, lymphatic malformation, rapidly involuting congenital hemangioma (not shown), or pyogenic granuloma (Figure 4c). It is interesting to note that CD83 labels the interior or luminal surface of the capillary-like structures and shows incomplete co-labeling with Von Willebrand factor (Figure

Figure 4. Hemangioma endothelial cells co-label with a dendritic cell marker, CD83. **a** and **b:** Proliferative hemangioma sections were labeled with an antibody against CD83 (green), and either anti-von Willebrand factor (vWF) (**a**) or *Ulex europaeus* lectin (ulex) (**b**) to label endothelial cells (red). Significant, although incomplete, co-localization was observed between CD83 and the endothelium. Each label is shown individually and at higher magnification to more readily visualize co-localization. **c:** Despite other histopathological similarities, this co-localization was not observed in pyogenic granuloma although a small number of CD83⁺ cells were observed in these lesions (green). Scale bar, 100 μ m.

Figure 5. Normal dendritic cells are present in proliferating hemangioma. Using two markers of dendritic cells HLA-DR (**a**) and DC-SIGN (**b**), we demonstrated that proliferating lesions contained significant numbers of these cells. Nuclei are shown in blue. Scale bar, 100 μ m.

4a). Similar to CD14⁺ cells, these CD83⁺ endothelial cells were reduced in number during involution, with the two negative cases appearing to be late in the involution process. Typical dendritic cells, which do not co-label with endothelial markers, were also present in significant number as evidenced by staining with antibodies directed to DC-SIGN and HLA-DR (Figure 5). These results are similar to a previously reported study.¹⁴

When proliferative hemangioma sections were stained for the myeloid marker CD15 (Lewis X), an endothelial-like distribution, similar to that of CD83 labeling, was observed (Figure 6a). When antibodies against CD15 and CD83 were used together, co-labeling of endothelial cells was observed (Figure 6b). As with CD83, arteriovenous malformation, lymphatic malformation, and pyogenic granuloma do not show an endothelial staining pattern with CD15, but rather the expected staining of myeloid leukocytes (Figure 6c). In this study 82% (14 of 17) of hemangiomas from all phases were positive for endothelial expression of CD15.

GLUT-1 has been described as a useful marker of hemangioma endothelium.¹⁵ Its expression in hemangiomas, in part, led to the speculation that these lesions

Figure 6. Hemangioma endothelial cells express the myeloid marker CD15. **a:** Labeling sections of proliferative hemangiomas with anti-CD15 (red) showed an endothelial-like distribution. **b:** Labeling sections with both anti-CD15 (red) and anti-CD83 (green), which was shown to label endothelial cells (Figure 4), shows that CD15 and CD83 are co-localized on many cells. **c:** Pyogenic granuloma did not show endothelial staining with anti-CD15, but rather the expected leukocyte staining (red). Scale bars: 100 μ m (**a**); 50 μ m (**b**); 10 μ m (**c**).

may be derived from placental cells because vessels in the placenta also express GLUT-1.¹⁶ CD32 (Fc γ RII) was also found to be expressed on hemangioma endothelium as well as placental vessels, providing more support for

the idea that hemangiomas may be of placental origin. Interestingly, GLUT-1 expression has been reported on monocytes^{17–20} and CD32 is a common marker of these cells.^{21,22} We found strong expression of both of these antigens on hemangioma endothelium, confirming the findings of North and colleagues.^{15,16} However, an alternative explanation for the presence of these antigens is suggested here by the finding that GLUT-1 co-localized with CD83 and Ulex lectin in proliferative hemangiomas (Figure 7), and CD32 co-localized with both CD14⁺ monocytes and the endothelial marker CD31 (Figure 8). Thus, when viewed as myeloid cell markers, GLUT-1 and CD32 co-labeling extends the myeloid-endothelial co-expression pattern in hemangioma. Once again, these co-labeled cells were not observed in other vascular anomalies studied.

Discussion

We have used immunohistochemical analysis of a number of myeloid cell markers to evaluate hemangioma tissue at varying stages of the disease and used this new information to formulate a hypothesis about the pathogenesis of these lesions. From this study, it is clear that hematopoietic cells of the myeloid lineage constitute a significant portion of the cells found in hemangiomas, particularly in the proliferative phase, suggesting a role for these cells in the promotion of hemangioma growth, possibly through expression of cytokines.

