CIRCULATING ACTIVATED ENDOTHELIAL CELLS IN SICKLE CELL ANEMIA

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ABSTRACT

Background The vascular wall participates in the pathogenesis of sickle cell disease. To determine whether the endothelium is activated in this disease, we studied the number, origin, and surface phenotype of circulating endothelial cells in patients with sickle cell anemia.

Methods We used immunohistochemical examination of buffy-coat smears to enumerate circulating endothelial cells, and we evaluated the surface phenotype by applying immunofluorescence microscopy to preparations of circulating endothelial cells. A panel of antibodies was used, including a specific anti–endothelial-cell antibody, P1H12.

Results Mean (±SD) numbers of circulating endothelial cells in normal blood donors, patients with sickle cell trait, and patients with hemolytic anemias not due to hemoglobin S were 2.6±1.6, 3.0±2.6, and 2.0±0.8 per milliliter of whole blood, respectively. Patients with sickle cell anemia who presented with acute painful episodes had 22.8±18.2 circulating endothelial cells per milliliter of blood ($P<0.001$ for the comparison with normal donors), and patients with no such events within one month before or after blood sampling had 13.2±11.8 circulating endothelial cells per milliliter of blood ($P=0.002$ for the comparison with normal donors and $P=0.019$ for the comparison with patients with acute events). Serial observations of three patients showed a tendency toward higher levels of circulating endothelial cells at the onset of acute painful crises. The average viability of circulating endothelial cells was 66±30 percent. In patients with sickle cell anemia, regardless of clinical status, the circulating endothelial cells were predominantly microvascular in origin (CD36-positive), and most of the cells expressed four markers of endothelial-cell activation: intercellular adhesion molecule 1, vascular-cell adhesion molecule 1, E-selectin, and P-selectin.

Conclusions Our studies suggest that the vascular endothelium is activated in patients with sickle cell anemia, regardless of the patients' clinical status. Adhesion proteins on activated endothelial cells may have a role in the vascular pathology of sickle cell disease. (N Engl J Med 1997;337:1584-90.)

THE endothelial cell participates in numerous functions of vascular physiology.[1-5] Many factors, such as cytokines, can alter the surface of the endothelial cell and thereby modulate the role of the endothelium in coagulation, inflammation, vaso-regulation, and adhesion.[5-7] The endothelial cell may also have a key role in the vascular pathology of sickle cell anemia,[5] including the vaso-occlusions that cause acute painful crises. However, research in this area has been hindered by the inaccessibility of vascular endothelium in patients. Circulating endothelial cells might provide useful material for the study of this problem. In previous investigations increased numbers of circulating endothelial cells have been found in sickle cell anemia[8,9] and other conditions with vascular injury, such as that due to cytomegalovirus infection,[10,11] rickettsial infection,[12,13] myocardial infarction,[14,15] intravascular instrumentation,[16,17] and endotoxinemia.[18] Because indirect evidence suggests perturbation and activation of vascular-wall endothelium in sickle cell disease,[5] we examined the viability, origin, and surface phenotype of circulating endothelial cells in patients with this disease.

METHODS

Subjects and Blood Collection

Blood donors were volunteers, as approved by the human-subjects review boards of the participating institutions. Adults gave informed consent; for minors, the consent of both the parents and the subjects was obtained, but only waste blood left over from clinical laboratory testing was used in the case of the subjects who were less than 14 years old. The subjects consisted of 14 normal persons, 18 patients with sickle cell anemia who presented with acute painful crises or were in a steady state (pain-free, with no acute clinical event within at least the month before and the month after blood sampling), 3 donors with sickle trait, and 4 control patients with high reticulocyte counts that were not due to sickle cell disease. The patients with sickle cell anemia ranged in age from 5 to 57 years (mean [±SD], 27±17) and included nine males and nine females; of these, six were less than 21 years old (mean, 12±7). Only two of these patients had received transfusions within three months before blood donation for this study. After the initial blood obtained from venipuncture was discarded, venous blood was drawn into tubes treated with silicon and EDTA and studied immediately.

