**Rickettsia rickettsii** Infection of Cultured Endothelial Cells Induces Release of Large von Willebrand Factor Multimers From Weibel-Palade Bodies

By Lee Ann Sporn, Rui-jin Shi, Sarah O. Lawrence, David J. Silverman, and Victor J. Marder

The clinical manifestations of Rocky Mountain spotted fever (RMSF) result from *Rickettsia rickettsii* (*R. rickettsii*) infection of endothelial cells and are mediated by pathologic changes localized to the vessel, including in situ thrombosis and tissue ischemia. This study uses in vitro infection of cultured human umbilical vein endothelial cells with *R. rickettsii* to test the hypothesis that such infection induces von Willebrand factor (vWF) release from Weibel-Palade bodies, a process that could contribute to thrombotic changes. At 24 hours postinfection, there was an increase in metabolically prelabeled large multimers of vWF in the culture medium, with a concomitant decrease of these forms in the cell lysate samples. This release reaction was specific for the large multimer pool of vWF, localized to Weibel-Palade bodies, because no change in the distribution of dimeric forms between cells and culture medium was detected. Double-label immunofluorescence staining showed an inverse corre-

ROCKY MOUNTAIN spotted fever (RMSF) is an acute febrile illness characterized by malaise, myalgia, and a maculo-petechial rash following about 4 days after exposure to the *Rickettsia rickettsii* (*R. rickettsii*) organism, usually transmitted by the wood tick (*Dermacentor andersoni*) or dog tick (*D. variabilis*). Untreated, the illness progresses to shock, coma, and lethal outcome in 14% of cases, and mortality even with appropriate antibiotic treatment is 6%.1,2 The major underlying pathology in humans and in experimental models such as the guinea pig is a diffuse vasculopathy caused by infection of endothelial cells, perivascular inflammatory response, and subsequent vascular thrombosis in many organs, especially the brain and heart.3,4 Although the plasma fibrinogen concentration initially increases as part of an acute phase reaction,1 the early appearance of thrombocytopenia7 and increased fibrin degradation products reflect the widespread consumption coagulopathy associated with endothelial damage.8 The early and integral feature of such consumption was shown in human volunteers receiving *R. rickettsii* challenge as part of vaccine assessment, in which fibrinopeptide A levels increased, prothrombin time was prolonged, and factor VII (FVII) levels decreased within 6 hours after fever onset with accompanying platelet activation and thrombocytopenia.6

*R. rickettsii* is an obligate intracellular bacterial parasite that has been studied by in vitro infection of cultured human umbilical vein endothelial cells.9,10 Such infection is characterized by adherence, entry, intracellular replication, and cell-to-cell spread, all of which can occur without endothelial cell death.11 Specific ultrastructural changes noted in chicken embryo fibroblasts and endothelial cells infected with *R. rickettsii* include dilatation of the endoplasmic reticulum and outer nuclear envelope and the formation of intracellular cisternae frequently containing large numbers of the organism.10,12 *R. rickettsii* infection is associated with increased adherence of platelets to endothelial cells and surrounding subendothelium by an as yet unknown mechanism.14

von Willebrand factor (vWF) is a principal adhesive glycoprotein synthesized and secreted by endothelial cells that mediates attachment of platelets to the vascular wall. Various physiologic or noxious stimuli can induce release of vWF from the specialized storage vesicles, the Weibel-Palade bodies. Thus, in response to soluble agents such as thrombin or histamine, to particulate fibrin clots, or to the calcium ionophore A23187, endothelial cells selectively release the largest multimers of vWF possessing maximal functional integrity.15 This process presumably contributes to the protective effect of hemostasis or serves as a harmful reaction to injury leading to thrombosis, whether such release occurs directly into the vessel lumen or abuminally into subendothelial matrix.16 The current study tests the hypothesis that *R. rickettsii* infection of human umbilical vein endothelial cells in culture induces the release of vWF from Weibel-Palade bodies.

