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## Anti-thrombotic treatment enhances antibiotic efficiency in a humanized model of meningococemia

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1                   **Anti-thrombotic treatment enhances antibiotic efficiency**  
2                   **in a humanized model of meningococemia**

3  
4   Running title: Antibiotic resistance during *Nm* sepsis

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6  
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21  
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## 23 **Abstract**

24 Meningococcal infections remain particularly difficult to treat. Despite antibiotic therapy, the  
25 state of the patients often rapidly deteriorates. Early clinical studies suggest that meningococci  
26 acquire a form of resistance to antibiotic treatments during infections. Taking advantage of a  
27 humanized animal model of infection, we confirm that adherent bacteria become highly  
28 resistant to antibiotic treatments as early as 3-6 hours post infection, although fully sensitive *in*  
29 *vitro*. Within this time frame, meningococci adhere to the endothelium via their type IV pili,  
30 proliferate and eventually fill the vessel lumen. Using intravital imaging, we show that rapidly  
31 upon infection blood flow is dramatically decreased, thus limiting antibiotic access to infected  
32 vessels. Concomitantly, fibrin is deposited inside infected vessels in proximity to bacterial  
33 aggregates. Pharmacologically impairing thrombin generation by inhibiting Factor X activity not  
34 only improves blood flow in infected vessels, but also enhances the efficacy of the antibiotic  
35 treatment. Our results indicate that the combined administration of anticoagulants together  
36 with antibiotics might represent a therapeutic approach to treat meningococcal sepsis more  
37 efficiently.

## 38 Introduction

39 Despite intensive medical care meningitis and *purpura fulminans* induced by *Neisseria*  
40 *meningitidis* remain a major concern worldwide, reflecting our limited understanding of the  
41 mechanisms underlying these diseases. Systemic forms of the infection are often the most  
42 life-threatening as they account for up to 90% of the mortality associated to invasive  
43 meningococcal diseases (IMD) (Brandtzaeg & van Deuren, 2012; van de Beek *et al*, 2006).  
44 Meningococcal sepsis is characterized by specific clinical manifestations such as the  
45 appearance of skin purpuric/petechial lesions and most importantly a particularly fast and  
46 severe progression (Stephens, 2007; Thompson *et al*, 2006). Otherwise healthy patients  
47 evolve from an absence of symptoms to being critically ill within 12 to 24 hours. Although  
48 made difficult by the relatively unspecific clinical features at disease onset, the early  
49 recognition and treatment of meningococcal systemic infections by parenteral administration  
50 of antibiotics is key in determining patient outcome (Dellinger *et al*, 2013). Remarkably,  
51 despite antibiotic administration, disease often progresses relentlessly towards septic shock  
52 making these infections particularly difficult to treat. While it is generally considered that  
53 treatments will be inefficient past a certain tipping point, the reasons for these failures remain  
54 largely unexplained. A possible explanation would be that bacteria acquire a form of  
55 resistance to the treatments at a certain point of infection. Accordingly, biopsies of skin  
56 lesions performed on patients suffering from meningococcal septic shock revealed the  
57 presence of live bacteria up to 13 hours after the initiation of the antibiotic treatment (de  
58 Greeff *et al*, 2008; van Deuren *et al*, 2000; van Deuren *et al*, 1993). The mechanisms  
59 underlying bacterial antibiotic resistance at play during meningococcal infections remains  
60 elusive. Due to the human-species specificity of the bacterium, the lack of reliable animal  
61 models of meningococcal infections has precluded any in-depth experimental studies of this  
62 process. Today, this limitation has been overcome by the development of a human skin  
63 xenograft mouse model that supports the anastomosis of dermal human blood vessels with  
64 the murine circulatory system (Melican *et al*, 2013). This model recapitulates the hallmark

65 features of meningococcal systemic infections, such as the adhesion of bacteria to the  
66 human endothelial surface and the occurrence of skin purpuric lesions, hence allowing the  
67 exploration of *Nm* systemic infections in the full complexity of the host tissue *in vivo* (Bonazzi  
68 *et al*, 2018; Melican *et al.*, 2013). Combining the use of such a model and intravital imaging,  
69 we show that bacteria become resistant to antibiotic treatments as early as 3-6h post  
70 infection and that this effect is due to a fast decrease in blood flow induced by the deposition  
71 of fibrin inside infected vessels.

## 72 **Results**

### 73 **Meningococci adhering along the vascular wall rapidly acquire antibiotic resistance**

74 We first sought to evaluate whether the humanized model of meningococcal infection could  
75 provide new information on the clinical observations pertaining to antibiotic resistance during  
76 infection. In this model, intravenously injected *Neisseria meningitidis* circulate in the blood  
77 stream and a fraction of bacteria specifically colonizes dermal human vessels in the grafted  
78 skin, forming tight bacterial aggregates that progressively fill the infected human vessels (Fig  
79 1A-B) (Manriquez *et al*, 2021; Melican *et al.*, 2013). Bacterial sensitivity to intravenously  
80 injected antibiotics was assessed in the animal model 3h, 6h and 16h post infection by  
81 treating mice with Cefotaxime (CTX, Fig 1C), a standard of care antibiotic for meningococcal  
82 infections in patients. Mice were treated with doses reflecting the concentration used in  
83 patients (200 mg/kg) (Griffiths *et al*, 2018). As for most clinical meningococcal isolates, the  
84 strain used for these experiments is sensitive to low doses of Cefotaxime when cultured in  
85 medium containing serum *in vitro* (Fig EV1). Antibiotic administration led to a strong  
86 reduction in the numbers of blood-circulating bacteria regardless the time post infection (Fig  
87 1D). Numbers of adherent bacteria also decreased upon antibiotic treatment 3h post  
88 infection. In contrast, 6 and 16h post infection, bacteria became strongly resistant to the  
89 antibiotic treatment. While bacterial counts were reduced by up to 11 000-fold 3h post  
90 infection ( $55\,410 \pm 12\,445$  versus  $5 \pm 3$  CFU/mg of skin in non-treated versus CTX-treated  
91 animals, respectively), 16h post infection bacterial counts only decreased by 12-fold ( $90\,731$   
92  $\pm 50\,772$  versus  $7\,642 \pm 2\,200$  CFU/mg of skin in non-treated versus CTX-treated animals,  
93 respectively) (Fig 1E). Similar results were obtained at 6h post infection (Fig 1E). Taken  
94 together, these data show that between 3h and 6h post infection, bacteria adhering to the  
95 endothelium experience a change, either intrinsic or in their environment, which leads to  
96 antibiotic treatment resistance. Given the ability of meningococci to occlude infected vessels  
97 combined with their pro-coagulant activity, a straightforward hypothesis would be that a  
98 reduction in microvascular patency could alter the access of antibiotics to bacteria.

99

100 **Meningococcal vascular colonization leads to a rapid and complete block of blood**  
101 **flow**

102 Using intravital imaging, we next assessed whether meningococcal vascular colonization  
103 alters vascular perfusion and consequently the proper access of antibiotics to sites of  
104 infection. Blood flow velocities were assessed using 1  $\mu\text{m}$  fluorescent microspheres injected  
105 intravenously. High temporal resolution imaging with a spinning disk confocal microscope  
106 allowed the visualization of beads freely circulating in human vessels of non-infected animals  
107 (Fig 2A and Movie EV1). Beads circulated at average speeds of  $1174 \pm 141 \mu\text{m/s}$  in the  
108 observed arterioles ( $n = 31$ , average diameter  $35 \mu\text{m}$ ) and  $505 \pm 219 \mu\text{m/s}$  in venules ( $n =$   
109  $13$ , average diameter  $54 \mu\text{m}$ ). Similar values were observed for the first 2 hours of infection  
110 (Fig 2B). In contrast, a steep reduction was observed at 3 hours post infection in all infected  
111 vessels, culminating in a complete absence of flow at 4 hours post infection (Fig 2B and  
112 Movie EV1). No variation in the blood vessel diameter was observed during the infection (Fig  
113 2C), indicating that blood flow reduction was not due to a global collapse of vessels. We  
114 recently showed that meningococci can infect dermal arterioles and venules (Manriquez *et*  
115 *al.*, 2021) and the same dramatic decrease in blood flow was observed in infected venules  
116 and arterioles (Fig 2D), suggesting that the effect observed is independent of the vessel type.  
117 Taken together, our results indicate that by preventing the perfusion of infected vessels  
118 starting at 3 hours post infection, vascular colonization by meningococci limits the  
119 bioavailability of antibiotics, thus restraining their ability to eliminate intravascular adherent  
120 bacteria. We then explored whether this blood flow reduction was due to the bacteria  
121 themselves or secondary to intravascular coagulation or thrombosis.