Endothelial-Cell Cultures

Preliminary studies to perfect and verify the techniques used in this investigation were performed on cultured human dermal mi-

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crovascular endothelial cells (MVEC) obtained from human foreskins[19] and large-vessel endothelial cells (HUVEC) obtained from human umbilical veins.[20]

Antibodies

We used the following antibodies: rabbit polyclonal antibodies against von Willebrand factor (Sigma Chemical, St. Louis) or thrombomodulin (Biplex, Richmond, Calif.); two murine monoclonal antibodies against CD36, OKM5 (Ortho Diagnostic Systems, Raritan, N.J.) and FA6-152 (Immunotech, Westbrook, Me.); fluorochrome-labeled murine monoclonal antibodies against intercellular adhesion molecule 1 (ICAM-1) or vascular-cell adhesion molecule 1 (VCAM-1) (South Biotechnology, Birmingham, Ala.); and murine monoclonal antibodies against E-selectin (Genzyme, Cambridge, Mass.) or P-selectin (Novocastra Laboratories, Newcastle upon Tyne, United Kingdom). The antibodies used as controls for nonspecific binding by these primary antibodies were polyclonal rabbit antichicken immunoglobulin for the rabbit polyclonal antibodies and antibodies with irrelevant binding specificities but the same isotype for the murine monoclonal antibodies (Sigma). As a positive control antibody for endothelial cells, we used a murine monoclonal antibody to beta$_2$-microglobulin (Sigma). Secondary antibodies were used as required: goat antimouse immunoglobulin conjugated to lissamine rhodamine (Jackson IRL, Westgrove, Pa.) or fluorescein isothiocyanate (Sigma), rhodamine-conjugated goat antirabbit immunoglobulin (Jackson IRL), and alkaline phosphatase–conjugated antimouse immunoglobulin (Sigma or Chemicon International, Temecula, Calif.).

Identification of Endothelial Cells

To identify circulating endothelial cells, we used the antibody P1H12. This murine IgG1 monoclonal antibody was obtained by immunizing mice with HUVEC, generating a hybridoma line, and separating IgG from supernatants of hybridoma-cell cultures with a protein G column. For some studies we used fluorescein isothiocyanate–labeled P1H12, prepared with the Fluoro Tag FITC Conjugation kit (Sigma).

P1H12 reacts specifically with endothelial cells. It stains primary HUVEC and MVEC cultures and the endothelial cells of all vessels in frozen sections of human skin, intestine, ovary, tonsil, lymph node, lung, and kidney. It does not stain any other type of cell in those tissues. It does not stain carcinoma cell lines HT-29 and COLO205, melanoma cell lines A-375 and M21, the T-cell lines Jurkat and HuT78, fibroblasts, HL-60 or Chinese-hamster-ovary cells, or Epstein–Barr virus–transformed B-cell lines. It does not stain monocytes, granulocytes, red cells, platelets, T cells, or B cells from marrow or peripheral blood; nor does it react with marrow megakaryocytes or the megakaryoblast line HU3. The peripheral-blood cells that do stain with P1H12 are also positive for both von Willebrand factor and thrombomodulin (the combined expression of which is limited to endothelium), and they stain for flt and flk (receptors for the endothelial-specific vascular endothelial growth factor). Subgroups of P1H12-positive blood cells also stain for CD34 and two endothelial-specific activation markers (VCAM and E-selectin, as reported here).

Quantitation of Circulating Endothelial Cells

We used immunohistochemical examination of buffy-coat smears to enumerate circulating endothelial cells. One milliliter of whole blood was diluted by a factor of 4 with Hanks' balanced salt solution without calcium and with 1 mM EDTA and 0.5 percent bovine serum albumin. Diluted blood was layered on one-half volume of Histopaque 1077 (Sigma) and centrifuged for 30 minutes at 250×*g*. The supernatant and the interface were pooled in polypropylene tubes (precoated with 0.5 percent bovine serum albumin) and centrifuged for 5 minutes at 1200×*g*. After the removal of the supernatant, the resulting "buffy-coat" pellet was gently resuspended and transferred to microslides in volumes containing the cells harvested from 0.25 ml of whole blood. Smears were air-dried overnight and fixed with 4 percent paraformaldehyde for 10 minutes.