**MATERIALS AND METHODS**

**Culture of endothelial cells and metabolic labeling.** Primary cultures of endothelial cells were established from human umbili-

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First passage cells (used for studies) were cultured in McCoy's 5a medium (Flow Laboratories, Inc, McLean, VA) with 20% fetal bovine serum, 50 μg/mL endothelial mitogen (Biomedical Technologies, Inc, Stoughton, MA), 100 μg/mL heparin (Sigma Chemical Co, St Louis, MO) and 25 μg/mL insulin in the absence of antibiotics. Endothelial cells were metabolically labeled with $^{35}$S-cysteine (25 kCi/mL, 600 Ci/mmol) (Amersham Corp, Arlington Heights, IL). VERO cells (kidney, African Green Monkey) were obtained from American Type Culture Collection (Rockville, MD). Tetracycline and Escherichia coli lipopolysaccharides (serotype 011:B4) were obtained from Sigma.

Measurement of cell viability and membrane integrity. Percent viable cells (control and infected) was determined by the ability of cells to exclude trypan blue. Cells were trypsinized and incubated for 1 minute with 0.2% Trypan Blue Stain (Sigma), and quantitated using a hemocytometer. For studies of $^{51}$Cr release, cell cultures were preloaded using 1 μCi/mL $^{51}$Cr (250 to 500 mCi/mg; Amersham Corp, Arlington Heights, IL), and quantitated using the following formula: cpm in medium - cpm in cells.

Infection of endothelial cells with R rickettsii. A plaque-purified seed of Rickettsia typhi with a titer of $10^7$ plaque-forming units (pfu)/mL was prepared in VERO (kidney, African green monkey) cells (American Type Culture Collection) as previously described and used to infect endothelial cells. Endothelial cells were infected by placing dilutions of R rickettsii in culture medium without antibiotics onto cultures of near-confluent endothelial cells and allowing them to incubate at 37°C in a 5% CO$_2$ atmosphere.

Antibodies and immunofluorescence staining. Endothelial cells cultured on glass coverslips were fixed and permeabilized as described previously. For quantitation of percent cells infected with R rickettsii or number of R rickettsii organisms per cell, endothelial cells were stained by placing 20 μL of fluorescein isothiocyanate (FITC)-labeled anti-R rickettsii rabbit globulin (undiluted) (Center for Disease Control, Atlanta, GA) on the coverslip for 30 minutes, rinsing in phosphate-buffered saline, then mounting in Fluoromount-8 (Fisher Scientific, Orangeburg, NY). Double-label immunofluorescence staining was performed as described, using mouse monoclonal anti-R rickettsii rabbit globulin (undiluted) (Syntoics, San Diego, CA) (1:10 dilution) followed by rhodamine-labeled goat antimouse Ig (Cappel Research Product, Durham, NC) then FITC-conjugated anti-R rickettsii rabbit globulin (undiluted). The preparation and characterization of monospecific antisera against human vWF used for immunofluorescence staining and immunopurification were previously described.

Cell lysis, purification of vWF, and gel electrophoresis. Media samples were collected at various timepoints and cells were lysed as previously described. Media samples were centrifuged to remove floating cells, and resulting cell pellets added to cell lysate samples. vWF was immunopurified from cell lysate and culture medium samples as previously described. Samples were resuspended in electrophoresis sample buffer and applied to 2% agarose gels or 5% polyacrylamide gels. Quantitation of vWF in samples was performed by densitometric scanning of autoradiographs of reduced gels.

Statistics. One-way analysis of variance (ANOVA) was performed to analyze the redistribution of metabolically labeled vWF occurring in the cell culture as a result of infection. Chi-square partitioning was used in the analysis of the relationship between numbers of Weibel-Palade bodies and numbers of R rickettsii in endothelial cells.

**RESULTS**

**Infection of endothelial cells with R rickettsii.** Figure 1 shows the progress of infections using five concentrations of R rickettsii, monitored over a 72-hour period. There was a progressive increase in percent cells infected with time at all concentrations of R rickettsii tested. With increasing concentrations of R rickettsii, the progress of the infection became more rapid. Based on these results, a concentration of $5.2 \times 10^4$ pfu/mL was used in subsequent experiments of slow, progressive infection, with 95% of cells infected within 72 hours, and a concentration of $5.2 \times 10^4$ was used for experiments of rapid and complete infection (100% infection within 6 hours).

At various times during the course of a slow, progressive infection ($5.2 \times 10^4$ pfu/mL), the number of R rickettsii organisms per cell was quantitated by microscopic observation after immunofluorescence staining using an R rickettsii antibody (Table 1 and Fig 2). There was an accumulation of R rickettsii organisms in the endothelial cells with time, with a mean of 1.6 organisms per cell at 4 hours postinfection, and greater than 20 organisms per cell at 48 hours postinfection. At intermediate timepoints (12 to 24 hours), the number of R rickettsii organisms in individual cells was highly variable.

