Chronic Meningococcemia
Cutaneous Lesions Involve Meningococcal Perivascular Invasion Through the Remodeling of Endothelial Barriers

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Chronic meningococcemia is a form of sepsis with frequent polymorphous skin lesions. Both in vivo and in vitro data suggest that, in these lesions, meningococci gain access from the capillary lumen to the peripheral extravascular compartment, in the absence of vascular dislocation, through a paraendothelial route.

Chronic meningococcemia (CM) is a form of meningococcal sepsis that involves, in the absence of meningitis, recurrent fever for at least 1 week, frequent arthralgia and polymorphous skin eruption with purpuric and nonpurpuric lesions [1]. Reports on invasive meningococcal infections showing the presence of Neisseria meningitidis in many organs have highlighted the ability of blood-conveyed N. meningitidis to interact with central or peripheral endothelia [2–4]. For meningitis, several hypotheses have been proposed for the opening of the blood-brain barrier by N. meningitidis. A transcytosis model based on results obtained with peripheral endothelial cells [5, 6] has recently been confronted with evidence, in a brain endothelial cell line model of infection, for a paracellular route for N. meningitidis through the blood-brain barrier [7, 8].

In the case of CM, bacteria are found in skin lesions, but so far there has been no evidence for the route by which N. meningitidis might gain access to the extravascular space. Taking advantage of a case of chronic meningococcemia, this report shows that, in vivo, purpuric and nonpurpuric CM cutaneous lesions are associated with the colonization of infected capillaries by piliated meningococci and that the translocation of N. meningitidis toward the perivascular compartment can occur in the absence of vascular dislocation. Besides internalization of N. meningitidis into endothelial cells, a combination of in vivo and in vitro data strongly suggests that the extravasation of N. meningitidis toward the extraluminal compartment relies on a paracellular route involving the remodeling of vascular endothelial (VE)–cadherin interendothelial junctions triggered by N. meningitidis.

PATIENT AND METHODS

A 51-year-old man was admitted for fever, tachycardia, lymphangitis on 4 limbs, and rash compound with multiple purpuric maculae of the extremities, some of them surrounded by pustules. The patient had been symptomatic for 4 days but showed no sign of meningitis nor of arthritis. He had a 20-year history of human immunodeficiency virus (HIV) infection, well controlled by highly active antiretroviral therapy. Skin biopsies were taken on the day of admission prior to administration of antibiotics, either from purpuric macular lesions or from red streaks on faint nonpurpuric lesions. Despite antibiotic treatment, the patient condition worsened with apparition of arthritis on 1 knee after 2 days, and with pleuritis after 4 days. Gentamicin was then added to ceftriaxone, and symptoms finally resolved within the next 2 days. The patient, who did not present with any detectable defect in the complement pathway, was discharged after 2 weeks with remission of skin lesions and of arthritis. Apart from skin contaminants, no viable bacteria were cultured from skin specimen or blood culture, but genomic amplification using N. meningitidis–specific primers eventually revealed the presence of a serogroup X meningococcal strain (Supplementary Data).

RESULTS

The tissular distribution of N. meningitidis was analyzed in both purpuric and nonpurpuric lesions with a combination of
hematoxylin-eosin-safran (HES), immunoperoxidase, immunofluorescence, and transmission electron microscopy (TEM) analysis. In the purpuric lesion, the epidermis appeared remodeled with pericapillary infiltration of lymphocytes and polymorphonuclear leukocytes and some blood vessels appeared disrupted with thrombus-like structures. Unlike the purpuric sample, HES staining of the nonpurpuric lesion showed no visible disruption of blood vessels and a moderate inflammatory infiltrate. However, for both lesions, immunoperoxidase staining of meningococcal type IV pili (tfp) showed the presence of *N. meningitidis* in blood capillaries and in the extravascular space, where they appeared as bacterial foci concentrically located around infected capillaries (Figure 1A and 1B). More infected capillaries were seen in the purpuric lesion, compared with the nonpurpuric one. In infected capillaries, immunofluorescence labeling of meningococcal tfp and of von Willebrand factor, as a marker of endothelial cells, showed intimate attachment of piliated meningococci to the endothelium (Figure 1C). TEM analysis further showed the intimate attachment of some extracellular meningococci to collagen fibers and the intraluminal organization of tfp as network-forming bundles (Figure 1D and 1E). These results suggest that, in CM, cutaneous lesions are associated with the extravasation of meningococci, and that dislocation of the endothelial barrier is not a prerequisite for *N. meningitidis* to gain access to the extravascular compartment.