For staining, the smears were rehydrated and pretreated with TRIS-buffered saline containing 2 percent bovine serum albumin for 30 minutes, after which 5 μg of P1H12 per milliliter of TRIS-buffered saline was applied. The samples were then washed with TRIS-buffered saline containing 2 percent bovine serum albumin and a secondary antibody, alkaline phosphatase–conjugated rabbit antimouse IgG, was applied. After the samples were washed, fast red substrate (Sigma) was added for color development. The samples were then counterstained with Mayer's hematoxylin (Biomeda, Foster City, Calif.) and examined by light microscopy. All nucleated circulating endothelial cells contained within the original 1-ml sample of whole blood were directly counted. Negative controls were provided by the white cells on the smears and by parallel slides prepared with control primary antibodies. Positive controls consisted of cultured MVEC and HUVEC.

Qualitative Studies of Circulating Endothelial Cells

Assessment of the surface phenotype of circulating endothelial cells required enrichment of the samples with Dynabeads carrying goat antimouse immunoglobulin (Dynal, Oslo, Norway). These were coated with P1H12 according to the manufacturer's instructions and were used after being washed with Hanks' balanced salt solution without calcium and with 1 mM EDTA and 0.5 percent bovine serum albumin. To isolate circulating endothelial cells from blood, we used two different methods.

To detect antigens that are constitutively expressed by endothelial cells, whole blood diluted by a factor of 4 with Hanks' balanced salt solution without calcium and with EDTA and 0.5 percent bovine serum albumin was mixed with 4 million P1H12-coated beads for each milliliter of undiluted blood and incubated for one hour at 4°C with gentle agitation. After incubation, beads with circulating endothelial cells were collected with a magnetic concentrator (Dynal). The harvested cells were washed with medium 199 and then transferred to slides with chambered coverslips (Nunc, Naperville, Ill.) at 37°C. The circulating endothelial cells were then washed with phosphate-buffered saline and fixed with 4 percent paraformaldehyde in phosphate-buffered saline for 10 minutes at room temperature. (For studies of von Willebrand factor, the circulating endothelial cells were made permeable by additional treatment with 0.4 percent Triton X-100 in phosphate-buffered saline for 10 minutes.) Before undergoing staining, cells were pretreated with phosphate-buffered saline with 3 percent bovine serum albumin for at least 30 minutes.

To detect antigens that endothelial cells express only or to a markedly greater extent on activation (P-selectin, E-selectin, VCAM, and ICAM), we fixed cells immediately after venipuncture by adding 0.25 percent paraformaldehyde to whole blood, incubating the sample for 10 minutes, and washing it three times with phosphate-buffered saline. Samples were restored to four times the initial volume with Hanks' balanced salt solution without calcium and with EDTA and 0.5 percent bovine serum albumin and mixed with P1H12-coated beads as described above. For P-selectin studies we made the cells permeable, as described for studies of von Willebrand factor.

We analyzed the phenotype of circulating endothelial cells using either direct or indirect immunofluorescence staining with antibodies in empirically determined concentrations (always between 1 and 10 μg per milliliter). Direct immunofluorescence was used to detect dual binding of fluorescein isothiocyanate–labeled P1H12 and R-phycoerythrin–conjugated antibodies to ICAM-1 or VCAM-1, and indirect immunofluorescence was used to detect the other antigens. After the staining procedures were completed, preparations of circulating endothelial cells were viewed with an inverted fluorescence microscope (Olympus, Tokyo, Japan). Specific positive controls were provided by cultured HUVEC and MVEC (stimulated in vitro if necessary with histamine or thrombin). Negative controls were provided by the white cells contaminating preparations of circulating endothelial cells and by smears