Increased secretion of large multimeric forms of vWF from endothelial cells during R rickettsii infection. The multimeric composition of cell-associated and secreted vWF from metabolically prelabeled endothelial cells is shown in Fig 3. Cells were infected after removal of labeled medium and samples were obtained 24 hours after the initiation of R
rickettsii infection. Cells infected with $5.2 \times 10^6$ pfu/mL $R$ rickettsii showed a striking decrease in the cell lysate and a corresponding increase in the culture medium of the very largest multimeric forms of vWF. On the other hand, there was no apparent change in the amount of vWF dimer in the infected cells or medium. These results indicate that vWF release occurred solely from the Weibel-Palade body storage compartment. Only a slight alteration in the multimeric composition was seen when $5.2 \times 10^5$ pfu/mL $R$ rickettsii was used to infect the cells (lane 2, Fig 3). Similar redistribution of multimeric forms between cells and culture medium was observed after stimulation of cells for 8 or 24 hours with bacterial lipopolysaccharides (1 μg/mL).

The quantitative redistribution of the various multimeric forms of vWF was studied in four experiments of endothelial cells infected with $5.2 \times 10^5$ and $5.2 \times 10^6$ pfu/mL, as compared with uninfected cells (Fig 4). At 24 hours, the percent of total vWF (multimer plus dimer) appearing in the medium was significantly increased as a result of infection and a linear trend in the means was shown with increasing level of infection ($P = .007$). Such a linear trend was shown when percent of total multimer ($P = .001$) but not dimer ($P = .42$) secreted was analyzed with increasing level of infection. The redistribution of vWF between cells and culture medium resulted in an absolute increase in the amount of vWF appearing in the medium due to infection. Infection with $5.2 \times 10^5$ pfu/mL for 24 hours resulted in a twofold increase in total vWF (dimers plus multimers) and a threefold increase in multimers when compared with uninfected controls.

Results obtained with a higher concentration of organisms ($5.2 \times 10^6$ pfu/mL) showed a faster rate and extent of infection with 72% of endothelial cells containing $>20 R$ rickettsii after only 8 hours of incubation.

Table 1. Number of Infected Endothelial Cells and the Number of $R$ rickettsii per Endothelial Cells as Related to Duration of Infection With $5.2 \times 10^5$ pfu/mL

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Percent Infected</th>
<th>No. of $R$ rickettsii per Cell (range)</th>
<th>No. of $R$ rickettsii per Cell (mean)</th>
</tr>
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<tbody>
<tr>
<td>4</td>
<td>8</td>
<td>1-3</td>
<td>1.6</td>
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<tr>
<td>12</td>
<td>20</td>
<td>1-8</td>
<td>3.1</td>
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<tr>
<td>24</td>
<td>45</td>
<td>2-20</td>
<td>3.9</td>
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<tr>
<td>48</td>
<td>90</td>
<td>&gt;20</td>
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After 72 hours of infection virtually all of the vWF (multimer plus dimer) was present in the medium, 88% and 97%, respectively, for cells infected with $5.2 \times 10^5$ or $5.2 \times 10^6$ pfu/mL, as compared with only 53% in the medium of uninfected cells. However, at this timepoint, there was evidence of lysis of the infected endothelial cells. First, there was an increase of vWF dimers in the culture medium. While only 40% of dimers were found in the medium of uninfected cultures, 74% and 85% of the dimers were found in the medium of cells infected with $5.2 \times 10^5$ and $5.2 \times 10^6$ pfu/mL, respectively. More importantly, the 260-Kd precursor subunit of vWF appeared in the culture medium of infected cells at 72 hours. This subunit was abundant in the cell lysate of 24-hour control and infected cultures, but was undetected in the medium of these cultures (Fig 5). Because the 260-Kd subunit is not secreted constitutively or with stimulation, its presence in the medium only at 72 hours is indicative of cell lysis at this time point.

