

Artificial Surface-Induced Inflammation Relies on Complement Factor 5: Proof From a Deficient Person

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Background. Exposing blood to artificial surfaces results in an inflammatory response, including complement activation and cytokine release. The aim of this investigation was to study complement-dependency and independency in artificial surface-induced inflammation in human whole blood from a patient with a genetic deficiency of complement factor 5 (C5).

Methods. Whole blood from a C5-deficient patient, C5 protein reconstituted blood, and blood from a control subject was used. The complement inhibitor compstatin (C3 inhibitor) and a C5a receptor antagonist were used to block complement. Blood was circulated in closed loops of polyvinyl chloride tubing. Leukocyte CD11b expression and release of granule enzymes (myeloperoxidase, elastase, lactoferrin), cytokines (interleukins, chemokines, and growth factors; $n = 27$) as well as complement activation were measured after incubation.

Results. In C5-deficient blood, there was no formation of the terminal complement complex, as opposed to reconstituted or control blood. Release of granule en-

zymes was partly dependent on C3, revealed by a compstatin-dependent effect in C5-deficient blood, and partly C5a-dependent as evident from the reconstitution and control blood. The chemokines interleukin-8 and monocyte chemoattractant protein-1 were also highly complement dependent, the effect being C5a-mediated, whereas platelet-derived and vascular endothelial growth factors were partly complement dependent. Interferon- γ increased in a complement-independent manner, whereas the rest of the cytokines did not respond to the surface. Leukocyte expression of CD11b was only marginally increased in deficient blood exposed to the surface, whereas reconstitution induced a considerable, C5a-dependent increase, comparable with that of the control.

Conclusions. The polyvinyl chloride surface induced a defined inflammatory response, which largely depended on C5.

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Exposing blood to an artificial surface, as in cardiopulmonary bypass, results in a systemic inflammatory response involving activation of leukocytes, platelets, and plasma cascades, including the complement system. Using specific monoclonal antibodies or peptides that attenuate or totally inhibit complement activation, it has previously been shown that complement activation is necessary for a number of the subsequent inflammatory reactions, since blocking complement activation prevents these secondary responses [1–6].

When studying interactions between inflammatory systems in whole blood, it is crucially important that the experimental conditions enable mutual interactions between the systems. In particular, the choice of anticoagulant in studies of complement involvement in inflammatory processes is crucially important, as several

anticoagulants such as calcium-binding agents and heparin interfere with complement activation and thus are unsuitable for this purpose. We have developed a model using human whole blood anticoagulated with the recombinant hirudin analogue lepirudin, a highly specific thrombin inhibitor that does not interfere with complement activation and therefore enables complement to interact in the inflammatory network [7].

The true role of complement in the inflammatory process can best be studied in blood from complement-deficient persons—nature's own knockouts. Using a broad panel of inflammatory readouts, we have recently examined the *in vitro* inflammatory process elicited by *Escherichia coli* or *Neisseria meningitidis* in whole blood from patients deficient in complement factor 2 or complement factor 5 (C5) [8]. To date, no studies on blood-

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Abbreviations and Acronyms

C3	= complement factor 3
C5	= complement factor 5
C5a	= complement factor 5a
C5aR	= complement factor 5a receptor
C5aRA	= complement factor 5a receptor antagonist
C9	= complement factor 9
EDTA	= ethylenediaminetetraacetic acid
IL	= interleukin
INF- γ	= interferon gamma
MCP	= monocyte chemoattractant protein
PBS	= phosphate buffered saline
PDGF-BB	= platelet-derived growth factor-BB
PVC	= polyvinyl chloride
TCC	= terminal complement complex
VEGF	= vascular endothelial growth factor

biomaterial interactions have been performed with whole blood from such persons. Thus, in the present study, using blood from 1 of these patients, we delineate the role of C5 in the inflammatory response induced by a polyvinyl chloride (PVC) artificial surface, using a model previously well described [9].