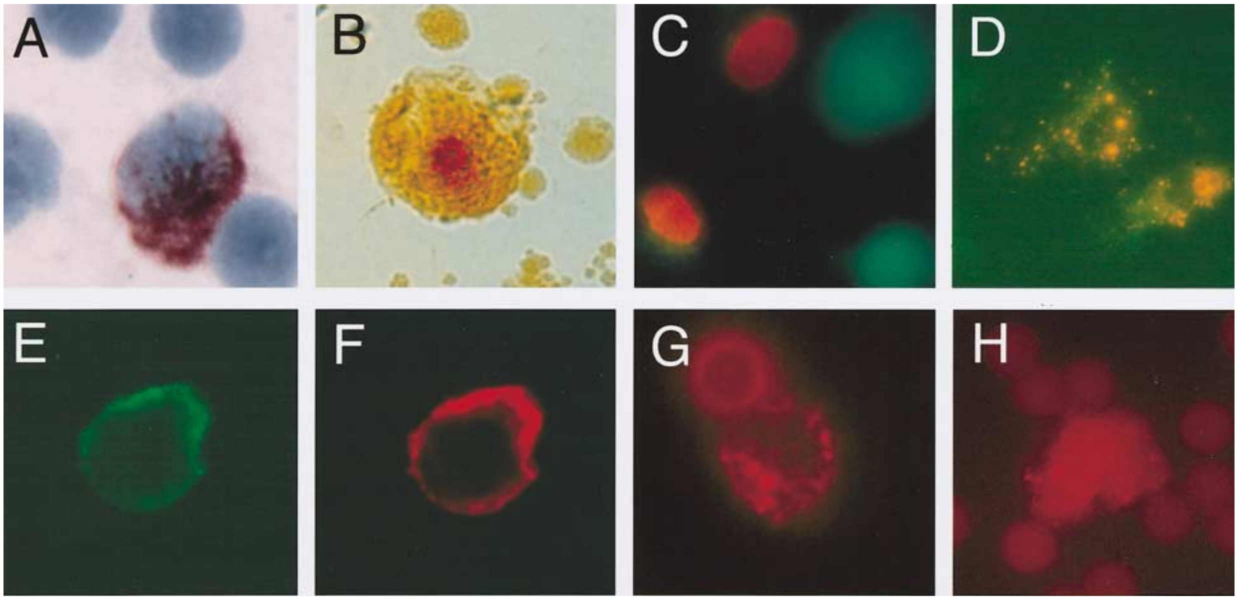

Figure 1. Examples of Immunohistochemical and Immunofluorescence Analysis of Circulating Endothelial Cells.

Each panel, except Panel C, shows circulating endothelial cells isolated from donors with sickle cell anemia. In Panel A, a buffy-coat smear stained with alkaline phosphatase–conjugated P1H12 has a single circulating endothelial cell (red staining). The nuclei were counterstained with hematoxylin (×1500). Panel B shows a circulating endothelial cell that is staining for both P1H12 (red area) and intracellular von Willebrand factor (brown area), obtained with peroxidase-conjugated antibody against von Willebrand factor (×1000). Panel C shows live HUVEC (green-stained cytoplasm) and dead HUVEC (red-stained nuclei) (×600). Panel D shows live circulating endothelial cells after 11 days in cell culture (×600). Before being added to the culture, circulating endothelial cells from blood were labeled with a cytoplasmic dye so they could be distinguished from the primary MVEC with which they were cultured. Live circulating endothelial cells stain with both P1H12 (green area) and the cytoplasmic dye (orange areas). A cell identified as an endothelial cell by staining with fluorescein isothiocyanate–conjugated P1H12 (Panel E; ×1000) is also positive for CD36 (Panel F; ×1000), detected with lissamine-conjugated anti-CD36. Panel G shows a circulating endothelial cell with the punctate pattern of expression of intracellular P-selectin that is typical of unstimulated cells (×1000), and Panel H shows the diffuse pattern of expression of surface P-selectin that is typical of activated cells (×900). The out-of-focus round object with the halo in Panel G is one of the immunomagnetic beads used to isolate circulating endothelial cells. Numerous beads are evident in Panel H.