122

123 **Platelets are not involved in the acquisition of antibiotic resistance**

124 Meningococcal systemic infections are associated with disturbed coagulation ranging from  
125 vessel occlusion and thrombus formation to disseminated intravascular coagulation (DIC), as  
126 evidenced by the post-mortem analysis of skin biopsies (Harrison *et al.*, 2002; Pathan *et al.*,

127 2003; Sotto *et al*, 1976). Similar observations have been recently made using the humanized  
128 mouse model of meningococcal infections (Manriquez *et al.*, 2021). We therefore tested  
129 whether infection-induced microvascular thrombosis might restrict the proper access of  
130 antibiotics to adherent meningococci.

131 We first assessed the role of platelets in the process. Visualization of platelets by intravital  
132 imaging showed limited platelet aggregation/accumulation, if any, at sites of bacterial  
133 adhesion (Fig EV2A). Platelets could be found in spaces where no bacteria were found, and  
134 small volumes of blood expected. Interestingly, no evidence of bacteria-platelet interactions  
135 could be found, in contrast to what has been observed with other pathogenic bacteria  
136 (Fitzgerald *et al*, 2006; Sun, 2006). We observed circulating platelets moving past bacterial  
137 aggregates without evidence of adhesive contacts (Movie EV2). We then evaluated the  
138 impact of platelet depletion on the efficiency of Cefotaxime treatment (Fig EV2B). To this  
139 end, grafted mice were administered an antibody targeting the platelet receptor  
140 GPIIb/IIIa/CD42b 6h prior to the infection, as previously described (Bergmeier *et al*, 2000;  
141 Nieswandt *et al*, 2000). This treatment led to the efficient depletion of platelets (Fig EV2C).  
142 Antibiotic treatment was then administrated 16h post infection and bacterial counts assessed  
143 3 hours later. As expected, low numbers of blood-circulating bacteria were found upon  
144 antibiotic treatment when compared to the infected but non-treated control grafted animals  
145 (Fig EV2D). Numbers of adherent bacteria following the antibiotic treatment were similar to  
146 the numbers observed in non-depleted control animals ( $6\ 835 \pm 3\ 495$  versus  $7\ 642 \pm 2\ 200$   
147 CFU/mg of skin in platelet-depleted versus non-depleted animals, respectively) (Fig EV2E to  
148 be compared to Fig 1E), suggesting that platelets do not limit the efficacy of antibiotic  
149 treatment upon vascular colonization by *Neisseria meningitidis*. Taken together, these results  
150 argue against a role for platelets in restricting the efficiency of antibiotic treatments upon  
151 meningococcal systemic infections.

152

153 **Coagulation inhibition restores antibiotic efficiency**

154 Clinical and experimental evidence point to the presence of fibrin clots in the vicinity of  
155 infected vessels (Faust *et al*, 2001; Guarner *et al*, 2004; Melican *et al.*, 2013). Using intravital  
156 imaging, we therefore monitored the deposition of fibrin inside infected vessels during  
157 vascular colonization by *Neisseria meningitidis*. In the first 3 hours following infection, no  
158 evidence of fibrin deposition could be found. In contrast, starting 3 hours post infection we  
159 observed the progressive accumulation of fibrin close to sites of bacterial adhesion (Fig 3A-  
160 B, Fig EV3A and Movie EV3). In absence of infection, fibrin deposition was not observed (n =  
161 17 vessels). Thrombus formation thus coincides with the time of blood flow decrease and the  
162 emergence of the resistance to the antibiotic treatment (Fig 1E and Fig 2B). Importantly,  
163 fibrin deposition occurs in most infected vessels, reaching 64% coverage at 4h30 post  
164 infection (Fig 3B). Interestingly, in patients, circulating monocytes have been shown to  
165 express Tissue Factor (TF) during meningococemia (Osterud & Flaegstad, 1983),  
166 suggesting a role for TF in triggering disseminated intravascular coagulation (DIC).  
167 Accordingly, TF expression on the surface of monocytes (CD115<sup>+</sup> Gr-1<sup>+</sup>) from infected mice  
168 increased with kinetics similar to microvascular fibrin deposition (Fig EV4A). In contrast,  
169 endothelial cells did not show any increase in TF expression, even though E-Selectin  
170 expression strongly increased, as shown previously (Manriquez *et al.*, 2021) (Fig EV4B).  
171 We next sought to determine whether infection-induced fibrin deposition negatively impacts  
172 the efficacy of antibiotic therapy by inhibiting the thrombin-dependent cleavage of fibrinogen  
173 into fibrin strands. Fondaparinux prevents the formation of fibrin clots by inhibiting Factor Xa,  
174 and thus the conversion of prothrombin into active thrombin (Bauer *et al*, 2002). To ensure  
175 the constant delivery of the anticoagulant treatment to grafted mice, Fondaparinux was  
176 infused subcutaneously using osmotic minipumps. Grafted mice were then infected, and the  
177 antibiotic treatment (Cefotaxime, CTX) was administrated 16h post infection (Fig 3C). As  
178 expected, Fondaparinux strongly reduced plasma Factor Xa (Fig 3D). Interestingly,  
179 Fondaparinux treatment *per se* did not alter, nor promote meningococci survival either in the  
180 blood or adhering to the vessel walls (Fig EV3B-C). The antibiotic treatment also efficiently  
181 decreased the numbers of circulating bacteria in conditions of Factor X inhibition (Fig 3E).

182 Strikingly, upon inhibition of coagulation and antibiotic treatment, numbers of adherent  
183 bacteria in the human grafts were decreased by more than 500-fold when compared to mice  
184 that did not received antibiotics ( $29\,591 \pm 23\,065$  versus  $52 \pm 45$  CFU/mg of skin in  
185 Fondaparinux- versus Fondaparinux+CTX-treated animals, respectively) (Fig 3F).  
186 Accordingly, the perfusion of infected vessels was partially restored upon inhibition of  
187 coagulation by Fondaparinux (Fig 3G-H and Movie EV4). Taken together these results  
188 strongly suggest that meningococcal-induced fibrin deposition reduces microvascular  
189 patency and thereby the tissue distribution of antibiotics, thus preventing their efficacy.

## 190 Discussion

191 Our results show that within a few hours of infection, meningococcal vascular colonization  
192 leads to a dramatic reduction in the perfusion of infected vessels. While bacterial colonization  
193 alone is sufficient to partially occlude vessels and reduce blood flow, ensuing thrombus  
194 formation leads to the complete cessation of blood flow in affected vessels. This, in turn, has  
195 important implications for disease progression. Complete absence of perfusion will result in  
196 severe ischemia and irreversible tissue injury, driving the rapid progression of the disease  
197 towards multiorgan failure. Another consequence of reduced microvascular perfusion,  
198 detailed in this study, is its impact on the access of antibiotics to target tissues. This is  
199 observed in the animal model described here but also in human cases where bacteria have  
200 been demonstrated to resist intensive antibiotic treatment (van Deuren *et al.*, 1993).