Material and Methods*Blood Samples*

The study was approved by the local Ethical Committee. The C5-deficient blood donor, the control person, procedures for blood sampling, and reconstitution of the deficient blood with purified C5 have all been described in detail elsewhere [8]. Briefly, blood sampling was arranged under standard conditions for blood donations and performed by trained intensive care personnel. Blood was collected simultaneously from the deficient patient and the corresponding control and processed in parallel throughout the experiments. To avoid untoward in vitro effects on the complement system, samples were drawn directly into tubes containing lepirudin, as previously described [7]. All incubations were performed twice, on 2 consecutive days.

Reagents

The equipment and reagents used in the experiments were endotoxin-free, as certified by the manufacturers. Lepirudin (Refludan), purchased from Pharmion ApS (Copenhagen, Denmark), was diluted to 2.5 mg/mL in phosphate-buffered saline (PBS) and used as anticoagulant at a final concentration of 50 μ g/mL whole blood. Ethylenediaminetetraacetic acid (EDTA), 10 mM final blood concentration was used to block further complement activation after the designated incubation periods. Sterile PBS with Ca^{2+} and Mg^{2+} (cat. no. D8662) and PBS without Ca^{2+} and Mg^{2+} (cat. no. D8537) were obtained from Sigma (St Louis, MO). The PBS for flow cytometry assays was purchased from Gibco, Invitrogen Corporation (Paisley, UK). A 4% stock solution made of paraformaldehyde was purchased from Sigma.

Bovine serum albumin 30% was purchased from Biotest (Dreieich, Germany).

For reconstitution experiments we used purified human complement protein C5 from Quidel (San Diego, CA). The protein was added to blood to give a final plasma concentration of 80 μ g/mL, corresponding to the concentration of C5 in normal persons. The functional activity of the complement system was tested with the Wielisa-kit COMPL 300 (Euro-Diagnostica, Malmö, Sweden). The Wielisa-kit is an enzyme immunoassay for simultaneous qualitative determination of the functional activity of the classical, lectin, and alternative complement pathways, using the incorporation of complement factor 9 (C9) into the terminal complex as a readout for all pathways [10]. The procedure was performed according to the manufacturer's instructions. The assay documented that the complement defect was restored to functional efficacy after reconstitution with the purified component [8].

COMPLEMENT INHIBITORS. The complement 3 (C3) inhibitor compstatin (Ac-I[CV(1MeW)QDWGAHRC]T), a 13-amino acid cyclic peptide that binds to C3 and inhibits its cleavage [11], and the C5a receptor antagonist AcF(OPdChaWR) [C5aRA] previously shown to efficiently block the C5a receptor [12], were produced as previously described [11, 12], and used at a final concentration of 25 mM and 10 μ M, respectively. A peptide (AcF[OPdChaAdR]) with no C5a receptor inhibitory activity was used as control at 25 mM. All complement components and inhibitors, as well as the PBS used in the blood incubations were analyzed for lipopolysaccharide contents using the LAL-assay QCL-1000 from BioWhittaker (Walkersville, MD) and were found to have endotoxin concentration in the low-picogram range.

EXPERIMENTAL SETUP. The model for artificial surface-induced inflammation has been described in detail previously [9]. The artificial surface used in the study was PVC, a surface regularly encountered in various clinical settings, such as cardiopulmonary bypass. The PVC tubing was provided by Carmeda AB (Stockholm, Sweden). Samples of blood were supplied with inhibitors or equal volumes of saline and incubated at 37°C for 4 minutes. A volume of 750 μ L blood was then transferred to segments of PVC tubing (length 30 cm, internal diameter 3 mm). Each segment was closed end to end and incubated by rotating slowly at 37°C. For analysis of CD11b, the incubation period was 15 minutes; for release of enzymes, cytokines, and complement activation it was 4 hours. After 4 hours, EDTA was added to halt further complement activation, and the blood was centrifuged for 15 minutes, 3,220g at 4°C. The plasma was frozen in aliquots at -70°C for later analysis.

ENZYME IMMUNOASSAYS. Assays for quantification of the granulocyte enzymes lactoferrin and elastase in plasma were purchased from Hycult biotechnology b.v. (Uden, Netherlands), and analysis of plasma concentration of myeloperoxidase was done using an assay from R&D

Systems (Minneapolis, MN). All assays were performed according to instructions from the manufacturer.