of circulating endothelial cells stained with primary or secondary control antibodies. Preliminary studies indicated that our enrichment method recovered more than 85 percent of the endothelial cells in the original sample of whole blood, suggesting that the results reflect the entire population of circulating endothelial cells. Control experiments using HUVEC and MVEC demonstrated that the sample-handling procedures caused no artifactual expression of the activation markers.

Viability of Circulating Endothelial Cells

Three independent criteria were used to assess the viability of circulating endothelial cells. First, we tested the responsiveness of circulating endothelial cells to histamine by incubating donor blood with or without 100 μM histamine at 37°C for 10 minutes, followed by immediate fixation with 0.25 percent paraformaldehyde. Viability was indicated by the inducibility of the expression of P-selectin on the surface of circulating endothelial cells in the histamine-treated sample.

Second, we identified live and dead cells with a kit (Molecular Probes, Eugene, Oreg.) that uses calcein AM, which stains the cytosol of live cells green, and ethidium homodimer, which stains the nuclei of dead cells red. Cultured endothelial cells stained according to the manufacturer's directions had either green cytoplasm or red nuclei, but not both. To assess the circulating endothelial cells, cells on slides were stained with P1H12 and with only the ethidium homodimer component of the kit, which allowed the identification of total and dead circulating endothelial cells. To determine the proportion of circulating endothelial cells with any given surface phenotype of interest that were dead, we combined staining with the ethidium homodimer with the detection of P1H12 and the second epitope of interest.

Third, we determined whether P1H12-positive cells isolated from the blood of patients with sickle cell anemia remain alive in cell culture. The freshly isolated population of cells containing circulating endothelial cells was first labeled with the intracellular fluorescent dye PKH26 (Molecular Probes) and then cultured with primary MVEC[19] for up to 28 days. Staining for both PKH26 and P1H12 was used as a marker to identify circulating endothelial cells that were alive in the culture.

RESULTS

Quantitative Studies

We enumerated circulating endothelial cells by using antibody P1H12 for immunohistochemical examination of buffy-coat smears (Fig. 1A). The P1H12-positive cells in these smears were also positive for both intracellular von Willebrand factor (Fig. 1B) and surface thrombomodulin (data not shown), demonstrating that they were endothelial cells. Even minimally manipulated samples often contained some small anucleate P1H12-positive cell fragments, but we report only the number of nucleated circulating endothelial cells. The circulating endothelial cells identified in the blood of patients with sickle cell anemia and the controls were in the form of single cells dispersed throughout the buffy-coat smears.

Blood from normal subjects contained a very small number of circulating endothelial cells (mean [±SD], 2.6±1.6 per milliliter of whole blood) (Table 1). The number of circulating endothelial cells was close to this value in three donors with sickle cell trait (3.0±2.6 per milliliter) and four patients with hemolytic anemias other than sickle cell disease (2.0±0.8 per milliliter), including two patients with persistently high reticulocyte counts after splenectomy, one with a microangiopathic hemolytic anemia, and one with paroxysmal nocturnal hemoglobinuria. In contrast, patients with sickle cell anemia who were not acutely ill (i.e., in a steady state) had significantly elevated numbers of circulating endothelial cells (13.2±11.8 per milliliter; P=0.002 for the comparison with normal subjects). Patients presenting on the first day of an acute painful crisis had even greater numbers (22.8±18.2; P=0.019 for the comparison with patients in a steady state; P<0.001 for the comparison with normal donors). Because of the large variation between patients in the number of circulating endothelial cells, we also examined three patients serially. Figure 2 shows the tendency in these patients for the number of circulating endothelial cells to increase further at the onset of a painful crisis.

