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What Blood Temperature for an Ex Vivo Extracorporeal Circuit?

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Abstract

Ex vivo circuits are commonly used to evaluate biomaterials or devices used for extracorporeal blood purification. However, various aspects of the ex vivo circuit, apart from the circuit materials, may affect inflammation and coagulation. One such aspect is temperature. The aim of this study was to evaluate the influence of different blood temperature conditions on inflammation parameters in an ex vivo circuit. Blood was collected from 20 healthy volunteers and run through three different experimental conditions for 4 h: a miniaturized ex vivo extracorporeal circuit equipped with a blood warmer set to 37°C, the same circuit without the warmer (23°C), and a tube placed in an incubator at 37°C (no circuit). We measured the granulocyte macrophage colony-stimulating factor, the tumor necrosis factor, and the interleukin (IL)-1 β , IL-6, IL-8, and IL-10 concentrations at baseline, 15, 60, 120, and 240 min. Human leukocyte antigen (HLA)-DR, CD11b, CD11a, CD62L, tumor necrosis factor alpha converting enzyme, annexin V expression, and NF κ B DNA binding were measured in monocytes and polymorphonuclear neutrophils (PMNs) using flow cytometry at baseline, 120 min, and 240 min. While cytokine production over time was very slight at room temperature, levels increased by more than 100-fold in the two heated conditions. Differences in the expression of some surface markers were also observed between the room temperature circuit and the two heated conditions (CD11b PMN, $P < 0.0001$; HLA-DR Mono, $P = 0.0019$; and CD11a PMN, $P < 0.0001$). Evolution of annexin V expression was also different over time between the three groups ($P = 0.0178$ for monocytes and $P = 0.0011$ for PMNs). A trend for a greater NF κ B DNA binding was observed in the heated conditions. Thus, for ex vivo studies using extracorporeal circuits, heating blood to maintain body temperature results in significant activation of inflammatory cells while hypothermia (room temperature) seems to suppress the leukocyte response. Both strategies may lead to erroneous conclusions, possibly masking some specific effects of the device being studied. Investigators in this field must be aware of the fact that blood temperature is a crucial confounding parameter and the type of “background noise” they will face depending on the strategy adopted.

Keywords

Blood temperature; Cytokines; Ex vivo extracorporeal circuit; Inflammation; Leukocyte surface markers

Extracorporeal therapies are widely used in numerous fields of medicine. Millions of patients around the world are treated with hemodialysis for chronic kidney disease, receive cardiopulmonary bypass during open heart surgery, or plasmapheresis for autoimmune

disease (1–3). In critical care, 5% of patients will undergo renal replacement therapy for acute kidney injury during their stay in the intensive care unit, and besides renal support, some of these extracorporeal therapies (high volume hemofiltration, super high-flux hemofiltration, hemoperfusion, coupled plasma filtration adsorption) are also proposed as a blood purification treatment for septic shock (4–6). Because of blood exposure to foreign materials, the extracorporeal circuit by itself is responsible for inflammation activation (7). Despite recent improvements in circuit biocompatibility, this additional production of locally formed inflammatory mediators is still considered a major adverse effect (8).

For research purposes, ex vivo circuits are commonly employed because they allow for evaluation of filters, dialyzers, sorbent cartridges, or other adsorption devices without any exposure to a patient or an animal, and they can be miniaturized, thus permitting rapid screening of materials (9–13). Blood is usually placed in a reservoir and then circulates through a closed loop with multiple passes through the device being studied. Circuit settings are not standardized and experimental conditions vary from one study to another (9–13). These ex vivo extracorporeal circuits are also under the influence of the inflammatory activation due to special environmental conditions such as artificial tubing, temperature, blood movement, and blood–air interface. However, in order to evaluate blood purification devices using ex vivo circuits, it is important to establish conditions that have the least inflammatory activation possible because this activation may interfere with or overshadow the effects of the device itself.

One such parameter that is known to have a significant impact on inflammatory cell activation is temperature. However, in ex vivo circuit experiments, it is unclear if work should be conducted at body temperature (37°C) or some other temperature (e.g., room temperature). One could argue that maintaining blood temperature at 37°C with a warmer could be physiological, but if maintaining body temperature is itself proinflammatory, then this effect will confound any attempt to evaluate artificial material. In addition, hypothermia (room temperature) is known to have anti-inflammatory effects by inhibiting leukocyte response following several tissue insults such as ischemic brain or liver injury (14–16). Therefore, the aim of this study was to evaluate the influence of different blood temperature conditions on cytokine production and leukocyte surface markers expression in a miniaturized extracorporeal ex vivo circuit.