Immunofluorescence labeling of tfp and of either von Willebrand factor (Figure 1C) or VE-cadherin, as a marker of endothelial junctions (Figure 2A), suggested the ubiquitous localization of piliated *N. meningitidis* in cutaneous lesions. The intraluminal, intraendothelial, and extravascular presence of *N. meningitidis* in morphologically conserved capillaries was confirmed by TEM analysis (Figure 2D and 2E). The presence of diplococcus-containing endocytic vesicles on the luminal side of endothelial cells sustained the hypothesis of an endothelial invasion by *N. meningitidis*. Intracytoplasmic bacteria were not contained in vacuoles, and no image of basolateral vesicle, which might have been evocative of meningococcal transcytosis, was observed.

In addition to meningococcal internalization into endothelial cells, TEM analysis of infected capillaries also showed the presence of *N. meningitidis* at interendothelial junctions (Figure 2F and 2G). Although remodeled, intercellular contacts were, at least apparently, not abolished, and endothelial lining of the capillary was conserved. Infected interendothelial junctions were also analyzed with immunofluorescent 3-dimensional reconstruction of capillary tangential cuts. This allowed for the spatial visualization of longitudinal intercellular VE-cadherin junctions and showed that, where piliated meningococci were present, intercellular VE-cadherin junctions were dismantled (Figure 2B and Supplementary Video 1).

In order to investigate if the remodeling of interendothelial junctions was specifically due to *N. meningitidis*, we used an in vitro infection model of human dermis microvascular endothelial cells (HDMECs). The in vitro infection of HDMECs with the piliated *N. meningitidis* 2C4.3 strain showed a selective redistribution of VE-cadherin junctions where meningococci were present (Figure 2C and Supplementary Video 2), thus supporting the hypothesis that the remodeling of interendothelial junctions observed in vivo was triggered by *N. meningitidis*. Because the patient’s infecting strain and the experimental strain *N. meningitidis* 2C4.3 belong to different
results suggest that, beside meningococcal internalization into peripheral endothelial cells, the pathophysiology of CM cutaneous lesions involves the remodeling of dermis capillary interendothelial junctions driven by \textit{N. meningitidis} and the invasion of the perivascular compartment by \textit{N. meningitidis} through a paracellular route.

**DISCUSSION**

With a combination of in vivo and in vitro analysis, these results show that, in the pathogenesis of CM cutaneous lesions, vascular dislocation is not a prerequisite for the dissemination of \textit{N. meningitidis} from blood vessels to the perivascular space and that, besides \textit{N. meningitidis} internalization into endothelial cells, \textit{N. meningitidis} triggers the remodeling of VE-cadherin interendothelial junctions.

Both host and bacteriological factors have been associated with invasive meningococcal diseases, and CM may be observed with strains that do not belong to major hyperinvasive genetic lineages such as serogroup X. Although being responsible for recent outbreaks in Niger, Burkina Faso, and Togo, this serogroup accounts for $<0.5\%$ of reported invasive meningococcal infections in France \cite{9, 10}.