We found no relation between the number of circulating endothelial cells and the sex or age of the patients (which ranged from 5 to 57 years). There was no relation between the number of circulating endothelial cells and the use of particular medications.

Viability of Circulating Endothelial Cells

Three independent measures indicated that the population of circulating endothelial cells included viable cells. First, preparations of circulating endothelial cells responded to histamine by shifting the distribution of P-selectin from the cytosol (Fig. 1G) to the surface in 19±16 percent of the cells (Fig. 1H). This shift is typical of activated endothelial cells. Second, circulating endothelial cells from four patients with sickle cell anemia remained alive when cultured with primary isolates of MVEC (Fig. 1D). These two qualitative tests gave no information about the actual proportion of viable circulating endothelial cells. However, fluorescent staining for live and dead cells (Fig. 1C) revealed that 66±30 percent of circulating endothelial cells in patients with sickle cell anemia were alive. The percentages varied among patients; the individual values for the 15 patients we tested were 29, 31, 38, 38, 40, 43, 47, 62, 67, 95, 100, 100, 100, 100, and 100 percent viable circulating endothelial cells. There was no correspondence between the proportion of live cells and clinical status (68±33 percent viability for eight patients in a steady state and 64±28 percent viability for seven patients with acute painful crisis).

Table 1. Quantitation of Circulating Endothelial Cells.

Subjects	No. of Samples	Circulating Endothelial Cells*
		cells/ml of whole blood (range)
Normal subjects	14	2.6±1.6 (0–5)
Patients with sickle cell anemia†		
Steady state	33	13.2±11.8 (0–56)‡
Acute painful crisis	24	22.8±18.2 (1–66)§
Patients with sickle cell trait	3	3.0±2.6 (1–6)
Patients with hemolytic anemias other than sickle cell trait¶	4	2.0±0.8 (1–3)

*Plus–minus values are means ±SD.

†A steady state was one in which the patient was pain-free with no acute painful crises within at least the month before and the month after blood sampling. Blood was obtained from patients on the first day of an acute painful crisis.

‡P=0.002 for the comparison with normal subjects.

§P=0.019 for the comparison with patients in a steady state.

¶One patient had paroxysmal nocturnal hemoglobinuria (reticulocyte count, 20 percent), one patient had microangiopathic hemolytic anemia due to thrombotic thrombocytopenic purpura (reticulocyte count, 15 percent), and two patients had persistently high reticulocyte counts (43 percent and 18 percent) after splenectomy.

Preliminary studies established that our enrichment and analysis protocols did not themselves adversely affect the viability of cultured endothelial cells, indicating that the dead cells found in the blood from our study subjects were not an artifact of the in vitro manipulations.

Phenotype of Circulating Endothelial Cells

Among the various types of endothelial cells, only microvascular endothelial cells express CD36. We used this marker to identify the vascular origin of circulating endothelial cells (Fig. 1E and 1F). About half (53±4 percent) of the circulating endothelial cells in samples of normal blood were positive for CD36 (Table 2). The proportion of circulating endothelial cells that were positive for CD36 was somewhat higher (78±15 percent) in samples from patients with sickle cell anemia (Table 2), and the absolute number of microvascular circulating endothelial cells in these patients was, on average, nine times normal. We found no difference in the proportion of CD36-positive circulating endothelial cells among patients at the onset of a painful crisis or in a steady state (76±16 vs. 80±16 percent). The increase in circulating endothelial cells during an acute