Myeloid cells (macrophages) have well established roles in promoting endothelial cell growth in a variety of conditions and hypoxia is known to stimulate secretion of angiogenic factors from macrophages. We propose that ischemia/hypoxia may have an important role in hemangioma pathogenesis through this mechanism. IGF2 expression is known to be induced under low oxygen conditions,²³ possibly explaining the high levels of this factor observed during the phase of rapid hemangioma proliferation. Continued growth of the lesion could quickly outrun blood supply and contribute to increasing hypoxia, this in turn increasing IGF2 expression and continuing proliferation. Indirect support for this mechanism comes from a recent study demonstrating that IGF2 imprinting patterns are maintained in hemangioma.²⁴ This study excluded loss of imprinting as a mechanism for IGF2 overexpression, but does not exclude the possibility that continuing hypoxia may play a role in hemangioma proliferation. Also observed on hemangioma endothelial cells is GLUT1, another hypoxia-inducible molecule.²⁵ The idea that hypoxia may initiate and maintain hemangioma growth is supported by a number of clinical observations, although direct experimental data has not yet been reported. For example, before the growth of a hemangioma, a blanched area of skin may be noticed by the family or clinician. This may be an area of local ischemia in the skin, caused by some unknown event, that creates a hypoxic environment and, thus, triggers growth factor expression.

We also observed that hemangioma endothelium co-expressed markers typically associated with myeloid

Figure 7. GLUT-1-positive hemangioma endothelial cells co-label with the dendritic cell marker, CD83. Sections were triple labeled with a marker for endothelium (ulex lectin) (**a**), hemangioma-specific endothelium (GLUT-1) (**b**), and dendritic cells (CD83) (**c**). As shown in the merged image (**d**), widespread co-localization is observed. Scale bar, 100 μ m.

Figure 8. The myeloid marker CD32 (Fc γ RII) labels both hemangioma-associated hematopoietic cells and hemangioma endothelium. **a:** When proliferative hemangioma sections were labeled with antibodies against the monocyte marker CD14 (blue), and the myeloid marker CD32 (red), co-localization is observed on some cells (purple). **b:** Co-staining sections with CD32 (red) and the endothelial marker CD31 (green) reveals co-expression of these two antigens on a portion of cells within the lesion (yellow). **c:** When **a** and **b** are merged, cells co-labeling with CD14 (blue) and CD32 (red) are seen in purple whereas cells co-expressing CD32 (red) and CD31 (green) are yellow. Note that some CD31⁺ cells (green) do not co-label with CD32. Scale bar, 100 μ m.

cells. Although both myeloid and endothelial cells share a common progenitor, it is not clear if the observed marker co-expression in these lesions reflects a maintained common lineage or if it reflects a contribution of myeloid cells to the development of hemangioma-associated endothelium. Taking into account the evidence for clonality in hemangiomas,^{8,9} we have speculated about the idea of a myeloid cell as the hemangioma progenitor cell, where the myeloid-endothelial co-expression might represent some intermediate differentiation step in the myeloid-to-endothelial transition, as has been described else-

where.^{26,27} Although we cannot rule out the possibility that this kind of *trans*-differentiation event is occurring, it seems unlikely to be occurring on a large scale, because we have shown that the majority of hematopoietic cells within the lesions are not proliferating (Figure 2). Proliferation of these cells would be expected to be necessary for clonal expansion from a single myeloid cell. It is possible, however, that the hematopoietic cells are clonal but the proliferation of these cells occurred before our analysis, or that myeloid cells recruited from the circulation are undergoing this transdifferentiation under the influence of conditions within the lesion. Although the significance of myeloid/endothelial marker co-expression on hemangioma cells to the etiology of hemangioma is not yet clear, it does serve to support the concept that hemangioma cells are distinct from normal endothelium and may be useful in the histopathological distinction of hemangioma from other vascular anomalies.

Other evidence may support the idea of myeloid involvement. Typical hemangiomas appear perinatally when they can enter a rapid growth phase. Overcoming the paradoxical situation between mother and the alloantigenic fetus relies on the suppression of maternal immune response against the fetus. This occurs in part through secretion of immunosuppressors by the placenta. There is evidence that pregnancy-related hormones have a specific inhibitory effect on monocyte cell cycle progression and induce their apoptosis.²⁸ Thus, birth removes the source of myeloid cell suppression and may allow the growth of a pre-established lesion, possibly explaining the observed perinatal growth of these tumors. Circulating maternal angiogenesis inhibitors have also been described during pregnancy, which is another idea that should be considered as a mechanism for the perinatal onset of hemangiomas.