Activation of the terminal complement pathway was determined in an enzyme immunoassay using the monoclonal antibody aE11 specific for a C9 neoepitope in the sC5b-9 complex. The assay used is a modification of an assay described in detail previously [13].

CYTOKINE ASSAY. Plasma samples were analyzed using a multiplex cytokine assay (Bio-Plex Human Cytokine 27-Plex Panel; Bio-Rad Laboratories, Hercules, CA) containing the following analytes: interleukin (IL) 1 beta (IL-1 β), IL-1 receptor antagonist (IL-1Ra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8 (CXCL8), IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin (CCL11), basic fibroblast growth factor, granulocyte colony stimulating factor, granulocyte-macrophage colony stimulating factor, interferon gamma (IFN- γ), chemokine (C-X-C motif) ligand 10 (IP-10 or CXCL10), monocyte chemoattractant protein 1 (MCP-1, or CCL2), macrophage inflammatory protein-1-alpha (MIP-1 α , or CCL3), macrophage inflammatory protein-1-beta (MIP-1 β , or CCL4), platelet-derived growth factor-BB (PDGF-BB), regulated upon activation T-cell expressed and secreted (RANTES, or CCL5), tumor necrosis factor- α , and vascular endothelial growth factor (VEGF). The analysis was performed according to the instructions from the manufacturer.

FLOW CYTOMETRIC STUDIES OF CD11B. Flow cytometric studies were performed on a FACScalibur from Becton Dickinson (San Jose, CA). Immediately after the 15-minute incubation, blood (100 μ L) was fixed with 100 μ L 0.5% paraformaldehyde for 4 minutes at 37°C, and 25 μ L fixed

blood was then incubated for 15 minutes at room temperature in the dark with anti-CD11b-PE or the isotype control IgG2a-PE. For gating, anti-CD14 PerCP was added to both mixtures. All antibodies were from Becton Dickinson. Samples were lysed for 5 minutes with 2 mL 0.16 M NH₄Cl, with 10 mM NaHCO₃, 0.12 mM EDTA, and 0.04% paraformaldehyde in the dark. The samples were centrifuged and washed once with PBS at 300g for 5 minutes at 4°C, then reconstituted with PBS containing 1% albumin and placed on ice until acquisition. Granulocytes and monocytes were gated in a SSC/anti-CD14-dotplot, and the median fluorescent intensity values for CD11b were calculated.

Statistics

Genetic complement deficiencies are rare, in particular C5 deficiency which has only been described in approximately 40 patients worldwide [14]. The design of the study thus precluded traditional statistical handling of the material [8].

Results

Complement Activation

Complement activation was assessed by measuring the formation of the soluble terminal complement complex (TCC). As expected, in blood from the C5-deficient patient, there was no formation of TCC in PVC loops (Fig 1). Reconstitution of the blood with purified C5 gave a marked increase in TCC formation. This increase was attenuated by the C3 convertase inhibitor compstatin,