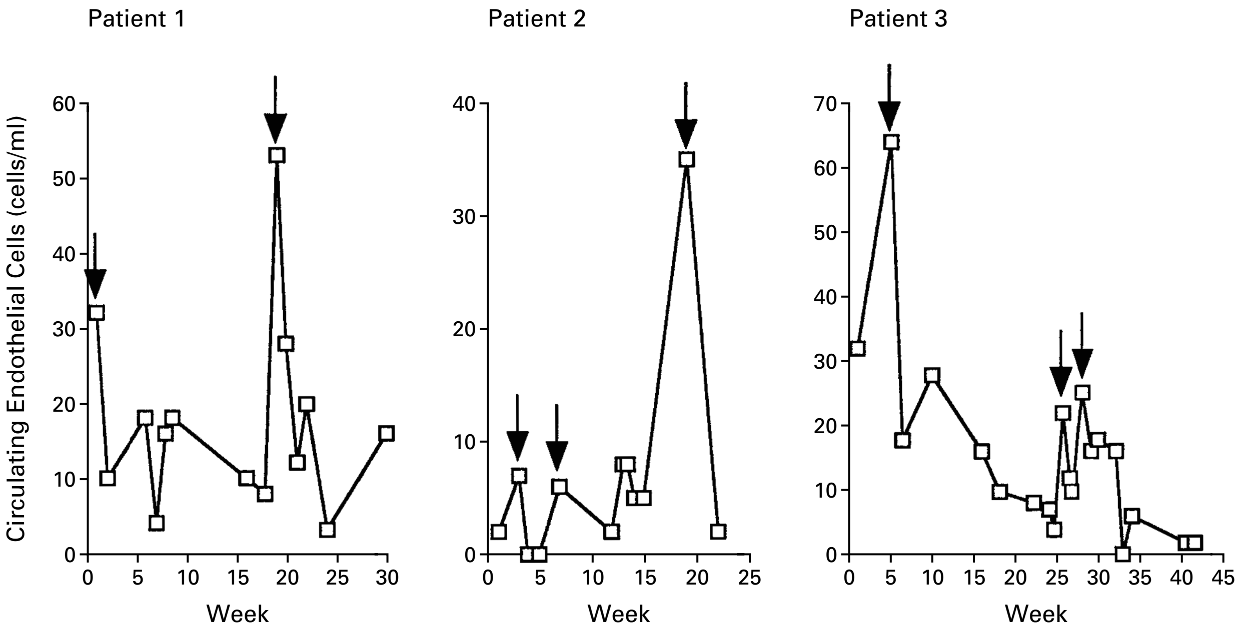

Figure 2. Longitudinal Quantitation of Circulating Endothelial Cells.

The numbers of circulating endothelial cells were monitored in three patients. The onset of every acute painful crisis that occurred during the study is indicated by an arrow.

painful crisis is due predominantly to microvascular endothelial cells.

We also examined circulating endothelial cells for dual expression of P1H12 and adhesion molecules that appear on activated endothelial cells. The percentage of circulating endothelial cells with surface expression of ICAM-1, VCAM-1, E-selectin, or P-selectin was markedly greater among patients with sickle cell anemia than among the normal controls (Table 2). The percentage of circulating endothelial cells that were positive for these activation markers was similar for samples obtained from patients in a steady state and patients at the onset of acute painful crises. In individual patients, the proportion of viable and dead circulating endothelial cells with these markers was exactly the same. The expression of the endothelial activation markers on circulating endothelial cells from the four control patients with hemolytic anemias other than sickle cell disease was in the normal range.

Table 2. Phenotype of Circulating Endothelial Cells in Patients with Sickle Cell Anemia and Normal Subjects.*

Variable	CD36	ICAM-1	VCAM-1	E-Selectin	P-Selectin
Normal subjects					
Value (%)	53±4	33±2	13±11	2±5	32±16
No. of subjects	5	3	3	4	3
Patients with sickle cell anemia					
Value (%)	78±15	88±10	85±10	82±16	62±31
No. of subjects	12	6	7	3	6
P value†	0.005	<0.001	<0.001	<0.001	NS

*Plus–minus values are the mean (±SD) percentages of P1H12-positive cells that were also positive for the indicated feature. The expression of E-selectin, ICAM-1, and VCAM-1 was identical whether circulating endothelial cells were prepared with immediate or delayed fixation.

†P values are for the comparison between groups. NS denotes not significant.