201  
202 The molecular process driving thrombin generation during meningococcal infection has been  
203 extensively studied clinically and *in vitro* but remains to be fully established (Lecuyer *et al.*,  
204 2017). Our results using a humanized model of *Neisseria meningitidis* systemic infection  
205 show extensive fibrin deposition in close proximity to bacterial aggregates early during  
206 infection and provide new insight into meningococci-induced thrombus formation. Our results  
207 suggest that the process is largely independent of platelets, which do not stably interact with  
208 meningococci, as opposed to many other bacterial infections (Fitzgerald *et al.*, 2006; Sun,  
209 2006). Rather, thrombus formation appears to be triggered by the extrinsic pathway of blood  
210 coagulation through the rapid expression of TF/CD142 by monocytes. Accordingly, in  
211 meningococemia cases (Osterud & Flaegstad, 1983) and the humanized model used here,  
212 monocytes express TF at early stages of infection. In contrast and despite massive local  
213 bacterial accumulation, human endothelial cells did not display TF expression *in vivo*.  
214 Interestingly, the contact (intrinsic) pathway of blood coagulation was also shown to be  
215 activated during meningococcal infections (Wuillemin *et al.*, 1995). The negative charges  
216 present on the bacterial LOS or capsule could be a trigger of the contact pathway (Oehmcke

217 & Herwald, 2010). This could provide an alternative explanation for fibrin deposition in  
218 proximity to bacterial colonies. In any case, vascular colonization by *Neisseria meningitidis*  
219 leads to the rapid formation of thrombi that restrict the efficacy of antibiotic treatment. Many  
220 pathogenic bacteria have evolved specific strategies to inhibit coagulation, a process that is  
221 thought to favor bacterial dissemination (Popov *et al*, 1991). Meningococci do not seem to be  
222 equipped to efficiently block thrombus formation, hence permitting the thrombotic occlusion  
223 of infected vessels and the sealing of the infected regions by the host. This likely reflects the  
224 main lifestyle of *Neisseria meningitidis* at the surface of the nasopharyngeal epithelium rather  
225 than in the vasculature.

226  
227 From the clinical point of view, our observations suggest that anticoagulation therapy could  
228 potentially provide a synergistic effect in conjunction with antibiotic treatment for  
229 meningococcal infections. A number of clinical studies have tested the benefits of  
230 anticoagulants such as activated protein C in combination with antibiotics. Activated protein  
231 C acts as a negative regulator of coagulation by proteolytically inactivating coagulation  
232 cofactors Factor Va and Factor VIIIa. Unfortunately, the use of recombinant Protein C has  
233 had variable success (Silva *et al*, 2010). Our study suggests that anticoagulation would  
234 mainly be effective in the very early stages of infection and that direct targeting of Factor Xa  
235 or thrombin may be more effective than Protein C. The efficacy of anticoagulation described  
236 here is likely to be linked to the pathogenesis of meningococcal infections and more  
237 specifically to the ability of this bacterium to adhere to the endothelium and colonize vessels.  
238 The utility of anticoagulation is thus likely to depend on the pathogen. Although the process  
239 described here is local, in close association with infected vessels, it is a host-driven response  
240 and a likely precursor of DIC. The induction of local coagulation appears to represent a  
241 tipping point in the progression of the disease after which antibiotic therapy is no longer as  
242 efficient.

243 While our study suggests the potential of anticoagulant therapy for increased efficacy of  
244 antibiotic treatment, it also argues that such therapy would have to be administered early in

245 the progression of the disease and that thrombolytic therapy is likely to be more efficacious  
246 once the bacteria have taken hold and thrombi are established. Importantly, the use of  
247 anticoagulant and fibrinolytic therapy is always associated with a potential risk of bleeding  
248 that will need to be carefully assessed. Although anticoagulation has been used in the  
249 context of sepsis in the past (Levi *et al*, 2001; Silva *et al.*, 2010), the strong implication of the  
250 cerebral vasculature render safety considerations particularly important for meningococcal  
251 infections. That said, experience from ischemic stroke shows that such risks can be  
252 managed by the use of strict inclusion criteria based on timing and individual risk/benefit  
253 assessment.

## 254 **Materials and Methods**

255 **Mice.** SCID/Beige (CB17.Cg-*Prkdc*<sup>scid</sup>*Lyst*<sup>tg-J</sup>/Crl) mice were purchased from Charles Rivers  
256 (France) and housed under specific pathogen-free condition at Institut Pasteur. Mice were  
257 kept under standard conditions (light 07.00-19.00h; temperature 22±1°C; humidity 50±10%)  
258 and received sterilized rodent feed and water *ad libitum*. All experiments were performed in  
259 agreement with guidelines established by the French and European regulations for the care  
260 and use of laboratory animals and approved by the Institut Pasteur committee on Animal  
261 Welfare (CETEA) under the protocol code DAP 180022. For all experiments, male and  
262 female mice between 9 and 16-weeks of age were used. Littermates were randomly  
263 assigned to experimental groups.

264 **Human skin.** Normal human skin was obtained from adult patients (20-60 years old), both  
265 males and females, undergoing plastic surgery in the *service de chirurgie reconstructrice et*  
266 *plastique* of Groupe Hospitalier Saint Joseph (Paris, France). In accordance with the French  
267 legislation, patients were informed and did not refuse to participate in the study. All  
268 procedures were approved by the local ethical committee *Comité d'Evaluation Ethique de*  
269 *l'INSERM* IRB 00003888 FWA 00005881, Paris, France Opinion: 11-048.

270 **Xenograft model of infection.** 6-13 weeks old mice, both males and females, were grafted  
271 with human skin as previously described (Melican *et al.*, 2013). Briefly, mice were  
272 anesthetized with a mixture of ketamine hydrochloride (100 mg/kg, Boehringer Ingelheim)  
273 and xylazine hydrochloride (8.5 mg/kg, Bayer) and a graft bed of approximately 1–2 cm<sup>2</sup> was  
274 prepared on their flank by removing the mouse epithelium and the upper dermis layer. A  
275 human skin graft (200 µm thick) comprising the human epidermis and the papillary dermis  
276 was immediately placed over the graft bed. Grafts were fixed in place with surgical glue  
277 (Vetbond, 3M, USA) and dressings were applied for 2 weeks. Grafted mice were used for  
278 experimentation 3-6 weeks post-surgery when the human dermal microvasculature is  
279 anastomosed to the mouse circulation without evidence of local inflammation, as previously  
280 described (Melican *et al.*, 2013). All efforts were made to minimize suffering.

281 ***Neisseria meningitidis* strains and mouse infection.** Experiments were performed using  
282 *N. meningitidis* 8013 serogroup C strain (<http://www.genoscope.cns.fr/agc/nemesys>)  
283 (Rusniok *et al*, 2009). Strains were streaked from -80°C freezer stock onto GCB agar plates  
284 and grown overnight in a moist atmosphere containing 5% CO<sub>2</sub> at 37°C. For all experiments,  
285 bacteria were transferred to liquid cultures in pre-warmed RPMI-1640 medium (Gibco)  
286 supplemented with 10% FBS at adjusted OD<sub>600nm</sub> = 0.05 and incubated with gentle agitation  
287 for 2 hours at 37°C in the presence of 5% CO<sub>2</sub>. Bacteria were washed twice in PBS and  
288 resuspended to 10<sup>8</sup> CFU/ml in 1x PBS. Prior to infection, mice were injected intraperitoneally  
289 with 8 mg of human transferrin (Sigma Aldrich) to promote bacterial growth *in vivo* as  
290 previously described (Melican *et al.*, 2013). Mice were infected by retro-orbital injection of  
291 100 µl of the bacterial inoculum (10<sup>7</sup> CFU total). When indicated, mice received a retro-orbital  
292 injection of 4 mg Cefotaxime (CTX, #C7912, Sigma-Aldrich) at 3h, 6h or 16h post infection  
293 and 3h prior to mouse sacrifice. No influence of mice sex on bacterial colonization have been  
294 observed.