MATERIALS AND METHODS

Study population

The study was approved by the University of Pittsburgh Institutional Review Board. After consent was obtained, 20 healthy volunteers donated blood for the purpose of these ex vivo experiments. Healthy volunteers were defined as being >18 years old and not pregnant, weighing at least 50 kg, having no history of anemia or hemophilia, and having no history of chronic medical illness or acute infection during the previous 2 weeks.

Ex vivo circuit

Blood was collected from healthy volunteers into standard vacuum-evacuated blood collection tubes containing sodium heparin. Venipuncture was performed in a dedicated room by trained personnel. The experiment consisted of running the blood through three different conditions over a period of 4 h: (i) a miniaturized ex vivo extracorporeal circuit equipped with a blood warmer set to 37°C; (ii) the same circuit without the warmer (blood kept at room temperature ~23°C); and (iii) blood samples placed into a rocking incubator at 37°C (no circuit). These three experimental conditions are represented in Fig. 1.

Components of the extracorporeal circuit were polyethylene tubing (Intramedic, Becton Dickinson and Company, Franklin Lakes, NJ, USA), a 15-mL conical bottom tube to serve as the 10-mL blood reservoir (Becton Dickinson), silicone tubing and a mini-pump (Control Company, Friendswood, TX, USA) set up with a blood flow rate of 0.75 mL/min. Unfractionated heparin (heparin sodium for injection, USP, Hospira, Inc., Lake Forest, IL, USA) was added to blood before the beginning of each experiment in order to obtain a heparin concentration of 10 UI/mL.

Sampling and cytokine assays

Blood samples were pipetted from the circuit blood reservoir or from the tube placed in the incubator at the following time points: baseline, 15 min, 1 h, 2 h, and 4 h. The samples were immediately centrifuged at 1500 rpm for 5 min (temperature of centrifugation = 4°C) and plasma was removed and stored in polypropylene microfuge tubes (Fisher Scientific, Pittsburgh, PA, USA) at -80°C until measurement. Granulocyte macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF), and interleukin (IL)-1 β , IL-6, IL-8, and IL-10 concentrations were measured in duplicate by Luminex bead technology using human inflammatory Five-Plex bead kits combined with human IL-10 bead kits (Invitrogen, Camarillo, CA, USA). Plates were analyzed on a Bio-Rad Bio-Plex 200 protein array system with Bio-Plex Manager 4.0 software (Bio-Rad Laboratories, Hercules, CA, USA). Detection limits were as follows: 3.1 pg/mL for GM-CSF; 6.7 pg/mL for IL-1 β ; 1.6 pg/mL for IL-6; 3.8 pg/mL for IL-8; 2.3 pg/mL for TNF; and 4.1 pg/mL for IL-10.

To assess if the time between blood withdrawal from the healthy volunteers and the start of the experiments (about 20–30 min) had any influence on the cytokine level measurements (blood temperature decreased just after the draw, then increased due to use of warming), we ran an additional experiment in which blood was withdrawn from healthy volunteers and separated in three tubes. One tube was placed immediately in the incubator at 37°C (for the purpose of this subexperiment, draw was performed at the laboratory, in front of the incubator), one tube was placed in the incubator after having been left at room temperature for 1 h, and the remaining tube was left at room temperature for the entire experiment. Samples for cytokine measurements were processed as described above over 4 h.

Flow cytometry assays

Blood samples were pipetted from the circuit blood reservoir or from the tube placed in the incubator at baseline, 2 h, and 4 h. Red cells were lysed with BD Pharm Lyse lysing solution (Becton Dickinson) and washed in 1% bovine serum albumin in phosphate buffered saline. Fc receptors were blocked with excess IgG. Cells to be stained for surface markers were incubated with appropriate antibodies and fixed in 1% paraformaldehyde before analysis on Beckman Coulter XL-MCL. All antibodies were from Becton Dickinson, except hTACE (R&D Systems, Minneapolis, MN, USA) and annexin V (Millipore, Billerica, MA, USA). NFkB cells were treated with Cycletest Plus Kit (Becton Dickinson) according to manufacturer instruction prior to staining with anti-NFkB antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). All data were analyzed with FCS Express (De Novo Software, Los Angeles, CA, USA). Cells were gated by distinctive sizes for polymorphonuclear neutrophils (PMNs) and monocytes. Human leukocyte antigen (HLA)-DR, CD11b, CD11a, CD62L, tumor necrosis factor alpha converting enzyme (TACE), and NFkB expression was measured by the geometric mean of fluorescence intensity. Apoptosis was measured as percent positive for annexin V staining, with propidium iodide excluding necrotic cells.