DISCUSSION

Evidence that perturbation of vascular-wall endothelium contributes to the vascular abnormalities of sickle cell anemia includes findings of histopathological changes in splenic and cerebral vasculatures[21-24]; the development of thromboses at sites of underlying intimal hyperplasia[22-24]; the abnormal presence of endothelial-cell adhesion molecules such as ICAM-1, VCAM-1, and E-selectin in the blood[25]; and increased numbers of circulating endothelial cells.[8,9] Several other abnormalities in the disease are probably related to the vascular endothelium. These include activation of the coagulation system, abnormal adhesion of red cells and white cells to endothelium, and disturbances of vasoregulation. Moreover, hypoxia and increased levels of interleukin-1, endotoxin, tumor necrosis factor, C-reactive protein, and thrombin tend to be present in the blood of patients with sickle cell anemia.[26] All these factors modulate the endothelial phenotype and may contribute to the development of vascular disease in sickle cell anemia by modulating the expression of various hemostatic and adhesion molecules on endothelial cells.[5]

Our data show that the increased number of circulating endothelial cells in patients with sickle cell anemia is not simply due to hyposplenia, high reticulocyte counts (i.e., hematopoietic stress), or both, because control patients with these abnormalities had normal numbers of circulating endothelial cells. We found that the number of circulating endothelial cells tends to increase at the onset of acute painful episodes, but the mechanism of this phenomenon is not known. It might reflect actual physical dislodgment of cells from vessels due to endothelial injury at the time of vaso-occlusion or it might be due to molecules such as thrombin that can cause the release of endothelial cells from underlying matrix proteins in vessels or bone marrow. Our data show that in patients with sickle cell anemia circulating endothelial cells tend to be viable. These cells tend to be microvascular in origin, as defined by the marker CD36.[27,28] The increased numbers of circulating endothelial cells at the onset of an acute painful crisis consist mostly of CD36-positive cells. We cannot say with certainty that CD36-negative circulating endothelial cells are not microvascular in origin, since CD36-negative endothelial cells have been identified in dermal microvessels.[29]

The circulating endothelial cells in patients with sickle cell anemia tend to have an activated phenotype, as evidenced by the expression of four adhesion molecules: ICAM-1, VCAM-1, E-selectin, and P-selectin. ICAM-1 is constitutively expressed in small amounts but increases after the activation of endothelial cells; the other three markers are expressed only on activated endothelium. Preliminary studies indicate that circulating endothelial cells in patients with sickle cell anemia express tissue factor abnormally,[30] suggesting that the endothelium has a procoagulant phenotype in addition to the proadhesive phenotype identified here. The proportion of circulating endothelial cells with activation markers was the same for live and dead cells, indicating that the activated phenotype is not an artifact due to cell death; indeed, it suggests that the circulating endothelial cells died after activation. If circulating endothelial cells truly represent in situ endothelial cells, our data imply that the vessel-wall endothelium is activated in patients with sickle cell anemia, whether they are in a steady state or an acute painful crisis. We do not know why circulating endothelial cells are increased and activated in patients in a steady state, but recent data indicate that markers of inflammation, such as C-reactive protein, are elevated variably or even chronically in sickle cell anemia.[26,31,32] Chronic activation of endothelial cells could be a risk factor for vaso-occlusion, and fluctuations in the activation of these cells (in response to biologic modifiers) might account for the apparently random attacks of vaso-occlusive crises.

It is likely that endothelial-cell activation increases the risk of vaso-occlusion.[26] In addition to (or instead of) the sickling process, vaso-occlusion may involve disturbances of endothelial function that influence hemostasis, or the adhesion of red cells and white cells to endothelium may promote vaso-occlusion.[26] The four adhesion molecules that we tested are involved in white-cell adhesion, and VCAM mediates the adhesion of sickle red cells. Whether endothelial-cell injury or dysfunction (as opposed to activation) also contributes to the vascular pathobiology of sickle cell disease is unknown.

Supported by a grant (HL 55174) from the National Institutes of Health.

We are indebted to Barrie Miller for assisting with monoclonal-antibody production and to Dr. Steve Nelson for providing samples from children with sickle disease.

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