295 **Wholemount immunofluorescent staining.** Following mouse infection with mCherry-  
296 expressing *Neisseria meningitidis* for 9 hours, the human grafts were harvested and fixed in  
297 4% PFA overnight at 4°C with gentle agitation. Skin samples were then washed and  
298 permeabilized/blocked overnight at 4°C with gentle agitation using a solution of 0.3% Triton-  
299 X100, 1% BSA, 1% normal goat serum in 1X PBS. The basal lamina of human blood vessels  
300 was stained using an AlexaFluor647-conjugated anti-human Collagen IV antibody (#51-  
301 9871-82, eBioscience, 1/100) diluted in permeabilization/blocking solution and incubated 3  
302 days at 4°C with gentle agitation. Samples were then extensively washed and tissue  
303 transparization and mounting were performed using RapidClear 1.47 (SunJin Lab) according  
304 to manufacturer's recommendations.

305 ***Neisseria meningitidis* growth curves.** Bacterial liquid culture was diluted to an OD<sub>600nm</sub> of  
306 0.05 and 150 µl of the bacterial suspension was transferred into wells of a 96-well plate.  
307 Bacteria were then treated or not with the indicated final concentration of Cefotaxime, each  
308 performed in quintuplicates. Plates were placed in a Cytation 5 multimode reader (Biotek) at

309 37°C with 5% CO<sub>2</sub> and continuous agitation and optical densities at 600 nm were recorded  
310 every 30 minutes for 20 hours.

311 **Colony-forming units (CFU) enumeration.** To assess bacteraemia (blood-circulating  
312 bacteria) in infected animals, 10 µl of blood was sampled at the time of sacrifice. Serial  
313 dilutions of blood were plated on GCB agar plates and incubated overnight at 37°C and in a  
314 moist atmosphere containing 5% CO<sub>2</sub>. Bacterial counts were expressed in colony-forming  
315 units (CFU) per ml of blood. To assess the extent of vascular colonization by meningococci  
316 (adherent bacteria) following mouse sacrifice at indicated times post infection, tissue biopsies  
317 were collected using a sterile dermatological biopsy puncher (approximately 4 mm<sup>2</sup>),  
318 weighted and placed in 500 µl 1x PBS. Skin biopsies were dissociated and homogenized  
319 using a BeadMill4 homogenizer (Fisher Brand) and serial dilutions of skin homogenates were  
320 plated on GCB plates incubated overnight at 37°C and in a moist atmosphere containing 5%  
321 CO<sub>2</sub>. Bacterial counts were expressed in colony-forming units (CFU) per mg of skin.

322 **Platelet depletion.** Platelets were depleted in grafted mice by retro-orbital injection of 40 µg  
323 of anti-mouse GPIIb/IIIa (CD42b) (#R300, Emfret Analytics) monoclonal antibodies diluted in  
324 100 µl 1x PBS 6 hours prior to infection, as previously described (Bergmeier *et al.*, 2000;  
325 Nieswandt *et al.*, 2000). The efficiency of platelet depletion was assessed by counting  
326 platelets on citrated blood using an automated hemocytometer (SCIL Vet ABC plus)  
327 according to manufacturer's instructions.

328 **Spinning disk confocal intravital imaging.** Intravital imaging of the human xenograft was  
329 adapted from (Ho *et al.*, 2000) and previously described (Manriquez *et al.*, 2021). Briefly, 30  
330 min prior to surgery, mice were injected subcutaneously with buprenorphine (0.05 mg/kg,  
331 CEVA) and anesthetized by spontaneous inhalation of isoflurane in 100% oxygen (induction:  
332 4%; maintenance: 1.5% at 0.3 L/min). A flap of skin supporting the human graft was  
333 immobilized onto a custom-made heated deck (36°C) and continuously moistened with  
334 warmed 1x PBS (36°C). Mouse body temperature was maintained at 37°C using a heating  
335 pad and oxygen saturation and heart rate were monitored using the pulse oximetry  
336 Physiosuit apparatus (Kent Scientific). Image acquisition was performed using a Leica DM6

337 FS upright microscope equipped with a motorized stage, a Fluotar 25x/0.95 objective (Leica),  
338 and coupled to a Yokogawa CSU-W1 confocal head modified with Borealis technology  
339 (Andor). Four laser excitation wavelengths (488, 561, 642, and 730 nm) were used in fast  
340 succession and visualized with the appropriate long-pass filters. Fluorescence signals were  
341 detected using a sCMOS 2048x2048 pixel camera (Orca Flash v2+, Hamamatsu).  
342 Metamorph acquisition software (Molecular devices) was used to drive the confocal  
343 microscope.

344 The human vessels were labelled using Dylight755-conjugated UEA-1 lectin (100 µg, Vector  
345 Laboratories), platelets using DyLight488-anti-mouse GPIIb/IIIa antibody (0.1 µg, #X488, Emfret  
346 Analytics) and fibrin deposits using DyLight488-anti-human/mouse fibrin antibody (8 µg,  
347 clone 59D8, Sigma-Aldrich). All antibodies and dyes were injected intravenously 15 min prior  
348 to intravital imaging. Five to ten fields of view of interest were selected per animal and time-  
349 lapse z-stack series (2-2.5 µm z-step, 50-80 µm range) were captured every 30-60 min for 4-  
350 5 hours following the intravenous injection of iRFP-expressing bacteria ( $10^7$  CFU/100 µl 1x  
351 PBS). In mice treated with anticoagulant, Fondaparinux was injected subcutaneously 5 min  
352 prior to imaging (80 µl, 1 mg).

353 The blood flow during vascular colonization was monitored by perfusing 1 µm diameter  
354 fluorescent microspheres (Yellow/Green Fluoresbrite carboxylate, Polysciences,  $10^7$   
355 microspheres/ml 1x PBS) at a rate of 15 µl/min before each z-stack series and images  
356 acquired on a single plane at high speed (50 frames per second, 300 frames). Blood flow  
357 was calculated from the vessel diameter and the centerline microsphere mean velocity,  
358 according to (Hidalgo *et al*, 2009), and normalized with respect to the basal blood flow.  
359 Platelet dynamics during vascular colonization was assessed by fast (50 frames/sec)  
360 simultaneous dual-color imaging of platelets and bacteria.

361 **Anticoagulant treatment.** The inhibition of coagulation was achieved by treating grafted  
362 mice with the factor Xa inhibitor Fondaparinux (ARIXTRA<sup>®</sup>, ASPEN) prior to and/or during  
363 the course of the infection. For short-term infection (6h and 9h), grafted mice received a  
364 subcutaneous injection of 80 µl Fondaparinux (corresponding to 1 mg) immediately after the

365 intravenous injection of bacteria and 3h and 6h post infection. For long-term infection (19h),  
366 grafted mice received a subcutaneous injection of 80  $\mu$ l Fondaparinux (corresponding to 1  
367 mg) immediately after the infection and the constant delivery (infusion) of Fondaparinux was  
368 then ensured by ALZET<sup>®</sup> osmotic pumps (200  $\mu$ l, 8  $\mu$ l/h during 24h, #2001D) filled with 200  
369  $\mu$ l Fondaparinux and placed subcutaneously, according to manufacturer's instructions. The  
370 efficiency of the anticoagulant treatment was assessed using a chromogenic enzymatic  
371 method (Biophen Heparin 6 kit, #221006, HYPHEN Biomed), according to manufacturer's  
372 instructions.

373 **Flow cytometry.** To monitor the surface levels of Tissue Factor and E-Selectin on human  
374 endothelial cells, biopsies of human xenograft harvested at the indicated time post infection  
375 were digested with 0.4 mg/ml Liberase TL (Sigma Aldrich) in CO<sub>2</sub>-independent medium for  
376 60 min at 37°C with gentle agitation (100 rpm). The resulting single-cell suspension was  
377 passed through a 70- $\mu$ m cell strainer (BD Bioscience), washed once with FACS buffer (1 $\times$   
378 PBS supplemented with 0.5% BSA and 2 mM EDTA) and stained in FACS buffer according  
379 to standard protocols. Briefly, human and murine Fc receptors were blocked using Human  
380 TruStain FcX (Biolegend, #422302, 1/20) and anti-mouse CD16/CD32 (FcBlock clone 2.4G2,  
381 BD Biosciences, #553141, 1/200), respectively, and cells were stained for 30 min at 4°C with  
382 the following anti-human antibodies: PerCP-Vio700-conjugated REAfinity recombinant anti-  
383 CD31 (Miltenyi Biotec, clone REA730, #130-110-673, 1/20), BV421-conjugated anti-Tissue  
384 Factor/CD142 (BD Biosciences, clone HTF-1, #744003, 1/20), and PE-conjugated anti-  
385 CD62E/E-Selectin (eBioscience, clone P2H3, #12-0627-42, 1/20).