Corticosteroids are the first-line therapy for the treatment of problematic hemangioma during the proliferative phase. Although some evidence suggests that corticosteroids induce apoptosis-associated proteins,^{29,30} more work needs to be done to clearly define the role of these proteins in hemangioma progression and which cells might be affected. Our work suggests a mechanism for the efficacy of corticosteroids on proliferating hemangioma, because these agents have established anti-inflammatory effects and have been shown to induce apoptosis in myeloid cells.³¹ The reduced numbers of myeloid cells in involuting lesions would remove the target of this therapy, thus explaining the reduced efficacy of these drugs in postproliferative hemangiomas. Still unaddressed is the failure of some proliferative lesions to respond to this mode of therapy, but perhaps further study of the role of myeloid cells in hemangioma may aid in our understanding of this phenomenon.

It should be mentioned that the hemangioma specimens under study here primarily represent the localized, focal type and not the plaque-like segmental variety, which is likely the case in most studies of this kind because the plaque-like lesions are rarely resected and, thus, not available for analysis. It is conceivable that there could be differences in the biochemistry and histopathology between the two types of lesions, which would be of

great interest. A thorough comparison of the different types may advance our understanding of the mechanisms regulating the evolution of these tumors.

Our findings suggest that the hemangioma pathogenesis is more complex than the simple idea of excessive local angiogenesis and suggests that myeloid cells may have roles in the growth of these tumors. Existing evidence indicates that hemangioma pathogenesis is multifactorial. The present work does not exclude from consideration other ideas such as placental involvement, developmental patterning, or endothelial progenitors, but may in fact represent additional factors that might be needed to trigger hemangioma growth. These studies provide a possible rationale for the efficacy of the most common therapy for hemangioma and identify myeloid cells as a potential target for the design of new, more specific treatments.

Acknowledgments

We thank the members of the Friedlander laboratory for valuable discussions about this work; David Friedlander and Dana Copeland for their technical excellence; and Drs. Bari Cunningham, Lawrence Eichenfield, Steven Cohen, Ralph Holmes, and Thomas Veccione all from Children's Hospital San Diego for providing specimens for this study.