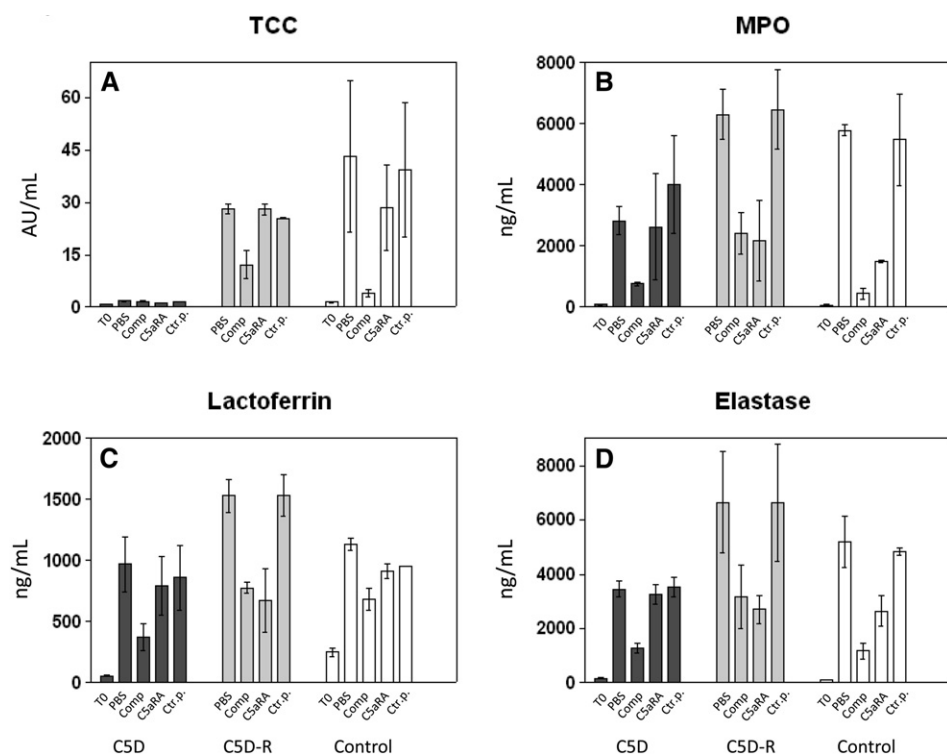


Fig 1. Complement activation and neutrophil degranulation in C5-deficient blood (C5D), C5-deficient blood reconstituted with C5 (C5D-R) and healthy control blood (Control) exposed to polyvinyl chloride. Blood was circulated for 4 hours in closed loops followed by quantification of the (A) terminal complement complex (TCC [AU/mL]), (B) myeloperoxidase (MPO [ng/mL]), (C) lactoferrin (ng/mL), and (D) elastase (ng/mL). Data are presented as mean and range of two separate experiments on two consecutive days. (T0 = baseline values; PBS = phosphate-buffered saline; Comp = compstatin [25 μ M]; C5aRA = C5a receptor antagonist [10 μ M]; Ctr.p. = control peptide [25 μ M].)

whereas the C5a receptor antagonist or the control peptide had no effect. The response in reconstituted blood was similar to that in blood from the control donor.

Neutrophil Degranulation

The concentration of myeloperoxidase, lactoferrin, and elastase increased markedly when blood was circulated in the PVC loops (Fig 1). The increase was most pronounced in reconstituted and control blood, but a substantial increase was also present in C5-deficient blood. Inhibition of C3 by compstatin attenuated the increase seen in C5-deficient blood, whereas the C5a receptor antagonist had no effect, consistent with a C3-dependent release of the enzymes. In reconstituted and control blood, however, the C5aRA also effectively attenuated the release, although not to the same extent as compstatin.

Cytokines and Chemokines

INTERLEUKIN-8. Circulation of C5-deficient blood for 4 hours in PVC loops induced no increase in the concentration of IL-8 (Fig 2). This was in contrast to reconstituted blood and control blood where there was a marked increase (approximately 10-fold compared with baseline) in IL-8 concentration. This increase could be almost completely blocked by compstatin and the C5aRA, reducing IL-8 concentrations almost to baseline values.

MONOCYTE CHEMOATTRACTANT PROTEIN-1. Circulation of C5-deficient blood for 4 hours in PVC loops induced no increase in the concentration of MCP-1 (Fig 2). This was in contrast to reconstituted blood where there was a marked (100-fold) increase in the MCP-1 concentration

after incubation. The increase was even more pronounced in control blood, and both in reconstituted and control blood the increase could be completely blocked by compstatin or C5aRA.

PLATELET-DERIVED GROWTH FACTOR-BB AND VEGF. The two growth factors PDGF-BB and VEGF increased after incubation compared with baseline (Fig 2). Reconstitution of deficient blood gave a modest increase in PDGF-BB, whereas VEGF increased markedly to levels of the control blood. Compstatin and C5aRA substantially inhibited the response, most pronounced for VEGF.