Cell viability, as measured by the ability of cells to exclude trypan blue, was monitored over the course of the infection (Fig 6). There was no decrease in cell viability when compared with uninfected controls at 4.5 or 24 hours. However, progressive loss of cell viability was observed at 48 and 72 hours, with both levels of infection. Consistent with this loss of cell viability was progressive detachment of the cells at 48 and 72 hours after the initiation of infection. Increase in $^{51}$Cr release from cells, a measure of membrane integrity, was not observed until 72 hours after the initiation of infection, and was seen only with the high level of infection ($5.2 \times 10^6$ pfu/mL). At this late timepoint, a 30% increase in percent $^{51}$Cr released from infected cells was observed when compared with uninfected controls incubated for the same time period.

We tested the possibility that culture medium conditioned by endothelial cells infected with $R$ rickettsii contained soluble factors that could induce release of multimers of vWF in uninfected endothelial cells. Endothelial cells were infected with $5.2 \times 10^6$ pfu/mL $R$ rickettsii for 24 hours, culture medium was collected, and $R$ rickettsii organisms removed by filtering through a 0.22-μm filter and then treating with 5 μg/mL tetracycline. Exposure of metabolically prelabeled uninfected endothelial cells to this “conditioned medium” for 24 hours did not increase the vWF.

Fig 2. Immunofluorescence staining of infected endothelial cells. Human umbilical vein endothelial cells cultured on glass coverslips were fixed and stained by fluorescence using anti-$R$ rickettsii antibody 24 hours after the initiation of infection. (a) Two cells infected with 4 and 12 $R$ rickettsii, respectively. (b) A cell infected with greater than 20 $R$ rickettsii. Bar = 10 μm.
multimer content in the medium (data not shown). Also, 24-hour exposure of endothelial cells to a suspension of uninfected VERO cells (which are used to prepare the *R. rickettsii* inoculum) at the same concentration as in a 5.2 × 10⁷ pfu/mL infection resulted in no multimer increase in medium (data not shown).

**Relationship between the presence of *R. rickettsii* organisms and number of Weibel-Palade bodies in endothelial cells.** The relationship between the number of *R. rickettsii* organisms per endothelial cell and the number of Weibel-Palade bodies in the same cell was studied in a cell population infected so as to achieve a slow, progressive infection. Figure 7 illustrates endothelial cells from a population infected for 24 hours with 5.2 × 10⁷ pfu/mL *R. rickettsii*, followed by double-label immunofluorescence staining. While uninfected cells in this population contained Weibel-Palade bodies (panels c and d), cells containing many *R. rickettsii* organisms were often devoid of Weibel-Palade bodies (panels a and b). Cells in the population were scored as to the number of *R. rickettsii* and number of Weibel-Palade bodies present (Fig 8). Cells containing no *R. rickettsii* had variable numbers of Weibel-Palade bodies. In these uninfected cells, approximately equal numbers of cells contained 0 (none), 1+ (1 to 3), 2+ (3 to 30), and 3+ (greater than 30) Weibel-Palade bodies. With only minimal numbers of *R. rickettsii* (1 to 2) per endothelial cell, there was an increase in percent of cells with 0 and 1+ amounts of Weibel-Palade bodies, and a concomitant decrease in 2+ or 3+ cells. With greater than three *R. rickettsii* per cell, there were more cells with no Weibel-Palade bodies, and a decrease in 1+, 2+, and 3+ cells. With greater than 20 *R. rickettsii* per cell, virtually no cells contained Weibel-Palade bodies. Chi-square analysis showed that the inverse relationship between the number of Weibel-Palade bodies and the number of *R. rickettsii* per cell was highly significant (*P* = .002), even when as few as 1 to 2 *R. rickettsii* were present per cell.
Fig 5. Subunit composition of vWF immunopurified from cell lysates and culture media after Rickettsia infection. vWF immunopurified from metabolically prelabeled endothelial cell cultures was analyzed, reduced, on 5% polyacrylamide gels, autoradiographs of which are shown. At 24 hours after infection with 5.2 × 10⁴ pfu/mL R. rickettsii, cell lysates (C) contained both the 260-Kd intracellular precursor form and the 220-Kd mature forms of vWF, whereas the culture medium (M) contained only the mature (220 Kd) form and a trace of slightly larger (275 Kd) precursor form with processed carbohydrates. The cleaved, 100-Kd propolypeptide of vWF was also present in cell lysate and medium samples. Seventy-two hours after infection, however, the intracellular precursor form of vWF (260 Kd) as well as processed forms (220 Kd and 275 Kd) appeared in the culture medium. Samples from uninfected cultures incubated for 72 hours contained 260-Kd and 220-Kd forms in cell lysates and only 220-Kd and a trace of 275-Kd forms in the culture medium.