The in vivo internalization of \textit{N. meningitidis} into endothelial cells is consistent with a transcytosis model for the crossing of the blood-brain barrier that was previously suggested from in vitro experiments using peripheral endothelial cells \cite{5, 6}. However, the in vivo material brought no further evidence for transcytosis as a major route for the crossing of peripheral endothelial barriers by \textit{N. meningitidis}. First, intracellular bacteria were not observed within vacuoles and the in vivo persistence of \textit{N. meningitidis} within peripheral endothelial cells remains unknown. Second, the absence of any exocytic vesicles, which one would expect to find on the basolateral side of endothelial cells, suggests that they might not exist unless as a rare occurrence. On the other hand, the analysis of in vivo specimen and in vitro experiments showed that (1) extravasation of \textit{N. meningitidis} into the dermis does not require the dislocation of the endothelial barrier; (2) pilated \textit{N. meningitidis} concentrate at intercellular endothelial junctions; (3) VE-cadherin junctions are remodeled in vivo at paracellular sites where \textit{N. meningitidis} is present; (4) the disruption of VE-cadherin junctions in peripheral dermis endothelial cells can be reproduced in vitro upon infection with \textit{N. meningitidis}; and (5) the remodeling of VE-cadherin junctions triggered by \textit{N. meningitidis} is not restricted to a particular serogroup. These data thus strengthen the hypothesis that, in CM cutaneous lesions, the paracellular route is essential for \textit{N. meningitidis} to gain access from the vascular lumen to the extravascular compartment. These results are consistent with those obtained in an in vitro model for

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**Figure 2.** Meningococcus internalization by endothelial cells and meningococcus-triggered effacement of VE-cadherin interendothelial junctions. **A**, In vivo infected capillary (purpuric lesion), transverse view. Immunofluorescence labeling of interendothelial junctions (VE-cadherin, green), meningococcal type IV pili (tfp, red) and nucleus (DAPI, blue). Pilated meningococci are located in the lumen as well as surrounding the nucleus of an endothelial cell, thus suggesting both extra- and intracellular locations. **B**, In vivo-infected capillary (purpuric lesion), tangential view. Immunofluorescence labeling of VE-cadherin (green), meningococcal tfp (red), and nucleus (DAPI, blue). Longitudinal intercellular junctions (plain arrows) are abolished where meningococcal foci are present (double arrowheads). **C**, In vitro infection of HDMECs with \textit{Neisseria meningitidis} 2C4.3. Nucleus and bacteria (DAPI, blue) and VE-cadherin (green). Interoendothelial VE-cadherin junctions appear linear in the absence of meningococci (plain arrow) but are specifically dismantled where meningococci are present (double arrowheads). **D**, Transmission electron microscopy (TEM) transverse view of an in vivo–infected capillary. Intraluminal meningococci are observed at the site of an interendothelial junction (white arrow) or being internalized into an endothelial cell (E). Nu, nucleus; RBC, red blood cell. **E**, Magnification of (D). Endocytosis of a diplococcus (white arrow) by an endothelial cell into an electron-dense vesicle (black arrow). **F** and **G**, TEM micrograph of in vivo interendothelial junctions in an infected capillary. Neighboring endothelial cells are delineated with colored lines. Meningococci (arrows) are seen in the lumen and at intercellular junctions (F and G), as well as inside endothelial cells (G). Abbreviations: Col, collagen fibers; Lu, lumen; Nu, nucleus.

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serogroups (ie, serogroups X and C, respectively), these results also show that the redistribution of VE-cadherin endothelial junctions in the dermis is not restricted to a particular meningococcal serogroup. Taken together, these
meningitis using hCMEC/D3 human brain microvascular endothelial cells showing that the \textit{N. meningitidis}–driven redistribution of components of endothelial intercellular junctions would open the way for a paracellular route for \textit{N. meningitidis} through the blood-brain barrier [7, 8]. Furthermore, the abundance of intraluminal tfp in infected peripheral capillaries supports the central role of these bacterial attributes for the attachment of \textit{N. meningitidis} to endothelial cells that was reported in experimental conditions of shear stress [11].

Further work will be needed to investigate the molecular mechanisms that rule the peripheral extravasation of \textit{N. meningitidis} and which bacterial or human factors influence the clinical evolution either as a chronic illness or as an acute, and sometimes fulminant, disease such as meningitis.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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References