386 To monitor the surface levels of Tissue Factor on circulating murine monocytes, blood was  
387 harvested at the indicated time post infection and treated with 1x RBC lysis solution  
388 (BioLegend). Murine Fc receptors were blocked using anti-mouse CD16/CD32 (FcBlock  
389 clone 2.4G2, BD Biosciences, #553141, 1/200) and cells were stained in FACS buffer for  
390 30 min at 4°C with the following anti-mouse antibodies: PacificBlue-conjugated anti-CD45  
391 (Biolegend, clone 30-F11, #103126, 1/100), PE-Cy7-conjugated anti-CD11b (BD  
392 Biosciences, clone M1/70, #552850, 1/200), APC-conjugated anti-CD115 (Invitrogen, clone

393 AFS98, #17-1152-82, 1/100), BV650-conjugated anti-Gr-1 (Biolegend, clone RB6-8C5,  
394 #108441, 1/50), and PE-conjugated anti-Tissue Factor/CD142 (R&D Systems, polyclonal,  
395 #FAB3178P, 1/50).

396 After staining, cells were washed twice in 1× PBS and fixed for 15 min at 4 °C with 1%  
397 paraformaldehyde in 1× PBS. Data were acquired using a CytoFLEX S flow cytometer  
398 controlled with the CytExpert software (Beckman Coulter). Data analysis was carried out  
399 using FlowJo software v10 (Tree Star).

400 **Statistics.** All graphs and statistical analyses were performed with GraphPad Prism 9  
401 (GraphPad Software). No statistical method was used to predetermine sample size.  
402 Kolmogorov–Smirnov test was used to assess the normality of all data sets. Scatter dot  
403 plots, bar graphs and values are provided as the mean ± sem. P-values were considered as  
404 statistically significant when inferior at 0.05. Statistical details of experiments (sample size,  
405 replicate number, statistical significance) can be found in the figures, figure legends and  
406 source data file.

407

## 408 **Data availability**

409 This study includes no data deposited in external repositories.

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415

## 416 **Author contributions**

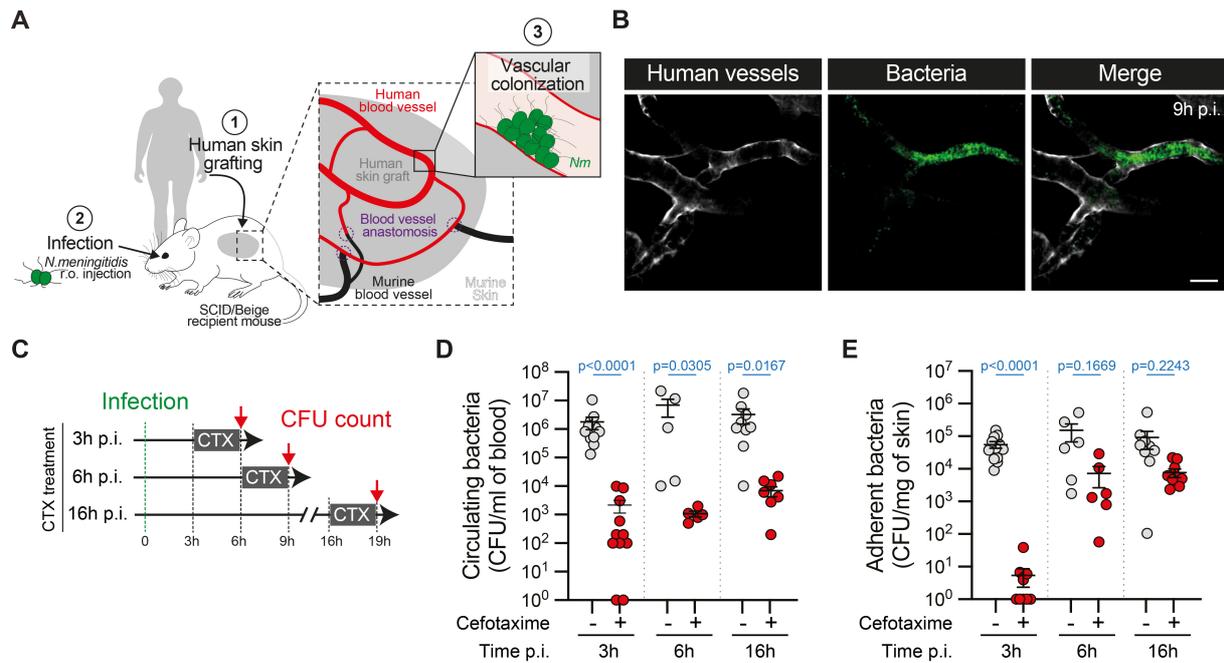
417 G.D. designed research and supervised overall data analysis; J.-P.C., P.N., A.Y. and D.O.  
418 performed and analyzed experiments; T.S. provided human skin samples; D.O. assembled  
419 figures; D.O. and G.D. wrote the manuscript.

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## 421 **Conflict of interest**

422 The authors declare no conflict interests.

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### Figure 1 - Limited antibiotic efficiency during *Neisseria meningitidis* vascular colonization

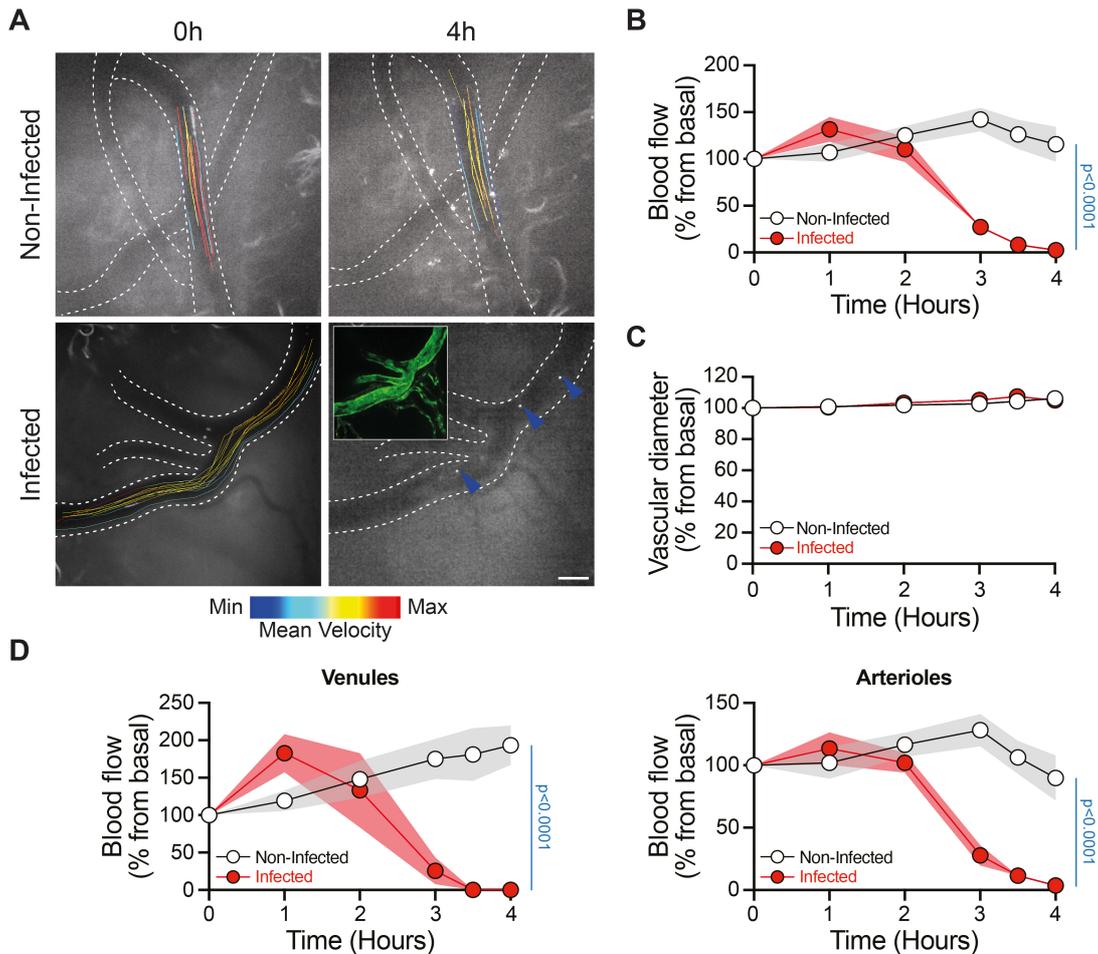
**A** Schematic representation of the human skin xenograft mouse model.