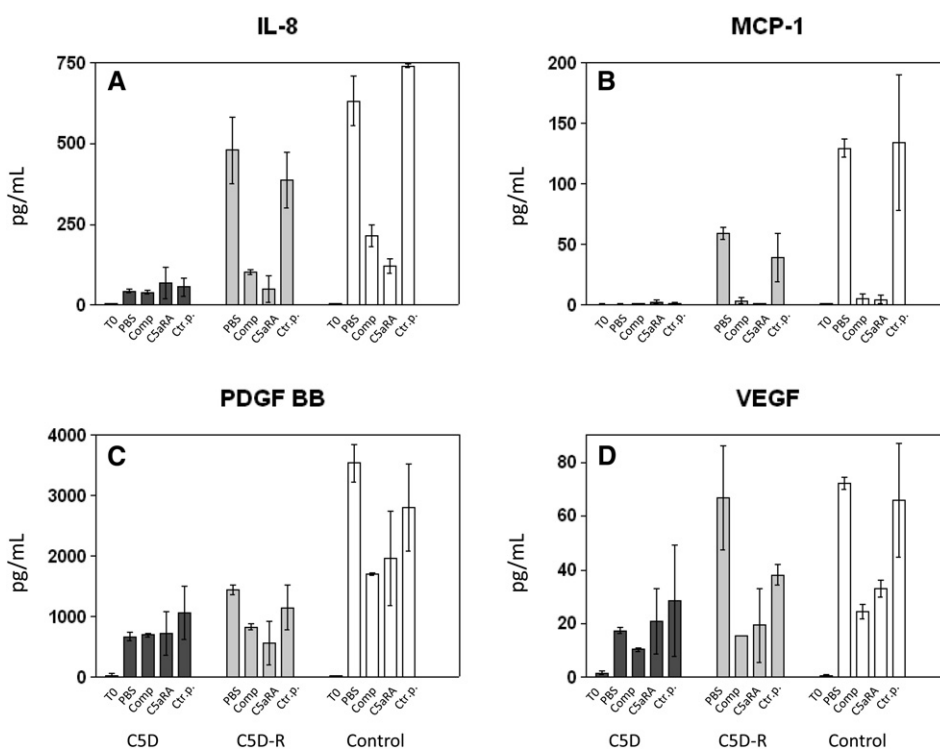
INTERFERON- γ . The concentration of IFN- γ increased fourfold to fivefold when blood was incubated in PVC loops for 4 hours (data not shown). That was true both for C5-deficient blood and for control blood. Reconstitution of deficient blood or complement inhibition did not affect this increase, indicating that the increase in IFN- γ was complement independent.

OTHER CYTOKINES. The concentration of rest of the cytokines (n = 22 of 27; see Material and Methods) were very low at baseline and, if measurable, did not increase after incubation in the PVC loops.

Leukocyte CD11b Expression

The expression of CD11b on granulocytes and monocytes in C5-deficient blood after circulating in PVC tubing for 15 minutes was very low (Fig 3). When reconstituted with C5, the PVC-induced expression in deficient blood increased to levels comparable to that of control blood. The CD11b expression in reconstituted blood was partly reduced by compstatin and completely with the C5aRA,

Fig 2. Release of cytokines: (A) release of IL-8 (interleukin 8), (B) MCP-1 (monocyte chemoattractant protein 1), (C) PDGF-BB (platelet-derived growth factor-BB), and (D) VEGF (vascular endothelial growth factor) in C5-deficient blood (C5D), C5-deficient blood reconstituted with C5 (C5D-R), and healthy control blood (Control) exposed to polyvinyl chloride. Blood was circulated for 4 hours in closed loops. Data are presented as mean and range of two separate experiments on two consecutive days. (T0 = baseline values; PBS = phosphate-buffered saline; Comp = compstatin [25 μ M]; C5aRA = C5a receptor antagonist [10 μ M]); Ctr.p. = control peptide [25 μ M].)