Statistical analysis

Cytokine measurements were compared between the three groups with the Kruskal–Wallis exact test for the overall difference. If there were significant overall effects, the Wilcoxon rank sum exact test was used for each pairwise comparison. For flow cytometry data, we performed the repeated measured analysis on the natural logarithm transformed data using generalized estimating equations (GEE) approach to account for the correlation between repeated measures. The models included the group, time, and their interactions as independent variables. If the group effect changed significantly over time (i.e., the interaction term was significant), difference between groups was compared at each time point. For the additional subexperiment comparing blood placed immediately at 37°C or after having been left at room temperature for 1 h, we conducted the Wilcoxon signed rank exact test for paired data to see the group effect. Finally, the Wilcoxon signed rank exact test was also performed for NFκB DNA binding comparison. Data are expressed as mean ± SD. A *P* value less than 0.05 was considered statistically significant. Statistical analysis was performed using the SAS software package (SAS Institute, Inc., Cary, NC, USA).

RESULTS

We experienced no clotting issues for any of the experimental conditions at any time in the study. For condition (i), the circuit with the warmer, blood temperature was measured between 28°C at the surface and 37°C at the bottom of the blood reservoir. For condition (ii), the circuit with no warmer, blood temperature was 23°C (room temperature). Finally, in condition (iii), no circuit, incubator, blood temperature was maintained at 37°C.

Cytokine levels increased over time in the three experimental conditions. At room temperature, the increase was insignificant for GM-CSF and IL-10 or very slight for other studied cytokines, from <6.7 to 8.9 ± 3.1 pg/mL for IL-1 β, from <1.6 to 3.3 ± 1.7 pg/mL for IL-6, from 4.5 ± 1.3 to 107 ± 84 pg/mL for IL-8, and from <2.3 to 11 ± 6 pg/mL for TNF. However, for the two other experimental conditions, there was a marked highly significant increase in cytokine levels, reaching at T4h 42 ± 15 pg/mL for GM-CSF, 221 ± 108 pg/mL for IL-1 β, 1126 ± 451 pg/mL for IL-6, 3902 ± 2360 pg/mL for IL-8, and 520 ± 392 pg/mL for TNF in the circuit equipped with the warmer. At T4h, the highest cytokine levels were observed for condition (iii) (incubator only). Cytokine levels were statistically different between conditions at T2h and T4h for all the studied cytokines except for IL-10 levels, which were different between conditions only at T4h (Fig. 2).

Differences in the expression of some surface markers were also observed between condition 2 and the two heated conditions. The difference between these conditions changed significantly over time for CD11b expression by PMNs ($P < 0.0001$), monocyte HLA-DR ($P = 0.0019$), and CD11a expression by PMNs ($P < 0.0001$). No difference was observed between the three conditions for the expression of CD62L and TACE. Evolution of annexin V expression was also different over time between the three groups ($P = 0.0178$ for monocytes and $P = 0.0011$ for PMNs). Leukocyte surface marker expression over time is represented on Fig. 3.

In the additional experiment conducted to determine if variation in temperature was a factor, we did not find any statistical difference in terms of cytokine production between blood samples placed immediately after the draw in the incubator at 37°C or the ones placed in the incubator after having been left at room temperature for 1 h, except for IL-8 and for IL-6 at T1h and T2h (Fig. 4). Again, the least activation was seen with blood maintained at room temperature.

In order to better understand the mechanisms responsible for heat-induced changes in cytokines, we measured NFkB DNA binding. NFkB expression was lower in the room temperature circuit compared with the two heated conditions but this was not statistically significant (Fig. 5).

DISCUSSION

In this ex vivo study, we obtained blood from 20 healthy volunteers and studied cytokine and cell surface marker expression with exposure to a miniaturized extracorporeal circuit. We investigated the effects of blood temperature and found that room temperature resulted in the least cytokine production. We also observed a modification of the profile of several leukocyte surface markers when blood was run through the room temperature circuit compared with blood run through the heated circuit.