**B** Representative whole-mount immunofluorescence pictures (maximum intensity z-projection) of the colonization of human vessels (grey, anti-human Collagen IV) by mCherry-expressing *Neisseria meningitidis* (green) in grafted animals 9 hours post infection (p.i.). Scale bar: 40  $\mu$ m. (n = 3 mice).

**C** Schematic representation of the experimental approach used to assess bacterial sensitivity to Cefotaxime (CTX) treatment at 3h, 6h and 16h post infection (p.i.).

**D, E** Bacterial colony forming unit (CFU) counts from **(D)** blood (circulating bacteria) and **(E)** dissociated human xenografts (adherent bacteria) collected from mice infected for the indicated time points and treated (+) or not (-) with Cefotaxime (CTX). (n = 12, 6 and 10 mice per group at 3h, 6h and 16h p.i., respectively, pooled from N = 3 independent experiments per time point).

Data information: In (D-E), data are presented as the mean  $\pm$  SEM. Kruskal-Wallis test with Dunn's correction for multiple comparisons.



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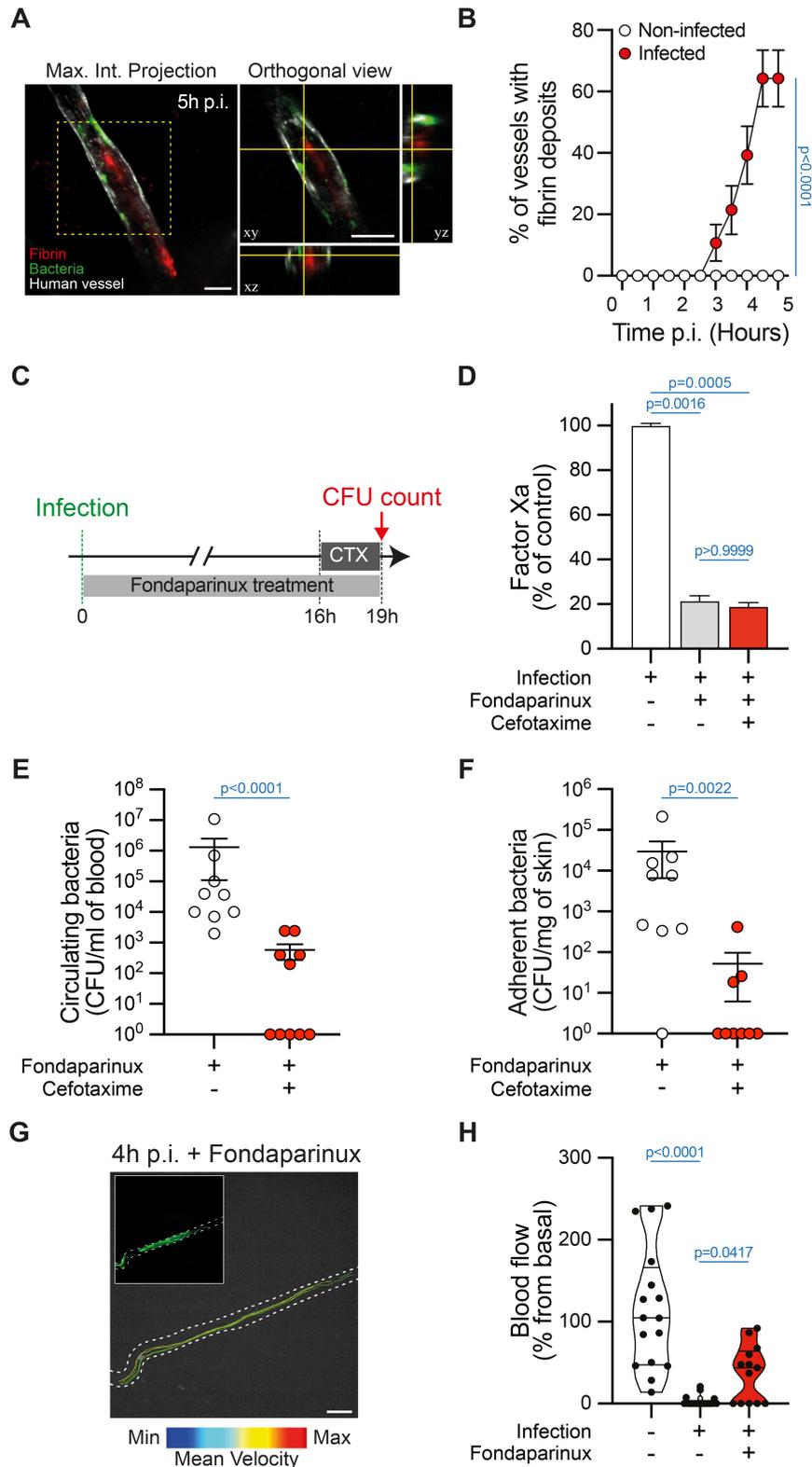
**Figure 2 – Vascular colonization by *Neisseria meningitidis* blocks the blood flow**

**A** Representative image of fluorescent microsphere tracking as a read-out of blood flow during the early phase of the infection (0h and 4h p.i.). Tracks were color-coded according to the mean velocity of the corresponding microsphere. Inset shows the extent of vascular colonization by iRFP-expressing *Nm* (green) 4h post infection. Blue arrowheads indicate immobilized microspheres. Scale bar: 50  $\mu$ m.

**B, C** Normalized blood flow (**B**) and vessel diameter (**C**) as a function of the time post infection. (n = 18 and 19 vessels for non-infected and infected animals, respectively, pooled from N = 2 mice per condition and imaged independently).

**D** Data in (B) were classified according to the vessel type: venules (left) and arterioles (right). (n = 13 and 14 arterioles, and 5 and 5 venules in non-infected and infected mice, respectively).

Data information: In (B-D), data are presented as the mean  $\pm$  SEM. Two-way ANOVA test.



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**Figure 3 - The efficiency of antibiotic treatments is restored upon inhibition of coagulation**

**A** Representative image (maximum intensity z-projection) and orthogonal view of fibrin deposition (red, anti-Fibrin) in human vessels (grey, UEA1 lectin) infected by iRFP-expressing *Neisseria meningitidis* (green). Scale bar: 50  $\mu$ m. (n = 28 infected vessels, pooled from N = 3 mice imaged independently).

465 **B** Percentage of vessels with fibrin deposition in control and infected animals (n = 28 infected  
466 vessels and 17 non-infected control vessels, pooled from N = 3 and 2 mice, respectively,  
467 imaged independently).

468 **C** Schematic representation of the experimental approach used to assess the impact of  
469 coagulation inhibition on bacterial sensitivity to Cefotaxime (CTX) treatment.

470 **D** Chromogenic-based quantification of the coagulation Factor Xa in the plasma of control  
471 (no Fondaparinux, no Cefotaxime), Fondaparinux-treated (no Cefotaxime) and  
472 Fondaparinux+CTX-treated mice. (n = 8, 10 and 10 mice for control, Fondaparinux-treated,  
473 and Fondaparinux+CTX-treated mice, respectively, pooled from N = 3 independent  
474 experiments).