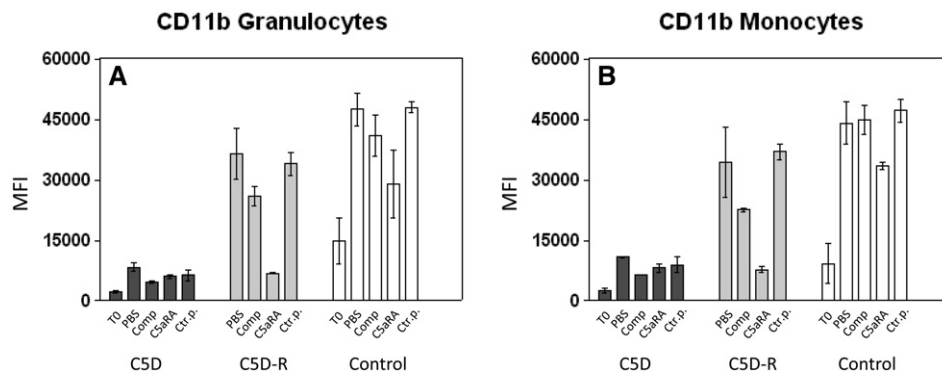


Fig 3. Leukocyte expression of CD11b was determined by median fluorescent intensity (MFI) in flow cytometry on (A) granulocytes and (B) monocytes in C5-deficient blood (C5D), C5-deficient blood reconstituted with C5 (C5D-R), and healthy control blood (Control) exposed to polyvinyl chloride. Blood was circulated for 15 minutes in closed loops. Data are presented as mean and range of two separate experiments on two consecutive days. (TO = baseline values; PBS = phosphate-buffered saline; Comp = compstatin [25 μ M]; C5aRA = C5a receptor antagonist [10 μ M]; Ctr.p. = control peptide [25 μ M].)

whereas these effects were less pronounced in the control blood.

Comment

It has previously been demonstrated that contact between blood and an artificial surface, such as PVC, induces an inflammatory response, including leukocyte expression of surface markers such as CD11b, release of neutrophil granule content, and synthesis and release of cytokines [15-18]. Through the use of specific complement inhibitors, it has also been shown that a number of these reactions are secondary to complement activation [1, 2, 6, 19, 20]. Applying another method, the use of blood from complement-deficient persons, we have recently delineated the role of complement in the *in vitro* inflammatory response to *E coli* and *N meningitidis* [8, 21]. In the present report, we confirm and extend a number of previous findings regarding artificial surface-induced inflammation through the use of this unique experimental approach—blood from a C5-deficient patient. Our findings confirm the crucial role of C5 in the artificial surface-induced inflammatory response.

It should be noted that the present model of artificial surface-induced inflammation is restricted to studying the interaction between blood and the artificial surface itself, and that the results cannot directly be extrapolated to an *in vivo* situation such as cardiopulmonary bypass, which in addition to the artificial surface includes several other inflammatory stimuli. It is, nevertheless, important to reveal the mechanisms by which a surface induces an inflammatory reaction, and for this purpose, we claim that the present model currently is the most reliable to investigate the interaction between inflammatory systems in human whole blood *in vitro*. Furthermore, the use of lepirudin as anticoagulant makes it possible to study in detail the role of the complement system in the inflammatory response, as this anticoagulant, in contrast to, for example, heparin, EDTA, or citrate, does not interfere with complement activation [7]. Blood from the

C5-deficient donor has previously been shown to lack complement activity in the Wielisa test [8]. In accordance with this, there was no formation of the terminal complement complex in C5-deficient blood circulated in PVC loops, as this formation requires the presence of C5. Formation of TCC in reconstituted and control blood was of the magnitude previously observed in this model [3], confirming the feasibility of this approach.

The artificial surface induced a marked release of granulocyte enzymes. This was true also for C5-deficient blood, although less pronounced. Notably, most of the release induced in C5-deficient blood could be inhibited by compstatin, indicating a C3-specific release. In reconstituted and control blood, the effect of compstatin was more pronounced than that of the C5aRA, consistent with this notion. This is partly, but not completely, in accordance with a recent finding using Gram-negative bacteria as inducers of inflammation, where the enzyme release was almost totally C3 dependent and could not be blocked by the C5aRA [8]. Neither in deficient nor in reconstituted or control blood could complement inhibition completely reverse the enzyme release, consistent with a certain complement-independent effect. Collectively, these data emphasize the importance of using diverse inflammatory stimuli when determining the role of complement in the inflammatory response, and interpret the data related to the model, as different stimuli may induce different activation patterns.