It has been known for many years that blood exposure to artificial biomaterials leads to leukocyte activation (7). Protein adsorption onto the material initiates a series of reactions including platelet adhesion, coagulation, and inflammation (17). This inflammatory reaction, part of circuit bioincompatibility, may be harmful by leading to different organ dysfunctions due to direct cytokine effects on tissues (7,8). During the last decade, medical research in this field has therefore been focused on developing different strategies to attenuate this inflammatory response. Heparin was first suggested as the circuit surface coating of choice because of its capacities to reduce cellular and protein activation (18,19). To date, polymeric biomaterials are used for the coating of cardiopulmonary bypass circuits because of their high biocompatibility properties (inhibition of protein adsorption to the interluminal surface of the circuit) (17,20). When a hemofilter is part of the extracorporeal circuit, it has also been proposed to increase the filter pore size to directly remove the additional locally formed cytokines (8,21).

The main goal of the present study was to develop an “optimal” ex vivo miniaturized extracorporeal circuit model that could be useful for further ex vivo studies by providing the least inflammatory activation possible, because this activation may also interfere with or hide the effects of the studied device itself. Hypothermia is a treatment with proven effectiveness for postischemic neurologic injury. Although inhibition of the immune response is not the only mechanism involved in hypothermia’s neuroprotective effects, numerous animal and clinical studies have reported that hypothermia suppresses ischemia-induced inflammatory reactions and release of proinflammatory cytokines (15,22–25). To date, it is still not elucidated how hypothermia is able to reduce cytokine production, but alteration of NFkB pathways has been suggested because NFkB plays a pivotal role in regulating the transcription of cytokines, adhesion molecules, and other mediators involved in the inflammatory response (16,24,26). In our study, the production of all cytokines was reduced in the room temperature circuit, and evolution of the expression of HLA-DR and adhesion molecules such as CD11b and CD11a on PMNs were different compared with heated conditions. Some of our findings can be compared with the ones reported by el Habbal et al. in their work that evaluated temperature effects on neutrophil activation in pediatric extracorporeal circuits. Results of this study were similar to our results finding that cooling decreased upregulation of CD11b and downregulation of L-selectin in this in vitro pediatric cardiopulmonary bypass model (27). Interestingly, we also found that when blood was kept at 37°C, cytokine production started 1 h after the beginning of the experiments, and this delay was the same reported after reperfusion of ischemic brain injury (15).

Studies investigating the effects of blood temperature on inflammation parameters when blood is run through an ex vivo extracorporeal circuit are lacking. Although there are several studies using ex vivo circuits to evaluate different devices such as dialyzers, hemofiltration

membranes, or hemoadsorption cartridges, very little information concerning the settings of the circuits is provided in these articles. Our data can therefore guide these types of experiments. Nevertheless, this study presents some limitations. First, blood temperature in the circuit with the warmer set to 37°C did not reach 37°C. The blood warmer only heated the bottom of the reservoir and therefore temperature observed at the surface of the reservoir was only 28°C. This is the most likely explanation for the difference in terms of cytokine levels between circuit with warmer and blood samples in the incubator, although these differences were not statistically significant (Fig. 2). Second, this study cannot elucidate the mechanistic relationship between cytokine elevation and the modification of leukocyte surface marker expression over time. Third, our data cannot be extrapolated to clinical conditions; however, as stated before this was not the purpose of this experiment. Last but not least, this study is not able to answer whether the observed effects are due to an enhancement of the cytokine response with the heating, or a suppressed response in the room temperature group.

CONCLUSIONS

With the use of this ex vivo model, maintaining body temperature (~37°C) resulted in significant activation of inflammatory cells with cytokine production and modulation of the expression of several cell surface markers involved in leukocyte adhesion or apoptosis. Our data suggest that alteration of the NFκB pathway could be partially responsible for these effects. Due to this “background noise,” heating blood may lead to erroneous conclusions regarding a device evaluation. On the other hand, working at room temperature is not optimal either because hypothermia appears to suppress the leukocyte response and therefore may also mask some specific effects of the device being studied. Consequently, in lieu of recommending one strategy over another, we believe that the main interest of this work resides in reminding investigators that blood temperature is a crucial factor to consider for ex vivo studies using extracorporeal circuits and demonstrating the type of “confounding factor” they will face depending on the blood temperature strategy adopted.