475 **E, F** Bacterial colony forming unit (CFU) counts from **(E)** blood (circulating bacteria) and **(F)**  
476 dissociated human xenografts (adherent bacteria) collected from mice treated or not with the  
477 Fondaparinux anticoagulant and infected for 19h and treated or not with Cefotaxime (CTX).  
478 (n = 9 mice per group, pooled from N = 3 independent experiments).

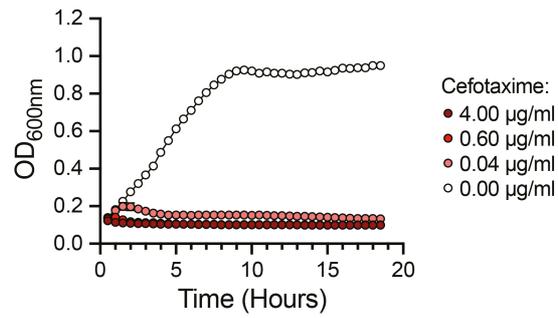
479 **G** Representative images (maximum intensity z-projection) of fluorescent microsphere  
480 tracking in human vessels (white dashed line) infected by iRFP-expressing bacteria (green,  
481 inset) in mice treated with Fondaparinux. Tracks were color-coded according to the mean  
482 velocity of the corresponding microsphere. Scale bar: 50  $\mu$ m. (n = 3 mice).

483 **H** Violin plot showing the distribution of the blood flow in vessels from non-infected, infected,  
484 and infected+Fondaparinux-treated animal 4h post infection. (n = 16, 19 and 13 vessels for  
485 non-infected, infected, and infected+Fondaparinux, respectively, pooled from N = 2, 2 and 3  
486 mice for control, infected, and infected+Fondaparinux-treated animals, respectively).

487 Data information: In (D), data are presented as the mean  $\pm$  SEM. Kruskal-Wallis test with  
488 Dunn's correction for multiple comparisons.

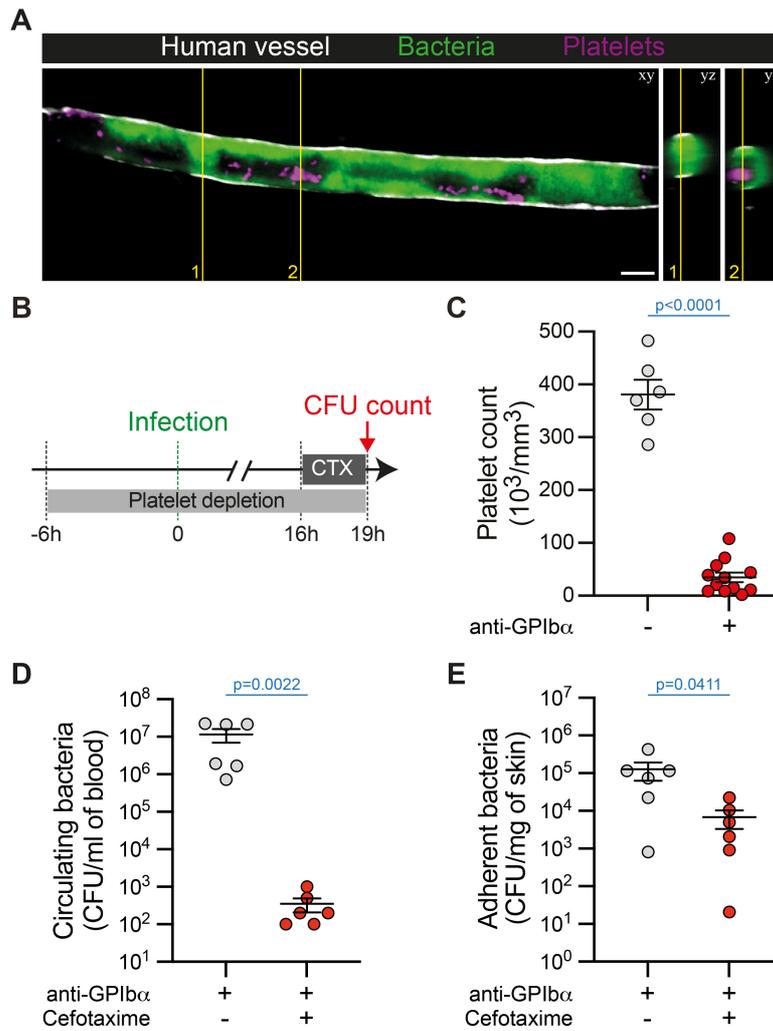
489 In (E-F), data are presented as the mean  $\pm$  SEM. Two-tailed Mann-Whitney test.

490 In (H), data are presented as violin plot showing the distribution of the dataset. Kruskal-Wallis  
491 test with Dunn's correction for multiple comparisons.



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**Figure EV1 - *Neisseria meningitidis* is sensitive to low doses of Cefotaxime *in vitro***  
Growth curves of *N. meningitidis* in the presence of the indicated final concentrations of Cefotaxime. (N = 1 experiment). Data are presented as the mean  $\pm$  SEM of quintuplicates.



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**Figure EV2 - Platelets do not prevent the efficiency of the antibiotic treatment**

500 **A** Representative image (maximum intensity z-projection) and orthogonal view of platelets  
501 (purple, anti-GPIIb/IIIa) in bacteria-free spaces in human vessels (grey, UEA1 lectin) infected by  
502 iRFP-expressing *Neisseria meningitidis* (green) for 4 hours. Scale bar: 25  $\mu\text{m}$ . (n = 3 mice).

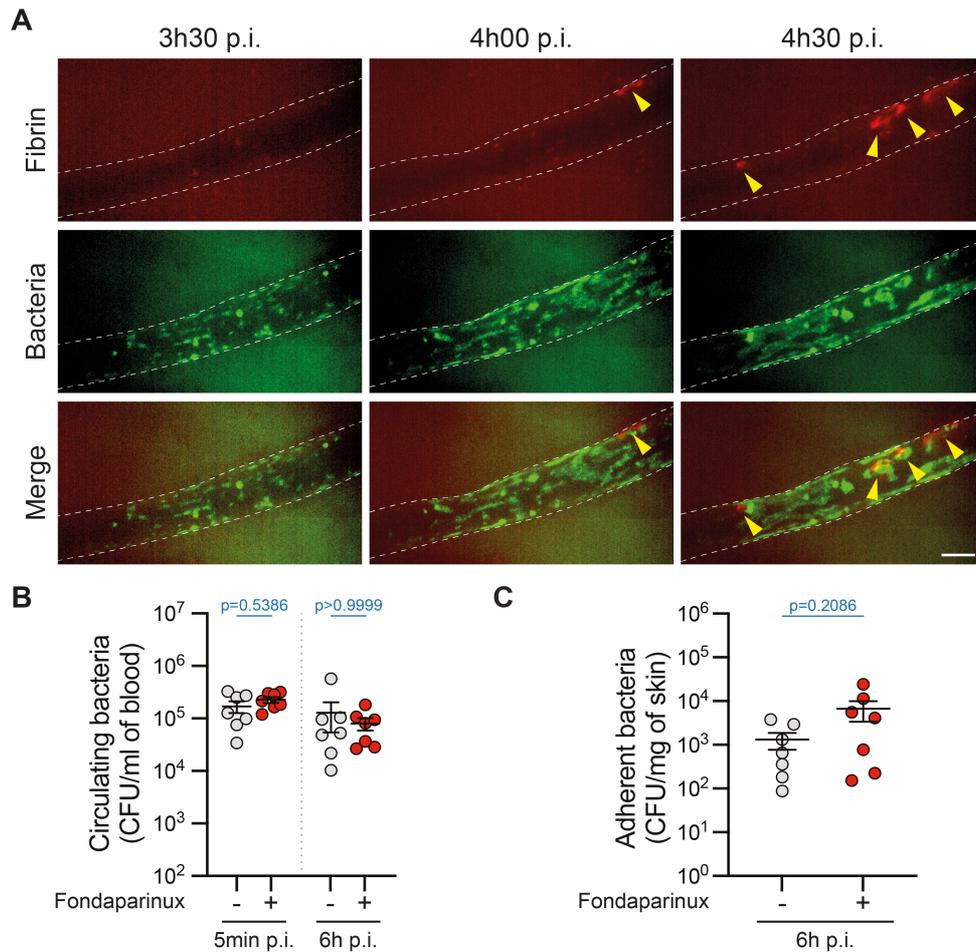
503 **B** Schematic representation of the experimental approach used to assess the impact of  
504 platelet depletion on bacterial sensitivity to Cefotaxime (CTX) treatment.