DISCUSSION

The Weibel-Palade bodies of endothelial cells contain a pool of only the largest multimeric forms of vWF composed of fully processed subunits (220 Kd) which can be readily released in response to an injury signal. Predominantly dimeric molecules and lower molecular weight forms are secreted constitutively from endothelial cells by a second, distinct secretory pathway. Rickettsia infection of metabolically prelabeled endothelial cells resulted in an increase in release of preformed large multimers of vWF into the culture medium, with a relative depletion of large multimeric forms from the cell lysate. There was no redistribution of the intracellular pool of dimeric molecules as a result of infection, indicating that infection specifically caused an increase in regulated secretion originating from the Weibel-Palade bodies. This release of Weibel-Palade body contents occurred without detectable cell lysis, as evidenced by the absence of the 260-Kd intracellular vWF precursor from the culture medium (Fig 5) at timepoints when large multimer release was observed.

Release of vWF from Weibel-Palade bodies induced by thrombin, the calcium ionophore A23187, histamine, or fibrin is characterized by a very rapid appearance of large multimers of vWF in the medium of cultured cells and a concomitant decrease in Weibel-Palade body number within the cells (occurring within minutes of cell stimulation). The release observed with R. rickettsii infection was much slower, minimally detectable at 8 hours after infection (not shown) and becoming statistically significant at 24 hours. Other agents have been shown to cause regulated secretion of vWF with a relatively long time course. Bacterial lipopolysaccharides (LPS) slowly increase the release of vWF from endothelial cells between 2 and 24 hours without causing detectable cellular lysis and without increasing vWF synthesis. This relatively slow release process was also characterized by depletion of intracellular stores and increased appearance of large multimeric forms in the culture medium. Irradiation also induces a gradual release of vWF from a preformed storage pool over a period of 1 to 3 days. After rapid release of Weibel-Palade body contents (such as with the calcium ionophore A23187 or thrombin), some released vWF remains associated with the external cell surface in “patches.” Release patches were absent from R. rickettsii-infected cells (not shown), likely because release occurred slowly and allowed for redistribution or recycling of membrane-associated vWF. Similar results occurred after the relatively slow release induced by irradiation.

Several lines of evidence suggest that the cellular association of R. rickettsii organisms is required to induce regulated secretion of vWF. Likely, the R. rickettsii organisms penetrated the cell membrane of endothelial cells almost imme-
Fig 7. Double-label immunofluorescence staining of endothelial cells infected with *R. rickettsii*. Endothelial cells from a population infected with 5.2 × 10^6 pfu/mL for 24 hours were fixed and stained by fluorescence using anti-vWF antiserum (b and d) and anti-*R. rickettsii* antiserum (a and c). Shown is an endothelial cell with greater than 20 *R. rickettsii* organisms (a), and the same cell in (b), showing lack of Weibel-Palade bodies. An uninfected cell in the population is shown in (c), and this cell contains numerous Weibel-Palade bodies (d). Bar = 10 μm.

Fig 8. The relationship between the number of *R. rickettsii* organisms and number of Weibel-Palade bodies in endothelial cells. Endothelial cells cultured on glass coverslips and infected with 5.2 × 10^6 pfu/mL for 24 hours were fixed, and double-label immunofluorescence staining was performed using anti-vWF and anti-*R. rickettsii* antibodies. Each cell was scored as to number of Weibel-Palade bodies present as follows: 0 ( ), no Weibel-Palade bodies, 1+ ( ), 1 to 3, 2+ ( ), 3 to 30, and 3+ ( ), greater than 30) Weibel-Palade bodies. The same cell was also scored as to number of *R. rickettsii* organisms present.