Acknowledgments

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References

1. Collins AJ, Foley RN, Herzog C, et al. Excerpts from the United States Renal Data System 2008 Annual Data Report. *Am J Kidney Dis.* 2009; 53:S1–S374.
2. Guillevin L, Pagnoux C. Indications of plasma exchanges for systemic vasculitides. *Ther Apher Dial.* 2003; 7:155–60. [PubMed: 12918937]
3. Mangano DT, Miao Y, Vuylsteke A, et al. Mortality associated with aprotinin during 5 years following coronary artery bypass graft surgery. *JAMA.* 2007; 297:471–9. [PubMed: 17284697]
4. Bouchard J, Khosla N, Mehta RL. Emerging therapies for extracorporeal support. *Nephron Physiol.* 2008; 109:85–91.
5. Cruz DN, Antonelli M, Fumagalli R, et al. Early use of poly-myxin B hemoperfusion in abdominal septic shock: the EUPHAS randomized controlled trial. *JAMA.* 2009; 301:2445–52. [PubMed: 19531784]
6. Uchino S, Kellum JA, Bellomo R, et al. Acute renal failure in critically ill patients: a multinational, multicenter study. *JAMA.* 2005; 294:813–8. [PubMed: 16106006]
7. Ueyama K, Nishimura K, Nishina T, Nakamura T, Ikeda T, Komeda M. PMEA coating of pump circuit and oxygenator may attenuate the early systemic inflammatory response in cardiopulmonary bypass surgery. *ASAIO J.* 2004; 50:369–72. [PubMed: 15307550]

8. Oudemans-van Straaten HM. Primum non nocere, safety of continuous renal replacement therapy. *Curr Opin Crit Care*. 2007; 13:635–7. [PubMed: 17975382]
9. Cole L, Bellomo R, Davenport P, Tipping P, Ronco C. Cytokine removal during continuous renal replacement therapy: an ex vivo comparison of convection and diffusion. *Int J Artif Organs*. 2004; 27:388–97. [PubMed: 15202816]
10. Cole L, Bellomo R, Davenport P, et al. The effect of coupled haemofiltration and adsorption on inflammatory cytokines in an ex vivo model. *Nephrol Dial Transplant*. 2002; 17:1950–6. [PubMed: 12401852]
11. Haase M, Bellomo R, Baldwin I, et al. The effect of three different miniaturized blood purification devices on plasma cytokine concentration in an ex vivo model of endotoxemia. *Int J Artif Organs*. 2008; 31:722–9. [PubMed: 18825645]
12. Tetta C, Cavaillon JM, Schulze M, et al. Removal of cytokines and activated complement components in an experimental model of continuous plasma filtration coupled with sorbent adsorption. *Nephrol Dial Transplant*. 1998; 13:1458–64. [PubMed: 9641176]
13. Uchino S, Bellomo R, Morimatsu H, et al. Cytokine dialysis: an ex vivo study. *ASAIO J*. 2002; 48:650–3. [PubMed: 12455777]
14. Kato A, Singh S, McLeish KR, Edwards MJ, Lentsch AB. Mechanisms of hypothermic protection against ischemic liver injury in mice. *Am J Physiol Gastrointest Liver Physiol*. 2002; 282:608–16.
15. Polderman KH. Mechanisms of action, physiological effects, and complications of hypothermia. *Crit Care Med*. 2009; 37:S186–202. [PubMed: 19535947]
16. Webster CM, Kelly S, Koike MA, Chock VY, Giffard RG, Yenari MA. Inflammation and NFκB activation is decreased by hypothermia following global cerebral ischemia. *Neurobiol Dis*. 2009; 33:301–12. [PubMed: 19063968]
17. Tanaka M, Motomura T, Kawada M, et al. Blood compatible aspects of poly(2-methoxyethylacrylate) (PMEA)—relationship between protein adsorption and platelet adhesion on PMEA surface. *Biomaterials*. 2000; 21:1471–81. [PubMed: 10872776]
18. Aldea GS, O'Gara P, Shapira OM, et al. Effect of anticoagulation protocol on outcome in patients undergoing CABG with heparin-bonded cardiopulmonary bypass circuits. *Ann Thorac Surg*. 1998; 65:425–33. [PubMed: 9485240]
19. Jansen PG, te Velthuis H, Huybregts RA, et al. Reduced complement activation and improved postoperative performance after cardiopulmonary bypass with heparin-coated circuits. *J Thorac Cardiovasc Surg*. 1995; 110:829–34. [PubMed: 7564452]
20. Courtney JM, Zhao X, Qian H. Biomaterials in cardiopulmonary bypass. *Perfusion*. 1999; 14:263–7. [PubMed: 10456780]
21. Clark WR, Gao D. Low-molecular weight proteins in end-stage renal disease: potential toxicity and dialytic removal mechanisms. *J Am Soc Nephrol*. 2002; 13(Suppl 1):S41–7. [PubMed: 11792761]
22. Aibiki M, Maekawa S, Ogura S, Kinoshita Y, Kawai N, Yokono S. Effect of moderate hypothermia on systemic and internal jugular plasma IL-6 levels after traumatic brain injury in humans. *J Neurotrauma*. 1999; 16:225–32. [PubMed: 10195470]
23. Dietrich WD, Chatzipanteli K, Vitarbo E, Wada K, Kinoshita K. The role of inflammatory processes in the pathophysiology and treatment of brain and spinal cord trauma. *Acta Neurochir Suppl*. 2004; 89:69–74. [PubMed: 15335103]
24. Kimura A, Sakurada S, Ohkuni H, Todome Y, Kurata K. Moderate hypothermia delays proinflammatory cytokine production of human peripheral blood mononuclear cells. *Crit Care Med*. 2002; 30:1499–502. [PubMed: 12130969]
25. Suehiro E, Fujisawa H, Akimura T, et al. Increased matrix metalloproteinase-9 in blood in association with activation of interleukin-6 after traumatic brain injury: influence of hypothermic therapy. *J Neurotrauma*. 2004; 21:1706–11. [PubMed: 15684762]
26. Yenari MA, Han HS. Influence of hypothermia on post-ischemic inflammation: role of nuclear factor kappa B (NFκB). *Neurochem Int*. 2006; 49:164–9. [PubMed: 16750872]
27. el Habbal MH, Carter H, Smith LJ, Elliott MJ, Strobel S. Neutrophil activation in paediatric extracorporeal circuits: effect of circulation and temperature variation. *Cardiovasc Res*. 1995; 29:102–7. [PubMed: 7534645]