We have previously shown that the induction of cytokine synthesis by the artificial surface is not random but that the chemokines and growth factors are generally more inducible than the classical cytokines [4, 6]. That was confirmed in the present study where 5 of 27 cytokines were induced by the surface. Thus, the present data confirm that induction of synthesis and release of inflammatory markers in human whole blood by the PVC surface is selective in the way that some markers show a considerable increase upon stimulation, some a more modest response, and some markers do not increase at all. This finding is in line with our previous findings that

IL-8 and MCP-1 increased, whereas IL-1 β , IL-6, IL-10, and tumor necrosis factor- α were virtually unaffected after incubation in PVC tubing [4]. We now show, using the broad panel of mediators, that this differential increase follows a pattern where, in particular, certain chemokines (IL-8, MCP-1) and growth factors (VEGF, PDGF-BB) were induced whereas most of the other cytokines were not. It is reasonable to speculate that the pattern-recognition receptors responding to microorganisms through Toll-like receptors, in particular the CD14/MD2/TLR4 complex, is not stimulated by this artificial surface. In contrast, complement is directly activated by the surface and generates anaphylatoxins, in particular C5a, which is most likely responsible for a major fraction of the inflammatory mediators generated in this system [2, 4, 20].

In addition to variations in inducibility, the inflammatory markers also differed with respect to the degree of complement dependence. Notably, however, there was a marked effect of complement inhibition on the majority of the mediators induced by the PVC surface. We have previously shown that compstatin effectively blocks complement activation in the present model [3, 20]. In this report, we confirm that compstatin is a very efficient inhibitor of complement, and through this, it was possible to block formation by nearly 100% for IL-8 and MCP-1. Formation of PDGF-BB and VEGF was also markedly inhibited (by 50% to 70%), whereas the release of IFN- γ was complement-independent. Expression of CD11b is a fast response in leukocytes challenged by an artificial surface, an incubation period of 15 minutes being sufficient for a considerable increase. There was only a minor increase in C5-deficient blood, whereas reconstitution markedly restored CD11b-expression both in granulocytes and monocytes to a response similar to that seen in control blood. In accordance with previous studies [3], the response in granulocytes was crucially dependent on C5a, as the C5aRA reduced CD11b expression close to baseline. The effect of C5aRA was more prominent than the effect of compstatin, and granulocytes seemed to be somewhat more complement dependent than monocytes. The latter observation is in accordance with similar findings using bacteria to induce inflammation [8]. The present study has an obvious limitation in the sense that the inflammatory stimulus is restricted to the artificial PVC tubing surface alone. Thus, the results are obtained in an in vitro model with numerous differences compared with a clinical situation with coronary bypass, and immediate extrapolation is not appropriate. However, this is also a strength of the study as it delineates the inflammatory effects of the surface itself and not the combined effects of surface, pump, oxygenator, and surgical trauma. If the perioperative inflammatory reaction is to be reduced, detailed knowledge regarding the contribution of the individual factors is important.

In conclusion, using whole blood from a C5-deficient patient, we present data that reveal a defined and complement-dependent inflammatory reaction upon contact with PVC, comprising chemokines (IL-8, MCP-1), growth

factors (VEGF, PDGF-BB), and leukocyte activation (enzyme release of CD11b expression). The data emphasize the central role of complement in general, and C5a in particular, in the inflammatory response to biomaterials. Furthermore, the data support the idea of complement inhibition as an interesting therapeutic approach in attenuating an inappropriate inflammatory reaction.