505 **C** Quantification of platelet numbers in the blood of mice treated (+) or not (-) with the  
506 platelet-depleting antibody directed against GPIIb/IIIa. (n = 6 and 12 mice for control and anti-  
507 GPIIb/IIIa-treated mice, respectively, pooled from N = 2 independent experiments).

508 **D, E** Bacterial colony forming unit (CFU) counts from **(D)** blood (circulating bacteria) and **(E)**  
509 dissociated human xenografts (adherent bacteria) collected from platelet-depleted mice (anti-  
510 GPIIb/IIIa) infected for 19h and treated (+) or not (-) with Cefotaxime (CTX). (n = 6 mice per  
511 group, pooled from N = 2 independent experiments).

512 Data information: In (C), data are presented as the mean  $\pm$  SEM. Unpaired t test.

513 In (D-E), data are presented as the mean  $\pm$  SEM. Two-tailed Mann-Whitney test.



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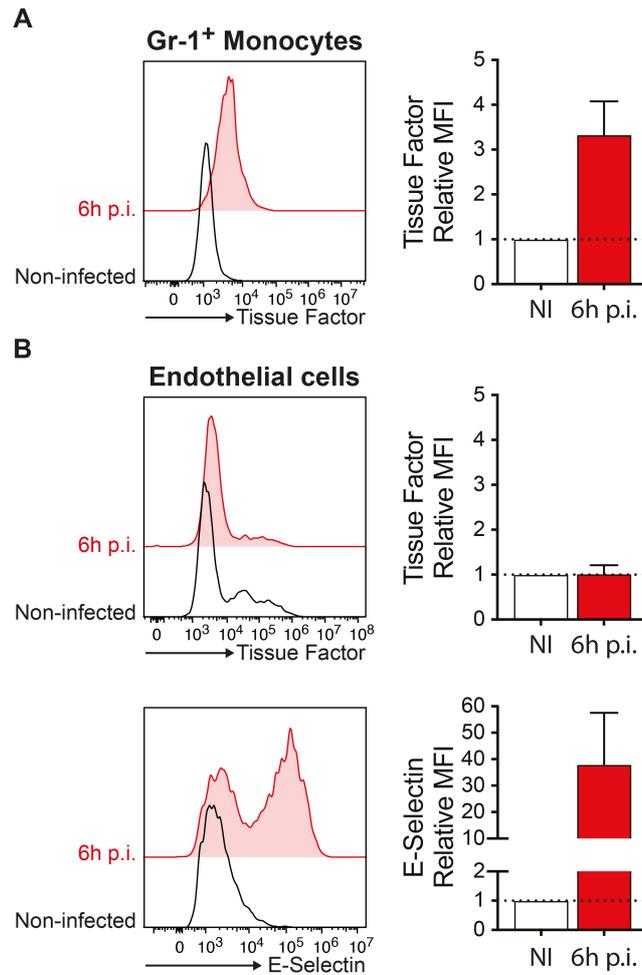
515

516 **Figure EV3 - The efficiency of the antibiotic treatment is restored upon coagulation**  
517 **inhibition**

518 **A** Representative time sequence (maximum intensity z-projection) of fibrin deposition (red,  
519 anti-Fibrin) in human vessels (dashed line) infected by iRFP-expressing *Neisseria*  
520 *meningitidis* (green). Scale bar: 50  $\mu$ m. (n = 28 infected vessels, pooled from N = 3 mice  
521 imaged independently).

522 **B, C** Bacterial colony forming unit (CFU) counts from (B) blood (circulating bacteria) and (C)  
523 dissociated human xenografts (adherent bacteria) collected from control (-) and  
524 Fondaparinux-treated (+) mice infected for 5 minutes or 6 hours. (n = 7 mice per group,  
525 pooled from N = 2 independent experiments).

526 Data information: In (B-C), data are presented as the mean  $\pm$  SEM. (B) Kruskal-Wallis test  
527 with Dunn's correction for multiple comparisons. (C) Two-tailed Mann-Whitney test.



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530 **Figure EV4 - Early upregulation of Tissue Factor at the surface of circulating**  
531 **monocytes upon meningococcal vascular colonization**

532 **A** Representative flow cytometry analysis (left) and quantification (right) of the cell surface  
533 expression of murine Tissue Factor (CD142) on blood-circulating monocytes (gated as  
534 CD45<sup>+</sup> CD11b<sup>+</sup> CD115<sup>+</sup> Gr-1<sup>+</sup>) in non-infected control mice (black) and mice infected for 6h  
535 (red). (n = 3 mice).

536 **B** Representative flow cytometry analysis (left) and quantification (right) of the cell surface  
537 expression of human Tissue Factor (CD142, Top) and human E-Selectin (CD62E, bottom)  
538 on CD31<sup>+</sup> human endothelial cells isolated from human skin xenografts in non-infected  
539 control mice (black) and mice infected for 6h (red). (n = 3 mice).

540 Data information: In (A-B), data are presented as the mean  $\pm$  SEM and were normalized with  
541 respect to the mean fluorescence intensity (MFI) of the non-infected controls.

542 **Movie legends**

543

544 **Movie EV1. Altered blood flow during *Neisseria meningitidis* infection**

545 High-speed (50 frames/sec) intravital imaging (single focal plane) of 1  $\mu\text{m}$  fluorescent  
546 microspheres (white) intravenously perfused allowing the visualization and quantification of  
547 the blood flow in human blood vessels (UEA1 lectin, gray) at the indicated time points in  
548 infected and non-infected control mice. The extend of vascular colonization by iRFP-  
549 expressing *Neisseria meningitidis* (green) is shown as a maximum intensity z-projection.  
550 Scale bar: 50  $\mu\text{m}$ .

551

552 **Movie EV2. Dynamics of platelets during *Neisseria meningitidis* infection**

553 Simultaneous dual color intravital imaging (single focal plane) of iRFP-expressing *Neisseria*  
554 *meningitidis* (green) and platelets (anti-GPIIb $\beta$ , magenta) in a human vessel (white dashed  
555 line) 2h post infection. Scale bar: 50  $\mu\text{m}$ .

556

557 **Movie EV3. Intravascular fibrin deposits in *Neisseria meningitidis* infected human  
558 vessels**

559 3D volume rendering of a fibrin deposit (anti-Fibrin, red) in a human vessel (UEA-1 lectin,  
560 gray) infected with iRFP-expressing *Neisseria meningitidis* (green) for 5 hours. Scale bar: 30  
561  $\mu\text{m}$ .

562

563 **Movie EV4. Coagulation inhibition restores the blood flow in *Neisseria meningitidis*  
564 infected vessels**

565 High-speed (50 frames/sec) intravital imaging (single focal plane) of 1  $\mu\text{m}$  fluorescent  
566 microspheres (white) intravenously perfused allowing the visualization and quantification of  
567 the blood flow in human blood vessels (UEA1 lectin, gray) before (t0h00) and after (t4h00)  
568 infection upon anticoagulant (Fondaparinux) treatment. The extend of vascular colonization  
569 by iRFP-expressing *Neisseria meningitidis* (green) is shown as a maximum intensity z-  
570 projection. Scale bar: 50  $\mu\text{m}$ .

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