FIG. 1.
The three studied experimental conditions: extracorporeal circuit with blood warmer, extracorporeal circuit without the warmer, tube placed in the lab incubator at 37°C (no circuit).

FIG. 2. Serum cytokine levels over time (pg/mL)

Black: Circuit at 23°C. Gray: Circuit at 37°C. White: No circuit, blood sample at 37°C. Data are expressed as mean $\pm$ SD. Statistics: * = $P < 0.05$ with the Kruskal–Wallis exact test for overall three groups effect.

† = $P < 0.05$ with the Wilcoxon rank sum exact test for the comparison between No warmer and Incubator.

‡ = $P < 0.05$ with the Wilcoxon rank sum exact test for the comparison between Warmer and Incubator.

†‡ = $P < 0.05$ with the Wilcoxon rank sum exact test for the comparison between Warmer and No warmer.

FIG. 3. Leukocyte surface markers expression measured by flow cytometry (geometric mean of fluorescence intensity)

Diamonds with continuous lines = Circuit at 23°C. Squares with dashed lines = Circuit at 37°C. Triangles with dotted lines = No circuit, blood sample at 37°C. Data are expressed as mean $\pm$ SD. Statistics: * = $P < 0.05$ for Time $\times$ Group interaction effect with GEE approach. † = $P < 0.05$ for the comparison between No warmer and Incubator at each time point. ‡ = $P < 0.05$ for the comparison between Warmer and Incubator at each time point. ↓ = $P < 0.05$ for the comparison between Warmer and No warmer at each time point.

FIG. 4. Serum cytokine levels over time (pg/mL)

Black: No circuit, 23°C. Gray: No circuit, 37°C immediately after draw. White: No circuit, blood sample left at room temperature (23°C) for 1 h and then incubated at 37°C. Data are expressed as mean $\pm$ SD. Statistics: * = $P < 0.05$ for the comparison between 37°C immediately after draw and blood sample left at 23°C for 1 h and then incubated at 37°C, using the Wilcoxon signed rank exact test.

FIG. 5. NFkB expression measured by flow cytometry (geometric mean of fluorescence intensity)
 Diamonds with continuous lines = Circuit at 23°C. Squares with dashed lines = Circuit at 37°C. Triangles with dotted lines = No circuit, blood sample at 37°C.
 Data are expressed as mean $\pm$ SD.
 Statistics: Wilcoxon signed rank exact test.