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<th>NUMBER OF <em>R. Rickettsii</em> PER CELL</th>
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<td>% ENDOThelial CELLS</td>
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The observed inverse correlation between number of *R. rickettsii* and number of Weibel-Palade bodies per individual cell also lends support to the notion that the cellular association of the organism is required for Weibel-Palade body release. However, the interpretation of this result is complicated by the fact that *R. rickettsii* infection decreases vWF biosynthesis and multimer formation (unpublished observations, July 1990). The decreased formation of multimers may have been due to an inability of infected cells to package vWF into Weibel-Palade bodies, which likely are sites of multimerization. Weibel-Palade body formation is, immediately on contact and were present intracellularly at all time points that were studied. Because vWF release induced by *R. rickettsii* infection occurred in a cell culture system in the absence of circulating inflammatory cells, release was independent of the presence of inflammatory mediators such as histamine, γ-interferon, interleukin-1, or the assembly of complement components C5b-9, all of which have been shown to induce or modulate vWF release. Because medium from infected cells did not induce Weibel-Palade body release from uninfected endothelial cells, it is unlikely that a secreted mediator, such as LPS, induced vWF release. Any involvement of rickettsial LPS would probably be limited to an intracellular effect, a conclusion that is in agreement with prior observations of limited or no presence of LPS in the blood of the patients with RMSF. The observed inverse correlation between number of *R. rickettsii* and number of Weibel-Palade bodies per individual cell also lends support to the notion that the cellular association of the organism is required for Weibel-Palade body release. However, the interpretation of this result is complicated by the fact that *R. rickettsii* infection decreases vWF biosynthesis and multimer formation (unpublished observations, July 1990). The decreased formation of multimers may have been due to an inability of infected cells to package vWF into Weibel-Palade bodies, which likely are sites of multimerization. Weibel-Palade body formation is, immediately on contact and were present intracellularly at all time points that were studied. Because vWF release induced by *R. rickettsii* infection occurred in a cell culture system in the absence of circulating inflammatory cells, release was independent of the presence of inflammatory mediators such as histamine, γ-interferon, interleukin-1, or the assembly of complement components C5b-9, all of which have been shown to induce or modulate vWF release. Because medium from infected cells did not induce Weibel-Palade body release from uninfected endothelial cells, it is unlikely that a secreted mediator, such as LPS, induced vWF release. Any involvement of rickettsial LPS would probably be limited to an intracellular effect, a conclusion that is in agreement with prior observations of limited or no presence of LPS in the blood of the patients with RMSF. The observed inverse correlation between number of *R. rickettsii* and number of Weibel-Palade bodies per individual cell also lends support to the notion that the cellular association of the organism is required for Weibel-Palade body release. However, the interpretation of this result is complicated by the fact that *R. rickettsii* infection decreases vWF biosynthesis and multimer formation (unpublished observations, July 1990). The decreased formation of multimers may have been due to an inability of infected cells to package vWF into Weibel-Palade bodies, which likely are sites of multimerization.
Indeed, a process easily disrupted by cellular perturbation such as with ammonium chloride, monensin, or microtubule-depolymerizing agents. This probable decreased ability of infected cells to form Weibel-Palade bodies could have contributed to the decrease in Weibel-Palade body content of infected cells. Weibel-Palade bodies have also been shown to slowly disappear (within 1 to 4 days) from endothelial cells infected with cytomegalovirus (CMV). However, this effect is likely due to decreased Weibel-Palade body formation alone because there was no stimulated release of vWF into the culture medium of CMV-infected endothelial cells.

The vascular thrombosis that appears in RMSF is likely due to a spectrum of changes that occur in Rickettsia-infected endothelial cells resulting in an increase in the procoagulant properties of the vessel wall. Our study shows that R. rickettsii infection of endothelial cells induces the release of Weibel-Palade body vWF. The vWF release observed in this study may not directly influence the pathologic changes occurring in RMSF, but such release may represent only a coincident or secondary manifestation of a wider disruption of antithrombotic endothelial cell biology. The modest elevation of plasma vWF concentration noted in volunteers inoculated with the R. rickettsii organism is not incompatible with our demonstration of vWF release, because such Weibel-Palade body release may be primarily abuminal, as shown for other secretagogues. The timecourse of our observations parallels the clinical studies and indicate that the changes described in this report are in the same time frame of infection, with increased plasma vWF being observed as early as 6 hours after fever onset. Disruption of endothelial cell metabolism by R. rickettsii infection could have other actions of greater relevence for vascular thrombosis, eg, by expression of tissue factor activity as shown for other endothelial cell infections, alterations in thrombomodulin regulation, exposure of cell attachment receptors, or other known relevant mechanisms. Further study is needed to better understand their relative roles in thrombosis induction of RMSF.

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