References

- Mulliken JB, Glowacki J: Hemangiomas and vascular malformations in infants and children: a classification based on endothelial characteristics. *Plast Reconstr Surg* 1982, 69:412-422
- Ritter MR, Dorrell MI, Edmonds J, Friedlander SF, Friedlander M: Insulin-like growth factor 2 and potential regulators of hemangioma growth and involution identified by large-scale expression analysis. *Proc Natl Acad Sci USA* 2002, 99:7455-7460
- Tan ST, Wallis RA, He Y, Davis PF: Mast cells and hemangioma. *Plast Reconstr Surg* 2004, 113:999-1011
- Yu Y, Flint AF, Mulliken JB, Wu JK, Bischoff J: Endothelial progenitor cells in infantile hemangioma. *Blood* 2004, 103:1373-1375
- Dadras SS, North PE, Bertoncini J, Mihm MC, Detmar M: Infantile hemangiomas are arrested in an early developmental vascular differentiation state. *Mod Pathol* 2004, 17:1068-1079
- Haggstrom AN, Frieden IJ: Hemangiomas: past, present, and future. *J Am Acad Dermatol* 2004, 51:S50-S52
- Waner M, North PE, Scherer KA, Frieden IJ, Waner A, Mihm Jr MC: The nonrandom distribution of facial hemangiomas. *Arch Dermatol* 2003, 139:869-875
- Boye E, Yu Y, Paranya G, Mulliken JB, Olsen BR, Bischoff J: Clonality and altered behavior of endothelial cells from hemangiomas. *J Clin Invest* 2001, 107:745-752
- Walter JW, North PE, Waner M, Mizeracki A, Blei F, Walker JW, Reinisch JF, Marchuk DA: Somatic mutation of vascular endothelial growth factor receptors in juvenile hemangioma. *Genes Chromosom Cancer* 2002, 33:295-303
- Sunderkotter C, Goebeler M, Schulze-Osthoff K, Bhardwaj R, Sorg C: Macrophage-derived angiogenesis factors. *Pharmacol Ther* 1991, 51:195-216
- Crowther M, Brown NJ, Bishop ET, Lewis CE: Microenvironmental influence on macrophage regulation of angiogenesis in wounds and malignant tumors. *J Leukoc Biol* 2001, 70:478-490
- Zhou LJ, Tedder TF: CD14+ blood monocytes can differentiate into functionally mature CD83+ dendritic cells. *Proc Natl Acad Sci USA* 1996, 93:2588-2592
- Ritter MR, Moreno SK, Dorrell MI, Rubens J, Ney J, Friedlander DF, Bergman J, Cunningham BB, Eichenfield L, Reinisch J, Cohen S, Veccione T, Holmes R, Friedlander SF, Friedlander M: Identifying potential regulators of infantile hemangioma progression through large-scale expression analysis: a possible role for the immune system and indoleamine 2,3 dioxygenase (IDO) during involution. *Lymphat Res Biol* 2003, 1:291-299
- Nguyen VA, Furhapter C, Romani N, Weber F, Sepp N: Infantile hemangioma is a proliferation of beta 4-negative endothelial cells adjacent to HLA-DR-positive cells with dendritic cell morphology. *Hum Pathol* 2004, 35:739-744
- North PE, Waner M, Mizeracki A, Mihm Jr MC: GLUT1: a newly discovered immunohistochemical marker for juvenile hemangiomas. *Hum Pathol* 2000, 31:11-22
- North PE, Waner M, Mizeracki A, Mrak RE, Nicholas R, Kincannon J, Suen JY, Mihm Jr MC: A unique microvascular phenotype shared by juvenile hemangiomas and human placenta. *Arch Dermatol* 2001, 137:559-570
- Daneman D, Zinman B, Elliott ME, Bilan PJ, Klip A: Insulin-stimulated glucose transport in circulating mononuclear cells from nondiabetic and IDDM subjects. *Diabetes* 1992, 41:227-234
- Estrada DE, Elliott E, Zinman B, Poon I, Liu Z, Klip A, Daneman D: Regulation of glucose transport and expression of GLUT3 transporters in human circulating mononuclear cells: studies in cells from insulin-dependent diabetic and nondiabetic individuals. *Metabolism* 1994, 43:591-598
- Korgun ET, Demir R, Sedlmayr P, Desoye G, Arikian GM, Puerstner P, Haeusler M, Dohr G, Skofitsch G, Hahn T: Sustained hypoglycemia affects glucose transporter expression of human blood leukocytes. *Blood Cells Mol Dis* 2002, 28:152-159
- Korgun ET, Demir R, Sedlmayr P, Desoye G, Arikian G, Puerstner P, Haeusler M, Dohr G, Skofitsch G, Hahn T: Physiological leukocytosis during pregnancy is associated with changes in glucose transporter expression of maternal peripheral blood granulocytes and monocytes. *Am J Reprod Immunol* 2002, 48:110-116
- Debets JM, Van de Winkel JG, Ceuppens JL, Dieteren IE, Buurman WA: Cross-linking of both Fc gamma RI and Fc gamma RII induces secretion of tumor necrosis factor by human monocytes, requiring high affinity Fc-Fc gamma R interactions. Functional activation of Fc gamma RII by treatment with proteases or neuraminidase. *J Immunol* 1990, 144:1304-1310
- Graziano RF, Fanger MW: Fc gamma RI and Fc gamma RII on monocytes and granulocytes are cytotoxic trigger molecules for tumor cells. *J Immunol* 1987, 139:3536-3541
- Feldser D, Agani F, Iyer NV, Pak B, Ferreira G, Semenza GL: Reciprocal positive regulation of hypoxia-inducible factor 1alpha and insulin-like growth factor 2. *Cancer Res* 1999, 59:3915-3918
- Yu Y, Wylie-Sears J, Boscolo E, Mulliken JB, Bischoff J: Genomic imprinting of IGF2 is maintained in infantile hemangioma despite its high level of expression. *Mol Med* (in press)
- Loike JD, Cao L, Brett J, Ogawa S, Silverstein SC, Stern D: Hypoxia induces glucose transporter expression in endothelial cells. *Am J Physiol* 1992, 263:C326-C333
- Fernandez Pujol B, Lucibello FC, Gehling UM, Lindemann K, Weidner N, Zuzarte ML, Adamkiewicz J, Elsasser HP, Muller R, Havemann K: Endothelial-like cells derived from human CD14 positive monocytes. *Differentiation* 2000, 65:287-300
- Havemann K, Pujol BF, Adamkiewicz J: In vitro transformation of monocytes and dendritic cells into endothelial like cells. *Adv Exp Med Biol* 2003, 522:47-57
- Thongngarm T, Jenkins JK, Ndebele K, McMurray RW: Estrogen and progesterone modulate monocyte cell cycle progression and apoptosis. *Am J Reprod Immunol* 2003, 49:129-138
- Hasan Q, Ruger BM, Tan ST, Gush J, Davis PF: Clusterin/apoD expression during the development of hemangioma. *Hum Pathol* 2000, 31:691-697
- Hasan Q, Tan ST, Xu B, Davis PF: Effects of five commonly used glucocorticoids on haemangioma in vitro. *Clin Exp Pharmacol Physiol* 2003, 30:140-144
- Schmidt M, Pauels HG, Lugerling N, Domschke W, Kucharzik T: Glucocorticoids induce apoptosis in human monocytes: potential role of IL-1 beta. *J Immunol* 1999, 163:3484-3490