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References

1. Rinder CS, Rinder HM, Smith BR, et al. Blockade of C5a and C5b-9 generation inhibits leukocyte and platelet activation during extracorporeal circulation. *J Clin Invest* 1995;96:1564-72.
2. Rinder CS, Smith MJ, Rinder HM, et al. Leukocyte effects of C5a-receptor blockade during simulated extracorporeal circulation. *Ann Thorac Surg* 2007;83:146-52.
3. Lappegard KT, Fung M, Bergseth G, et al. Effect of complement inhibition and heparin coating on artificial surface-induced leukocyte and platelet activation. *Ann Thorac Surg* 2004;77:932-41.
4. Lappegard KT, Fung M, Bergseth G, Riesenfeld J, Mollnes TE. Artificial surface-induced cytokine synthesis: effect of heparin coating and complement inhibition. *Ann Thorac Surg* 2004;78:38-44.
5. Fitch JC, Rollins S, Matis L, et al. Pharmacology and biological efficacy of a recombinant, humanized, single-chain antibody C5 complement inhibitor in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass. *Circulation* 1999;100:2499-506.
6. Lappegard KT, Bergseth G, Riesenfeld J, et al. The artificial surface-induced whole blood inflammatory reaction revealed by increases in a series of chemokines and growth factors is largely complement dependent. *J Biomed Mater Res A* 2008;87:129-35.
7. Mollnes TE, Brekke OL, Fung M, et al. Essential role of the C5a receptor in *E coli*-induced oxidative burst and phagocytosis revealed by a novel lepirudin-based human whole blood model of inflammation. *Blood* 2002;100:1869-77.
8. Lappegard KT, Christiansen D, Pharo A, et al. Human genetic deficiencies reveal the roles of complement in the inflammatory network: lessons from nature. *Proc Natl Acad Sci USA* 2009;106:15861-6.
9. Mollnes TE, Riesenfeld J, Garred P, et al. A new model for evaluation of biocompatibility: combined determination of neoepitopes in blood and on artificial surfaces demonstrates reduced complement activation by immobilization of heparin. *Artif Organs* 1995;19:909-17.
10. Seelen MA, Roos A, Wieslander J, et al. Functional analysis of the classical, alternative, and MBL pathways of the complement system: standardization and validation of a simple ELISA. *J Immunol Methods* 2005;296:187-98.
11. Katragadda M, Magotti P, Sfyroera G, Lambris JD. Hydrophobic effect and hydrogen bonds account for the improved activity of a complement inhibitor, compstatin. *J Med Chem* 2006;49:4616-22.
12. Finch AM, Wong AK, Paczkowski NJ, et al. Low-molecular-weight peptidic and cyclic antagonists of the receptor for the complement factor C5a. *J Med Chem* 1999;42:1965-74.

13. Mollnes TE, Lea T, Harboe M. Detection and quantification of the terminal C5b-9 complex of human complement by a sensitive enzyme-linked immunosorbent assay. *Scand J Immunol* 1984;20:157-66.
14. Delgado-Cervino E, Fontan G, Lopez-Trascasa M. C5 complement deficiency in a Spanish family. Molecular characterization of the double mutation responsible for the defect. *Mol Immunol* 2005;42:105-11.
15. Asberg AE, Videm V. Activation of neutrophil granulocytes in an in vitro model of a cardiopulmonary bypass. *Artif Organs* 2005;29:927-36.
16. Edmunds LH. Inflammatory response to cardiopulmonary bypass. *Ann Thorac Surg* 1998;66(Suppl):12-6.
17. Solberg R, Scholz T, Videm V, Okkenhaug C, Aasen AO. Heparin coating reduces cell activation and mediator release in an in vitro venovenous bypass model for liver transplantation. *Transpl Int* 1998;11:252-8.
18. Videm V, Mollnes TE, Fosse E, et al. Heparin-coated cardiopulmonary bypass equipment. I. Biocompatibility markers and development of complications in a high-risk population. *J Thorac Cardiovasc Surg* 1999;117:794-802.
19. Lappegard KT, Bergseth G, Riesenfeld J, Sexton J, Mollnes TE. Role of granulocytes and monocytes in the polyvinyl chloride-induced synthesis of interleukin 8, monocyte chemoattractant protein 1, and leukotriene B4. *J Biomed Mater Res A* 2005;74:230-6.
20. Lappegard KT, Riesenfeld J, Brekke OL, Bergseth G, Lambris JD, Mollnes TE. Differential effect of heparin coating and complement inhibition on artificial surface-induced eicosanoid production. *Ann Thorac Surg* 2005;79:917-23.
21. Hellerud BC, Aase A, Herstad TK, et al. Critical roles of complement and antibodies in host defense mechanisms against *Neisseria meningitidis* as revealed by human complement genetic deficiencies. *Infect Immun* 2010;78:802-9.

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